NATIONAL EYE INSTITUTE

ANNUAL REPORT

FISCAL YEAR 1992

Cover Photo

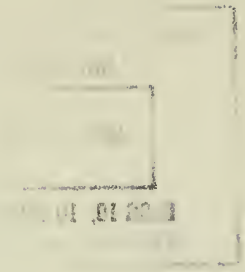
Photomicrograph of a rhopalium within a formalin-fixed cubomedusan jellyfish (*Tripedalia cystophora*). The rhopalium is attached to a stalk within a depression on the surface of the jellyfish. It contains a larger (top) and smaller (right) complex eye at right angles to one another as well as a statocyst (balancing organ). Each eye has a protruding cellular lens, retina, and pigmented epithelium. The picture was taken by Dr. Toichiro Kuwabara and was described in Piatigorsky, Horwitz, Kuwabara, and Cutress (1989) *J. Comp. Physiol. A* 164:577–587.

NATIONAL EYE INSTITUTE

ANNUAL REPORT of Program activities

FISCAL YEAR 1992

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health



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Statement of the Institute Director

Statement of the Institute Director

Carl Kupfer, M.D.

Vision researchers supported by the National Eye Institute (NEI) once again have had a highly productive year. They have made outstanding progress in our effort to improve the prevention, diagnosis, and treatment of diseases and disorders that affect the eye and vision. Results from important clinical trials were reported this year, and several basic research studies have provided information that greatly improves our understanding of some of the pathological processes associated with blinding eye diseases.

Clinical trial results were released recently from the NEI-supported Herpetic Eye Diseases Study (HEDS), a randomized, controlled clinical trial designed to evaluate whether oral acyclovir, given to patients in combination with steroid and antiviral eye drops, improves the management of active herpes simplex stromal keratitis. HEDS investigators reported that oral acyclovir is no better than a placebo in treating the condition. However, the role of acyclovir in preventing recurrences and progression to a more severe form of the disease is still being examined by the HEDS research group.

Results also were reported from the NEI-sponsored Collaborative Corneal Transplantation Study (CCTS), which was designed to evaluate whether donor-recipient tissue typing helps to prevent transplant rejection. CCTS researchers found that people who received corneal transplants with well-matched antigens did not fare significantly better than those people receiving transplants without such a match. This will eliminate the need for costly tissue typing.

Recently, researchers supported by the NEI demonstrated that α-crystallin belongs to a class of proteins called molecular chaperons. Laboratory tests have demonstrated that the biological function of α-crystallin may be to maintain the lens structural proteins in a transparent state. With this important discovery, it is now possible to investigate a number of long-standing questions in lens biology and cataractogenesis and to examine directly whether certain age-related lens changes can be prevented or reversed.

The NEI-sponsored Optic Neuritis Treatment Trial (ONTT) compared oral corticosteroid (ie, prednisone), intravenous corticosteroid methylprednisolone followed by oral prednisone, and placebo for the treatment of new cases of optic neuritis. ONTT investigators found that oral prednisone, the most common treatment for the disease, is ineffective when used alone in treating the disease and actually increases a person's risk for future attacks.

The Age-Related Eye Disease Study has begun and will provide information on the development (incidence), course, and progression of age-related macular abnormalities and lens opacities. The study also will evaluate various possible risk factors for the development and/or progression of the two disorders, while another arm of the study will assess the effects of nutritional supplements and/or minerals on the development and progression of age-related macular degeneration and cataract.

The first two National Eye Health Education Program public service campaigns were released in January. The glaucoma campaign—consisting of print, radio, and television public service announcements (PSAs)—and the diabetic eye disease campaign—using print PSAs—targeted potential audiences at risk for these diseases totaling more than 60 million people. The glaucoma television PSAs were aired more than 25,000 times in 47 States by 239 stations, representing more than $2.4 million in air time. The radio PSAs were aired more than 30,000 times in 49 States by 360 stations, representing $2.7 million worth of air time.

Intramural investigators have developed the ability to produce large numbers of retinal pigment epithelial (RPE) cells that have been used in ongoing transplantation experiments. They also have developed a microinjection technique that allows placement of RPE cells into the proper space beneath the retina. Studies are currently under way to develop molecularly altered RPE cells that do not evoke an immune response and that, therefore, could be used as universal donors.

The role of various modifications of lens crystallins in the development of cataract is being investigated by NEI investigators. The primary focus is on metal-catalyzed oxidation, and gamma crystallin is being used as a model system. Using this system, these scientists have isolated a protein from bovine lenses that protects from this type of oxidation. Information from these types of studies may ultimately lead to the development of the means to prevent cataract formation in humans.

Intramural scientists are using subtractive cloning to identify genes that are predominantly or exclusively expressed in ocular tissues. Retina-specific genes located on the short arm of the X chromosome are being localized to determine whether they are disease linked.

These are but a few of the highlights of the activities of NEI-supported extramural and intramural scientists during Fiscal Year 1992. The following reports provide more detail on these and other activities and accomplishments of each of the NEI's sections, branches, laboratories, offices, and programs during this past year.

Carl Kupfer, M.D.

Extramural and Collaborative Program

Report of the Associate Director for the Extramural and Collaborative Program

Jack A. McLaughlin, Ph.D.

Extramural research activities supported by the Vision Research Program address the leading causes of blindness and impaired vision in the United States, including retinal diseases, corneal diseases, cataract, glaucoma, strabismus, and amblyopia. The program seeks to increase understanding of the normal development and function of the visual system; to understand the causes of, as well as better diagnose, prevent, and treat, sight-threatening conditions; and to enhance the rehabilitation, training, and quality of life of individuals who are partially sighted or blind.

In Fiscal Year 1992 the National Eye Institute (NEI) supported 1,241 research grants, 262 training awards, and 15 contracts for a total of $229,330,000. Individual expenditures in those areas were as follows:

Research Grants	$216,957,000
Research Training Awards	7,294,000
Research Contracts	5,079,000
Total	$229,330,000

The NEI continues to emphasize investigator-initiated research with this group of mechanisms, accounting for 78% of the extramural budget. The following are highlights of the activities and accomplishments of NEI-supported extramural investigators.

Retinal and Choroidal Diseases

The retina, a delicate, light-sensitive tissue at the back of the eye, initiates the process of vision and transmits visual messages to the brain via the optic nerve. Collectively, diseases and disorders of the retina are the leading cause of blindness in the United States.

Diabetic Retinopathy

Approximately one-half of the estimated 14 million people in the United States who have diabetes experience at least early signs of diabetic retinopathy. Of this group, about 700,000 have serious retinal disease, and 8,000 people are blinded by this disorder annually. Generally, the longer one has had diabetes, the greater is one's chances of developing diabetic retinopathy.

Laser surgery is now being used successfully to treat two forms of diabetic retinopathy: (1) macular edema, in which a central portion of the retina swells and blurs vision, and (2) proliferative retinopathy, in which abnormal, easily ruptured, new blood vessels grow onto the surface of the retina. The series of clinical trials that proved the value of laser treatment for diabetic retinopathy were supported by the NEI. The cost savings to the Federal Government associated with improved detection and treatment of diabetic retinopathy and diabetic macular edema have been estimated to exceed $100 million annually.

Opportunities for research include studies of newly discovered growth factors that control blood vessel growth, regulation of retinal blood flow, and new drugs and molecular genetic techniques to control retinal metabolism.

Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is an important and growing public health problem; it is the leading cause of legal blindness for older Americans. By 1995 it is estimated that 1.7 million Americans will have decreased vision from AMD and 100,000 will be blind from the disease. The prevalence of decrease vision from AMD is expected to rise to 2.6 million by the year 2020, when an even greater percentage than now of our population will enter its retirement years. AMD destroys photoreceptor cells in the macula, the small specialized area of the central retina that is responsible for sharp, crisp vision. As the disease progresses, the acute vision that is needed for reading, driving a car, and

other daily activities is diminished, sometimes gradually, sometimes catastrophically. Unfortunately, there is no proven way to either prevent AMD or treat the vast majority of people who have the disease.

An important new goal of the Age-Related Eye Disease Study is to evaluate the effect of high-dose antioxidant vitamins and zinc on the progression of AMD. Patients will be randomized to a high-dose dietary supplement or placebo and followed for a minimum of 7 years to assess AMD progression and cataract formation.

NEI-funded basic scientists are searching for the cause of AMD. Molecular genetic approaches seem most likely to provide useful information about AMD, since there are no precise animal models for this disease.

Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a diverse group of hereditary retinal diseases that causes a progressive degeneration of the rod and cone photoreceptors and loss of visual function. RP affects approximately 100,000 people in the United States. Currently, no effective treatments are available for the common types of RP.

Molecular genetic techniques have enabled the identification of gene defects responsible for several forms of RP. NEI-supported scientists have found that some RP patients carry mutations in the gene encoding rhodopsin, the visual pigment critical for initiating the visual process. Other forms of RP can be investigated using these techniques. In parallel studies, transgenic mice have been produced that carry the human gene for one form of RP. Expression of this gene in mice causes a retinal degeneration that is similar to that seen in humans. Further study of this animal model has enormous potential for delineating the precise molecular and cellular mechanisms involved in the pathogenesis of RP. These types of studies have begun to provide the first real clues to understanding the mechanisms by which RP causes blindness. They are important first steps in the development of rational treatment strategies.

Ocular Complications of AIDS

Cytomegalovirus (CMV) retinitis is a potentially blinding disease of the retina that affects about 20% of people with AIDS (acquired immunodeficiency syndrome). The NEI supports a network of investigators with expertise in AIDS clinical research, retinal diseases, and clinical trial methodology to expedite the testing of new treatments for CMV retinitis and other ocular complications seen in AIDS patients. This network is called the Studies of the Ocular Complications of AIDS (SOCA).

The CMV Retinitis Retreatment Trial is the second multicenter clinical trial conducted by SOCA investigators. The study's primary objective is to compare the safety and efficacy of three therapeutic regimens for AIDS-related CMV retinitis in patients who were treated with foscarnet or ganciclovir and whose retinitis progressed or recurred. The three regimens under investigation are (1) high-dose foscarnet, (2) high-dose ganciclovir, and (3) combination treatment with standard doses of foscarnet and ganciclovir.

Retinal Detachment

The Silicone Oil Study, a NEI-sponsored multicenter clinical trial, was designed to evaluate the benefits and risks of using a long-acting gas or silicone oil as an aid in reattaching the retina after intraocular surgery for severe proliferative vitreoretinopathy (PVR). The study found that use of silicone oil is superior to use of long-acting gas in PVR treatment, resulting in a higher rate of successful retinal reattachment.

Corneal Diseases

Diseases and injury to the cornea are the leading causes of visits to clinicians for eye care in the United States and rank among the most painful of eye conditions. NEI-supported research on corneal diseases includes studies of ocular infections and inflammation, problems with tears and the ocular surface, refractive errors, corneal transplantation, and corneal wound healing.

Ocular Herpetic Infection

Second only to trauma as the leading cause of corneal blindness in the United States today, ocular herpes simplex virus keratitis accounts for 500,000 reported cases yearly. Ocular herpes infections often begin as painful sores on the surface of the cornea

(ie, the transparent tissue at the front of the eye). Like herpes cold sores, these ocular lesions may recur periodically. Over time, the herpes virus may cause an inflammation deep inside the cornea. This deep infection is known as herpes simplex stromal keratitis, a condition which can lead to severe corneal scarring, inflammation of the interior of the eye, and even blindness. This disease affects otherwise healthy, productive individuals, leading to long periods of disability and the need for chronic care. The costs associated with ocular herpetic infection are significant, totaling approximately $100 million per year in direct medical costs, not counting the costs associated with loss of work.

The NEI-supported Herpetic Eye Diseases Study (HEDS) is a randomized, controlled clinical trial designed, in part, to evaluate whether oral acyclovir, given to patients in combination with steroid and antiviral eye drops, improves the management of active herpes simplex stromal keratitis. HEDS investigators recently reported that oral acyclovir is no more effective than a placebo in treating the condition. This indicates that the significant costs associated with the use of oral acyclovir can be avoided. The role of acyclovir in preventing recurrences of herpetic eye diseases over the course of 1½ years and the role of acyclovir in preventing progression of superficial (ie, epithelial) keratitis to the more severe stromal keratitis or iritis are currently being examined by the HEDS research group.

Corneal Wound Healing

The cornea's vulnerable position at the front of the eye places it at great risk for damage from infection, injury, and toxic agents. Such problems can lead to ulceration of the cornea and the formation of opaque scar tissue, with permanent visual impairment. Therefore, an important program goal is to understand and enhance the processes that lead to normal healing of the ocular surface. Such knowledge may lead to better ways to promote normal healing of corneal wounds. The role of various growth factor cell-cell and cell-matrix interactions is an important area for research.

Corneal Transplantation

More than 40,000 corneal transplant operations are performed annually in the United States; however, about 1 in 10 patients receiving a corneal transplant is at high risk of rejecting the transplant. The NEI-sponsored Collaborative Corneal Transplantation Study (CCTS) was designed to evaluate whether donor-recipient tissue typing helps to prevent transplant rejection. Previous studies had suggested that closely matching the donor's HLA with those of the recipient increases the likelihood that the patient's immune system would accept, rather than reject, the donor tissue. CCTS researchers have found, however, that people who received corneal transplants with well-matched antigens did not fare significantly better than those people who received transplants without such a match. These findings indicate that tissue typing was not an important factor in transplant survival. Had donor-recipient tissue typing become standard practice in corneal transplantation, it would have significantly increased the waiting period and the cost (by at least $1,000) for this operation.

Cataract

Cataract, a clouding of the eye lens, is the leading cause of blindness worldwide, disabling approximately 20 million people. In the United States cataract is responsible for more than 3.3 million cases of visual impairment and accounts for 1.5 million surgical procedures for extraction annually. Cataract surgery is the most frequent operation performed among older Americans. Reimbursement for cataract surgery (totaling more than $5 billion) currently accounts for approximately 12% of the entire Medicare Part B budget. The enormous burden of cataract will worsen in coming decades as the American population ages. Consequently, the three major goals of this program are as follows: (1) to determine the causes and mechanisms of cataract formation, (2) to search for ways to slow or prevent the progression of cataract, and (3) to develop and evaluate new diagnostic and therapeutic techniques in cataract management.

Function of α-Crystallins

The α-crystallins are major structural proteins of the lens which contribute to its structure and transparency. Recently researchers supported by the NEI demonstrated that α-crystallin belongs to a class of proteins called molecular chaperons. Chaperons stabilize protein conformations, mediate the folding

and correct assembly of new proteins, catalyze the membrane translocation of secretory proteins, and prevent protein aggregation in the face of heat denaturation or other environmental stresses. Laboratory tests have demonstrated that the biological function of α-crystallin may be to maintain the lens structural proteins in a transparent state. With this important discovery, it is now possible to investigate a number of long-standing questions in lens biology and cataractogenesis and to examine directly whether certain age-related lens changes can be prevented or reversed.

Glaucoma

Glaucoma is a group of eye disorders in which a distinct type of optic nerve damage leads to blindness. As well as being a major public health problem, glaucoma is a leading cause of blindness in the United States and the number one cause of blindness in African-Americans. Approximately 3 million Americans have glaucoma, but one in two is unaware of his/her condition. As many as 120,000 are now blind from this disease. Blindness from glaucoma costs the United States Government more than an estimated $1.5 billion annually in health care expenditures, lost tax revenues, and Social Security benefits. The NEI's extramural program activities in glaucoma are directed toward understanding the mechanisms of the disease through basic research, identifying risk factors, and preventing blindness through the development of improved methods for early detection and treatment.

Treatment for Ocular Hypertension

Elevated intraocular pressure (IOP) is a key risk factor for the development of open-angle glaucoma. However, there is no consensus on whether early treatment of elevated IOP prevents or delays the onset of open-angle glaucoma. The Ocular Hypertension Treatment Study (OHTS) is an NEI-supported, randomized, multicenter clinical trial to determine whether medical reduction of IOP prevents or delays the onset of glaucomatous optic nerve and/or visual field damage in ocular hypertensive subjects. The OHTS will enroll 1,500 subjects who have elevated IOP in at least one eye with normal visual fields and optic discs in both eyes; these individuals are considered to be at moderate risk for the development of open-angle glaucoma. Study participants will be assigned randomly to receive medical treatment to both eyes or no treatment to both eyes. The central OHTS units have been funded, and the protocol is nearing completion. Patient recruitment is projected to begin in early 1994.

Treatment for Open-Angle Glaucoma

The Collaborative Initial Glaucoma Treatment Trial, also in a stage of protocol refinement, will compare medications with a surgical filtration procedure as the initial treatment for newly diagnosed open-angle glaucoma. This study will not only compare the two modes of treatment but will carefully evaluate the quality of life among patients receiving the different therapies.

Juvenile Glaucoma

Several NEI-supported research groups are exploring molecular genetic techniques in studies of childhood glaucomas. These diseases are the result of congenital abnormalities that lead to morphological defects in the anterior segment of the eye. One type is autosomal dominant juvenile glaucoma (ADJG). This disease lends itself to molecular biologic techniques for identifying the gene(s) responsible for this condition. As a first step in mapping the ADJG gene(s), laboratories have established pedigrees of families in which the disease occurs. A group of investigators at the University of Michigan has identified a kindred large enough to establish a linkage map, and work has begun to determine the chromosomal location of the gene(s).

Strabismus, Amblyopia, and Visual Processing

Developmental disorders such as strabismus (ie, misalignment of the eyes) and amblyopia (ie, commonly known as "lazy eye") affect 2-4% of the U.S. population. Correction of strabismus is one of the most frequently performed of all surgical operations (about 700,000 operations each year). Blindness from amblyopia is preventable if the condition is detected and treated early in life. In addition to research on strabismus and amblyopia, the program supports research on neuro-ophthalmological dis-

orders, presbyopia, and refractive errors. This program also supports research on more effective means to improve the quality of life for people with visual impairments by helping them maximize their use of remaining vision or by devising aids to assist those without useful vision. Currently 3 million Americans have chronic visual conditions that are not correctable by eye glasses or contact lenses and that impair everyday function. About 900,000 Americans are legally blind, and approximately 100,000 are totally blind or have only the ability to perceive light but not formed images. Understanding visual processing and its disorders require new knowledge of the visual nervous system, including the molecular, genetic, chemical, cellular, and integrative processes that underlie perception and the control of eye movements.

Optic Neuritis

Optic neuritis is an acute debilitating inflammation of the optic nerve that affects more than 25,000 Americans annually, primarily women between the ages 18 and 45. People with the disease usually experience rapid vision loss. Left untreated, most patients regain normal vision after several months of gradual improvement, but some are left with at least some visual deficit. Because a significant number of people who have an initial attack of optic neuritis later develop multiple sclerosis, many physicians consider optic neuritis as a precursor or manifestation of that disease. The NEI-sponsored Optic Neuritis Treatment Trial (ONTT) compared oral corticosteroid (ie, prednisone), intravenous corticosteroid methylprednisolone followed by oral prednisone, and placebo for the treatment of new cases of optic neuritis. ONTT investigators found that oral corticosteroid, the most common treatment for the disease, is ineffective when used alone in treating the disease and actually increases a person's risk for future attacks. The investigators also found that 27% of people taking oral prednisone had at least one new attack of optic neuritis during the followup period. In contrast, patients in the intravenous methylprednisolone group had a 13% rate of new attacks, and those given a placebo had a 15% rate of subsequent optic neuritis.

The ONTT study points the way to potential annual savings of $26 million in the treatment of acute optic neuritis. About $4 million will be saved due to decreases in the optic neuritis recurrence rate when prednisone is no longer used as the common treatment modality. Another $4 million in savings will result if intravenous and other less common, but expensive, treatment methods are discontinued. The remaining $18 million in potential annual savings will be realized if costly and unnecessary pretreatment magnetic resonance imaging and blood testing are not included as part of the pretreatment evaluation.

Development and Regeneration of the Visual System

Critical to our understanding of the regenerative process is knowledge of the factors that control the elongation and guidance of visual nerve axons. Research over the last decade shows that adult visual neurons do not synthesize all of the constituents that they did during their development. A class of molecules known as growth-associated proteins (ie, pretreatment), for example, is found in the developing central nervous system but is "down-regulated" as development ends. Recent work by NEI grantees has demonstrated that experimental interruption of optic nerves can lead to a reexpression of some pretreatment. Investigators are now trying to elucidate the factors that regulate the genetic expression of pretreatment and related molecules with the goal of enhancing regenerative capacity.

Computational Neuroscience

Stereopsis, or the process by which we see in three dimensions, remains a difficult phenomenon to understand. Most individuals see objects from two slightly different perspectives due to the lateral separation of the two eyes. The brain has the ability to somehow combine these two different monocular views and provide depth perception. How does this happen? Developments in computational neuroscience have provided new theoretical insights into the nature of vision. With these concepts, neurophysiologists now have a highly sophisticated framework by which to guide their experimental approach.

Eye Movements and Neurological Disease

One type of eye movement is the vestibular-ocular reflex (VOR). Normally, a head turn in one direction causes the eyes to rotate in the opposite direction so that the eyes continue to point at the same place in the stationary world. This is referred to as VOR. Without VOR, vision would appear blurred

during head turns because the eyes would move with respect to the surroundings.

VOR is being studied by NEI-supported investigators in monkeys fitted with telescopic spectacles that alter the visual scene. The investigators have found that, after several days, VOR becomes modified to compensate for the altered visual input. The immediate goal of this research is to better understand the neural basis for this behavioral plasticity. The continued assimilation of advances from basic research has led to many new tests for diagnosing neurological disease in humans and localizing its site through various oculomotor symptoms. VOR studies appear exceptionally promising in this regard. Researchers recently have found specific sites in the nervous system that appear to be associated with the behavioral changes in animals. Thus, VOR research provides us with a real opportunity to study the dynamics of how these plastic changes occur at the behavioral, synaptic, and even molecular levels.

Biometry and Epidemiology Program

Report of the Acting Associate Director for the Biometry and Epidemiology Program

Roy Milton, Ph.D.

The primary mission of the Biometry and Epidemiology Program (BEP) is to plan, develop, and conduct human population studies concerned with the causes, prevention, and treatment of eye diseases and vision disorders. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures. The BEP also conducts an education program in biometric and epidemiologic principles and methods for the vision research community, as well as provides biometric and epidemiologic assistance to the National Eye Institute's (NEI's) scientific staff.

The BEP began a prospective cohort study this fiscal year called the Age-Related Eye Disease Study (AREDS) that is designed to provide information on the development (ie, incidence), course, and progression of age-related macular abnormalities and lens opacities. The study also will evaluate various possible risk factors for the development and/or progression of the two disorders. In addition, a multicenter, randomized clinical trial will assess the effects of nutritional supplements and/or minerals on the development and progression of age-related macular degeneration (AMD) and cataract. Eleven clinical centers are now enrolling eligible participants—with the goal of recruiting 4,600 persons—to be followed for at least 7 years. Randomization and followup begins in early 1993. The information resulting from AREDS should provide important stepping stones toward preventing AMD and cataract.

A study of patients with a variety of retinal diseases continues to yield important information on the role of environmental and nutritional factors in the causation of eye disorders. In the first component of the study, patients with higher blood levels of nutrients with antioxidant characteristics, such as carotenoids, vitamin C, and vitamin E, were found to be at decreased risk of the most visually disabling form of macular degeneration. This leading cause of blindness also was seen to share many risk factors with cardiovascular disease. A second component of the study now suggests that about 55% of nontraumatic retinal detachments are attributable to myopia and that systemic factors play little role in the pathogenesis of retinal detachment. The study also is identifying risk factors for the following other retinal disorders that result in severe vision loss: branch retinal vein occlusion, central retinal vein occlusion, and macular holes.

The NEI joined with the U.S. National Cancer Institute and the Cancer Institute of the Chinese Academy of Medical Sciences in conducting eye examinations in central China in 1991 at the end of a 5-year randomized trial of vitamin/mineral dietary supplements. Almost 6,000 adults were examined to see if that nutritional intervention might reduce the occurrence of age-related cataract and AMD. A manuscript describing the results has been prepared.

In collaboration with Boston University, BEP is investigating familial relationships for age-related cataract and AMD using Framingham Study cohorts. Examination of 1,600 offspring of parents who were examined in the 1972-74 Framingham Eye Study was completed in late 1991. Analyses designed to identify genetic relationships have begun.

Office of International Program Activities

Report of the Acting Assistant Director for International Program Activities

Terrence Gillen, M.B.A., M.A.

Over the past year, the National Eye Institute (NEI) has continued to support investigations of important blinding eye diseases with worldwide impact. These studies have been implemented through bilateral agreements with the U.S. Government, other types of country-to-country programs (such as those supported by the U.S. Agency for International Development), and through collaborative activities with the World Health Organization (WHO), the Pan-American Health Organization, and other foundations and private and voluntary organizations (such as Lions Clubs International). The following are highlights of the ongoing activities and accomplishments of this office during the last year.

Because cataract is responsible for about one-half of the developing world's curable blindness and is a major problem for the United States as well, the NEI has developed a collaborative research program that includes projects to prevent blindness from cataract with collaborating groups in India, Italy, and Latin America. Additionally, health services research expertise from the NEI is made available to selected collaborating partners through training activities and the conduct of joint research projects.

Open-angle glaucoma is the leading cause of blindness in African-Americans and is a major cause of visual impairment and disability. The incidence of glaucoma has not been measured precisely in any population, and the risk factors related to its development are largely unknown. Since 1988 the Barbados Eye Study has examined a cohort of more than 4,600 persons ages 40 to 86 as part of a population-based study to determine the prevalence and risk factors for glaucoma and other eye disorders, such as age-related macular degeneration, cataract, diabetic retinopathy, and visual impairment. In 1992 the Barbados Incidence Study was initiated to estimate the incidence of glaucoma and other ocular disorders from individuals free of disease in the Barbados prevalence survey. Risk factor analysis also will be conducted for associations with development of glaucoma and to characterize those who have progressive eye disease.

Although not a major problem in the United States, the leading cause of blindness among preschool-age children in Asia is vitamin A deficiency. The NEI supports a great deal of basic research on the interaction of nutrients, such as vitamins A, C, and E, on retinal and eye tissue development. Such investigations can lead to clinical interventions that may help alleviate morbidity from malnutrition eye disease. In addition, the NEI has provided technical consultation for a study in South India, which has shown an impressive reduction in childhood mortality associated with improved vitamin A nutritional status, and other efforts to transfer this technology to alleviate world blindness are under way.

The NEI has continued its collaboration with the National Institute of Allergy and Infectious Diseases and three Brazilian scientific organizations in Sao Paulo to develop a research program on the basic mechanisms, epidemiology, and immunology of toxoplasmosis in Southern Brazil. The prevalence of ocular toxoplasmosis in Southern Brazil was found to be more that 30 times higher than previous estimates for the same condition elsewhere. In this population ocular toxoplasmosis appears to be a sequela of postnatal, rather than congenital, infection.

The NEI and the Indian Council of Medical Research (ICMR) have developed a collaborative blindness research program under the 1983 Indo-U.S. Science and Technology Initiative. This program includes projects to reduce blindness in India from cataract, Eales' disease, and vitamin A deficiency. Indian government funds for the work come through the ICMR, and U.S. Government funds are funneled through the National Science Foundation and the NEI. In addition, the NEI collaborates with Indian scientists under the U.S.-Indo Subcommission Program.

NEI's Director, Deputy Director, and Special Advisor to the Director have participated as consultants to the World Bank in the development of a proposal by the government of India for a major new initiative in cataract blindness control. Technical meetings have been held in New Delhi and Madurai to provide the knowledge base upon which training and surgical guidelines can be developed for a twofold expansion of cataract surgery, with explicit attention to the quality and extent of vision restoration.

An NEI-supported randomized clinical trial to compare intracapsular cataract surgery plus aphakic spectacles versus extracapsular cataract extraction plus implantation of an intraocular lens is being conducted at the Aravind Eye Hospital in Madurai. The trial's primary comparison concerns operative and postoperative complications. Secondary evaluation endpoints include measurement of vision function assessed by interview using a multi-item questionnaire and appraisal of economic impact in terms of direct and indirect costs associated with blindness and cataract surgery.

NEI staff continue to provide technical advice to Lions Clubs International in the development of its new $100 million SightFirst initiative, a global sight-conservation program aimed at substantially reducing the prevalence and incidence of preventable and curable vision loss.

In Fiscal Year 1992 the NEI continued its activities as a WHO Collaborating Center for the Prevention of Blindness. The NEI Director has continued to serve on the WHO's Special Advisory Panel in the Prevention of Blindness, and the Assistant Director for International Program Activities has been appointed to the Global Advisory Committee. Other NEI staff have, on request, provided consultation to the WHO program.

Office of Science Policy and Legislation

Report of the Acting Associate Director for Science Policy and Legislation

Michael P. Davis

The Office of Science Policy and Legislation is responsible for all National Eye Institute (NEI) program planning, analysis, and evaluation activities, including the development and maintenance of a computerized management information system; legislative and other program coordination activities; and public information and scientific reporting, including health education, media relations, and scientific reporting services in support of the NEI's research programs.

Policy, Legislation, Planning, and Evaluation Section

One of the major activities of the Policy, Legislation, Planning, and Evaluation Section (PLPES) this fiscal year has been the finalization and preparation for publication of the latest in the series of NEI long-range plans, *Vision Research—A National Plan: 1994-1998.* Section staff were responsible for finalizing draft panel reports and sending them to recipients of NEI Merit awards for reviewing the science in their particular area of expertise. Comments also were solicited from special reviewers from the optometric community. The PLPES staff worked with staff from the NEI Extramural and Collaborative Program on incorporating comments and suggestions and in updating some sections of the reports. These revised reports were then sent to the panel chairs and each program planning panel member for final review and approval. Final comments from the panel members and chairs have been received and are being incorporated into the reports. Introductory narrative material and an executive summary are currently being drafted. Design work and internal layout for publication should be completed by January 1993 for publication in February 1993.

This PLPES also has served as the primary contact point for the NEI's involvement in the National Institutes of Health (NIH) strategic planning process. This involvement has included attendance by Section personnel at the National Task Force meeting and the NIH Director's Retreat on the NIH Strategic Plan. In addition, PLPES staff have been responsible for reviewing and providing comments on the various drafts of the NIH Strategic Plan. Section staff also have prepared material for the draft NEI plan detailing the development of the NIH plan and the initiatives proposed by the NEI for incorporation and implementation.

One of the primary activities of the PLPES staff is the assignment of scientific, disease-related, and other codes to awarded grants. Nearly 540 grants were coded during Fiscal Year (FY) 1992 using a state-of-the-art, computer-based coding system and a data base that allows quick and accurate storage, retrieval, and reporting of scientific, programmatic, and fiscal information concerning the Institute's research grants, contracts, and intramural projects. Because of this activity, the PLPES is the focal point within the Institute for responding to a variety of requests for specific program and expenditure information and for the extensive reporting of NEI program developments and accomplishments to the NEI, the U.S. Public Health Service, the U.S. Department of Health and Human Services, Congress, and nongovernmental organizations and individuals.

The PLPES staff also are engaged in writing, editing, contributing to, and commenting on correspondence, reports, legislative analyses, public testimony, and other written materials from within and outside of the NEI.

Management Information Systems Section

Over the past fiscal year the Management Information Systems Section (MISS) staff implemented client-server data base systems to allow

extramural staff to enter and retrieve grants information directly. The MISS staff also installed internetworking software, allowing high-speed communications between NEI personal computers and other NIH staff, mainframe systems (such as the administrative data base, WYLBUR, and TSO), and other BITNET and INTERNET users worldwide.

MISS personnel have enhanced the Institute's computer hardware configuration with the installation of an optical scanner, film recorder, automatic backup power supply, high-speed network printer interface cards, and some new computer workstations. The MISS staff have smoothly implemented a major upgrade of its network operating system to a MicroSoft local area network (LAN) manager and have added custom enhancements to facilitate log-on procedures. Full remote access capability for the network has been added, as well as enhanced daily backup procedures.

MISS staff have upgraded software packages for spreadsheets, graphics, remote printing, calendar functions and scheduling, FAX interface, Windows applications, menus, and the NEI data base server. Custom spreadsheets for use in grants management have been developed, and MISS personnel have conducted special training seminars in the use of some of these software packages. All equipment has been ordered, and software configuration has begun to support a LAN at the new site for the Extramural and Collaborative Program. This system will be fully compatible with the NEI's building of 31 LANs.

MISS staff also have been active on several trans-NIH computing committees and activities, including the following: the Office Technical Coordinators and its LAN subcommittee, the Extramural ADP Coordinating Committee and its steering committee, the Technical LAN Coordinators, the NIH Lead Users, the WordPerfect Working Group, the Procurement Decentralization Task Force, the IMPAC Database Conversion Advisory Group, and the Database Technology Group. The head of MISS has made presentations on client-server data base technology, and his technical recommendation for the new IMPAC data base system was incorporated as a policy recommendation by the NIH contractor for that effort.

Scientific Reporting Section

The Scientific Reporting Section (SRS) develops and disseminates information and education activities and programs for the public and health professionals. Responsibilities of the SRS include the following: the National Eye Health Education Program (NEHEP); development of publications and other information materials; preparation of responses to inquiries received from the press, public, and health professionals; the NEI exhibit program; preparation of reports to Congress; maintenance of a computerized data base of eye health-related educational materials; and dissemination of the results of NEI-supported clinical trials.

The SRS staff responded to more than 9,000 inquiries from the general public, patients and their families, students, health professionals, legislators, and the media in FY 1992, including 40 pieces of controlled correspondence representing congressional inquiries and Presidential greetings and proclamations. This is a significant increase over the 6,000 inquiries received last year. In addition, the SRS disseminated clinical trial results through clinical alerts and mass media activities for the Foscarnet-Ganciclovir Cytomegalovirus Retinitis Trial of the Studies of the Ocular Complications of AIDS and the Optic Neuritis Treatment Trial. The SRS staff also developed the Eye Health Education (EY) Subfile on the Combined Health Information Database, which now includes more than 400 abstracted items.

National Eye Health Education Program

The NEHEP was formally launched at a press conference in December 1991, reaching more than 65 million people with important health information through television, radio, newspapers, and magazines. The NEHEP Partnership now includes more than 40 public and private organizations that implement activities designed to reach individuals at high risk for glaucoma and diabetic eye disease.

The first two NEHEP public service campaigns were released in January 1992. The glaucoma campaign—consisting of print, radio, and television public service announcements (PSAs)—and the diabetic eye disease campaign—using print PSAs—

targeted potential audiences at risk for these diseases totaling more than 60 million people. The glaucoma television PSAs were aired more than 25,000 times in 47 States by 239 stations, representing more than $2.4 million in air time. The radio PSAs were aired more than 30,000 times in 49 States by 360 stations, representing $2.7 million worth of air time.

Three education kits were produced: the *Educating People With Diabetes Kit*, the *Glaucoma Community Education Kit*, and the *Information Kit for Pharmacists*, each providing a variety of materials designed to help health professionals and community leaders implement eye health education programs. Since becoming available in April, more than 16,000 kits have been distributed. To support local efforts of the NEHEP Partnership members, the NEHEP staff provided technical assistance by linking groups with additional resources in their area and assisting them in designing programs for their specific target audiences. The NEHEP also provided funds to the Healthy People 2000 Project of the National Medical Association to evaluate eye health education strategies in two cities aimed at reaching African-Americans and providing them with important eye health information.

Grants for three applied research projects on diabetic eye disease education were awarded this year. These projects—designed to evaluate cost-effective education strategies that increase awareness and knowledge of diabetic eye disease and encourage actions to prevent loss of vision—will be aimed at three different target audiences: (1) low income African-Americans, (2) Native Americans, and (3) all people with diabetes in a defined community.

Office of the Scientific Director

Report of the Scientific Director

Robert B. Nussenblatt, M.D.

This past year has been one of continuing change and achievement in the Intramural Research Program of NEI. During this year work has progressed in both clinical and basic research spheres. Continued heavy emphasis in the area of AIDS (acquired immunodeficiency syndrome) has resulted in the initiation of a clinical study to evaluate the effectiveness of a sustained-release device for ganciclovir, placed in the eye for the treatment of cytomegalovirus (CMV) retinitis. A study to immunomodulate uveitis and the diseases in uveitis patients without the use of medications has begun as well. In the area of basic research, there are exciting new concepts concerning gene regulation, and the initial steps of a gene therapy approach to eye disease have been taken. The following are a few highlights of research achievements by NEI intramural scientists in Fiscal Year 1992.

Laboratory of Retinal Cell and Molecular Biology (LRCMB)

The mission of the LRCMB is to uncover new aspects of functioning of the retinal pigment epithelium (RPE) complex in health and disease. The focus has been on the elucidation of new genes and biochemical mechanisms as well as learning the underlying causes of ocular disease. This work has been performed in both clinical and basic research. In collaboration with Dr. Muriel Kaiser-Kupfer (Ophthalmic Genetics and Clinical Services Branch), LRCMB researchers are investigating fatty acid uptake and metabolism in Bietti's crystallin retinopathy and a tubulin acetylation defect in a form of Usher's syndrome in the hope of elucidating specific defects. Candidate genes for hereditary retinal diseases have been evaluated. The approach has included a method that uses subtractive cloning. Retina-specific genes and genes located on the short arm of the X chromosome are of special interest. These genes are being chromosomally localized to see whether they are disease linked.

Other work performed in the Laboratory includes the search for RPE-specific genes. The RPE is essential for the photoreceptive process. A major effort has been in the cloning of genes unique to the RPE and its function. A new, specific 65-kD protein of potential immunologic importance has been isolated and cloned from human RPE. Several transgenic studies have been initiated. A gene analysis system in transgenic mice and in transient transfections in cultured human retinal blastoma cells have been established for interphotoreceptor retinoid-binding protein (IRBP).

Another LRCMB area of interest has been the use of ribozymes as a mode of gene therapy. These ribozymes are specifically constructed RNA species that can control the expression of proteins within the cells. Linking these simplified gene forms to appropriate promotors and utilizing a suitable transfer vector enables construction of new therapeutic modalities. It is hoped that this approach may be used in the future to treat autosomal dominant disorders that cannot now be managed.

Laboratory of Ocular Therapeutics (LOT)

The LOT's focus is on the development, evaluation, and mechanism of action of new ophthalmic drugs to treat eye diseases. The major emphasis has been in examining aldose reductase inhibitors and anticataract agents. Developing and pursuing the more effective and less toxic therapies, the LOT scientists have discovered an inhibitor unrelated to previous aldose reductase inhibitors. They are now conducting studies to characterize this inhibitor.

In addition, LOT work has centered on elucidation of the specific mechanisms of aldose reductase-initiated diabetic complications. In these studies, the use of galactose-fed dogs has permitted the investigators to follow the progression of retinal changes associated with diabetic retinopathy and to see the

dose-dependent arrest of these changes with aldose reductase inhibitors.

Laboratory of Mechanisms of Ocular Diseases (LMOD)

The LMOD's primary emphasis in the past year has been on cataract formation and the ocular complications of diabetes. The Laboratory has had a longstanding interest in the evaluation of the vascular network of the retina during diabetes. Using elastase, LMOD researchers have developed a new procedure for the isolation and evaluation of the entire vascular network of the retina. Some in the group have continued to investigate the role of the polyol pathway in diabetic complications. They have determined the primary sequence of human sorbitol dehydrogenase. Others in the group have developed an in vitro system to assay the effectiveness of anticataract agents. The development of such an organ culture system will allow initial screening of compounds before they are moved to animal models of cataract for the next stage of testing.

More clinically oriented studies have also continued. They include investigation of the modifications that lens crystallins undergo as they become cataracts. The primary focus has been on metal-catalyzed oxidation using γ-crystallin as a model system. Dr. Fielding Hejtmancik and his coinvestigators have successfully mapped to chromosome 11 two genes causing Usher's syndrome type I.

Laboratory of Sensorimotor Research (LSR)

This Laboratory of international standing focuses its research efforts on the brain mechanisms underlying visual sense and visually controlled movement. The group, which has concentrated on the processing of the visual input to the brain, has made several advances this past year. This work has indicated that different visual areas in the brain may communicate via temporally modulated messages. Neurons in the visual areas of the cerebral cortex carry information about the form and color of the stimulus in the temporally modulated code. The results suggest that cortical neurons can convey information about many different features without confounding them.

LSR scientists have also evaluated the effects of selective attention on visual processing, obtaining results that suggest the brain treats voluntary attention differently from involuntary attention to salient and novel stimuli. In other work, they have studied the rapid or saccadic eye movements that shift the focus of the eye from one object of interest in the visual field to another. This year LSR investigators found that, while many neurons in the brain may give bursts of cell discharge before the onset of these saccades, other types of cells lying just below these bursts cells are related to the generation of saccades. This spread of activity may represent a type of neuroprocessing not previously recognized in the brain.

Laboratory of Molecular and Developmental Biology (LMDB)

The continuing focus of the LMDB is on understanding the fundamentals of gene expression and cellular differentiation in the eye, particularly in the lens. The group has ventured into the complex area of protein-DNA interactions involved in the expression of crystallin genes. Some of their experiments have shown that putative regulatory regions appear to be involved in the heat-shock response of these genes and in their expression in the lung and brain, both of which use a different promotor than those expressed in lens and other tissues.

Others in the LMDB group have investigated the crystallins in invertebrates with cellular lenses. These models have shown a convergent evolution that we hope will elucidate basic aspects of crystallins and their genes required for optical properties of the transparent lens. Of particular interest was their finding that the ectopic expression of interferon-γ in the lens induced major histocompatibility complex class II gene expression in the eye disrupted the developmental program of the lens and retina. The transgenic mouse developed may become a useful model for the study of ocular autoimmune disease. It promises to provide insight into the tight control of gene expression and cell differentiation in the eye.

Proto-oncogenes in the lens have been of ongoing interest to the LMDB. Recent work has revealed

unexpected patterns that shed light on the mechanisms of cell cycle rest during differentiation. Insights have also been gained on the cellular mechanisms that regulate proto-oncogene expression in the lens.

Ophthalmic Genetics and Clinical Services Branch (OGCSB)

The purpose of the Branch is to conduct clinical and laboratory research on gene expression and molecular interaction important to the eye and to apply clinically relevant research findings to the prevention, diagnosis, and treatment of diseases affecting the eye and visual system. The OGCSB is also responsible for the essential psychophysical and electrophysiological diagnostic testing of visual function required by clinical Intramural Research Programs of all of the Institutes.

Work in the field of cataract has demonstrated that with aging there is acidic shift in proteins and an increased number of polypeptide species in the molecular weight range of the crystallins. These aging changes need to be differentiated from changes occurring in cataract formation.

Other OGCSB work has centered on the disease gyrate atrophy. There appear to be many different single-point mutations in the ornithine aminotransferase gene in gyrate atrophy patients. Dietary intervention studies utilizing an arginine-deficient diet have been quite promising, especially in delaying the onset of pathologic changes in young patients. Furthermore, foveal cone sensitivity was found to be abnormal in a group of patients with gyrate atrophy, suggesting that foveal cones have altered orientation and sensitivity before the encroachment on the foveal area by gyrate atrophic lesions.

Other OGCSB researchers, looking at visually evoked potentials (VEP) in albinism, have noted two different patterns of VEP asymmetry in albinism that may be explained by differences in the reorganization of the geniculo-cortical pathway. Also this year, after confirming the usefulness of 0.5% cysteamine eyedrops in young patients with cystinosis, the Branch has expanded its study to include older patients, producing strikingly similar results.

Laboratory of Immunology (LI)

The Laboratory of Immunology is dedicated to the evaluation, diagnosis, and treatment of ocular inflammatory diseases. Work by this group has been both in clinical and basic research fields. In the clinical area, studies continue to center around the evaluation of the Molteno glaucoma implant and 5-fluorouracil, combined with trabulectomy.

In addition, the LI group has made a major clinical investment in the study of AIDS. This study has dealt with better ways to diagnose the presence of CMV retinitis, including noninvasive mechanisms such as increased protein content in the anterior chamber, as well as therapeutic studies on the use of such anti-CMV retinitis drugs as foscarnet. Recently the laboratory began a randomized study to evaluate the usefulness of an intraocular slow-release device containing gancyclovir, over either a 4- or 8-month period to treat CMV retinitis.

Other areas of interest to the group include the development of new aspects of immunosuppression. They have worked in developing rapamycin as a potential therapeutic aid in the treatment of uveitis. The Laboratory has also studied various virologic and immunopathologic processes that occur when viruses replicate in the ocular microenvironment. This project has used a coronavirus model for retinal degeneration. It is of interest that the virus is capable of inducing an acute infection in the presence of a mild inflammation, with the virus then being cleared and a subsequent retinal degeneration ensuing with no virus present.

The Laboratory has also developed a reproducible method for performing RPE cell transplants. The ultimate goal is to use molecularly altered RPE cells to develop universal donors, as well as to develop a reproducible animal model for the study of RPE interactions from both biochemical and immunologic points of view.

Experimental uveitic models have been thoroughly studied by the LI. Over the past year, researchers have developed an in vitro system to investigate mechanisms of specific unresponsiveness toward uveitogenic peptides. The group has also initiated a study to evaluate the effectiveness of oral administra-

tion of various antigens on the ocular immune response. This approach, which has been tested in the animal model for severe intraocular inflammatory disease induced by uveitogenic antigens, is quite successful. A randomized masked trial to evaluate more fully the usefulness of S-antigen feeding in patients with intraocular inflammatory disease has now begun.

Other areas of immunoregulation under investigation include evaluation of adhesion molecules and their role in the induction of disease. Over the past year, one section has devoted itself to an NEI priority, that of gene therapy. Work has centered on the development of a construct of ornithine aminotransferase that can be used for gene therapy in patients who have gyrate atrophy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00065-15 OSD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Studies of the Primate Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Francisco M. de Monasterio M.D., D.Sc. Medical Officer OSD, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves study of the physiological organization of neurons of the visual system of primates, with emphasis on the chromatic properties of color-opponent ganglion cells. The cells studied are from the lateral geniculate nucleus and the primary visual cortex of macaques.

Project Description

Objectives

This project is designed to study the neural organization underlying the processing of visual information at different levels of the primate visual system.

Methods

The research includes intracellular and extracellular recordings from single neurons, extracellular recordings of mass responses, computer video stimulation, and tangent screen chromatic and spatial stimulation.

Major Findings

The researchers attempted to continue studying the responses from single ganglion cells and neurons of the primary visual cortex of macaque monkeys. The continuation of these studies, however, was impeded by underground construction work along the wall separating the laboratory rooms from the street as well as delays in obtaining needed additional equipment. This construction work, which has lasted for several months, shakes the ground to the extent of making microelectrode recordings impossible, and even resulted in damage to computer hard disks. Cell recordings have been suspended until the end of this construction.

Significance to Biomedical Research and the Program of the Institute

Numerous behavioral, psychophysical, and electrophysiological studies show that the visual performance and characteristics of macaques and humans are extremely similar to one another, so that an understanding of nonhuman primate physiology provides a useful animal model for human visual function.

Proposed Course

These studies will be continued.

NEI Research Program

Strabismus, Amblyopia, and Visual Processing—Visual Processing and Amblyopia (Structure and Function)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00122-12 OSD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anatomical Studies of the Primate Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Francisco M. de Monasterio M.D., D.Sc. Medical Officer OSD, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the anatomical properties and organization of cells in the primate visual system with emphasis on the retina and the visual cortex. The studies include (1) the anatomical association of outer-retinal cells selectively stained with tissue-reactive dyes and (2) the distribution pattern of cones in the retina of human donors who have a diagnosis of diabetic retinopathy.

Systematic light-microscopy of the anatomical association of macaque retina blue cones and horizontal and bipolar cells, with selective staining by several tissue-reactive dyes, has provided information on the probable circuitry of the blue-sensitive cone pathway of primate retina.

Retinal cone studies of human eye donors with a history of diabetes continue. There is evidence that the cone population from the retinas of these donors has a point pattern resembling that of cones selectively stained by tissue-reactive dyes. Although such dyes were not injected in the eyes from diabetic donors, in vitro staining with more conventional dyes shows a differential labeling of the receptors. Some cones are more densely stained than other cones, and the point pattern is similar to that of the blue-sensitive cones of the primate retina.

Project Description

Objectives

The purpose of this project is to study the anatomical properties and neural organization of the primate visual system.

Methods

Included in this research are retinal histological processing, intravitreal injection of dyes, computer modeling and spatial statistical analyses of point and area patterns, silver cell and myelin staining, histological processing of the cerebral cortex, deoxyglucose labeling, autoradiography, and cytochrome oxidase labeling.

Major Findings

While both studies continue from Fiscal Year 1991, their continuation has been severely hampered by underground construction work along the wall separating the laboratory rooms from the street, as well as difficulties in obtaining new equipment. This construction work, which has lasted for several months, shook the ground to the extent of making tissue sectioning and microphotography impossible.

Evidence of a cone population with a point pattern distribution resembling that of the cones identified as blue-sensitive ones (ie, absence in the central-most region of the fovea, peak density in the parafovea, and regular though slightly disordered spacing in which stained cones are separated by two to three unstained cones) has been obtained in initial studies of the retinas of human donors with clinical history of diabetes and diabetic retinopathy. Due to the need for eyes fixed within 3 hours of death, additional material has been difficult to obtain. Results consistent with the above findings were obtained in two additional diabetic cases; however, the eyes were fixed several hours after death, and the retinal preservation was less than adequate. Because of the unpredictability of obtaining properly fixed tissue from donors with documented retinopathy, slow progress is expected in this study.

Systematic examination of thin serial sections of macaque retinas stained with a tissue-reactive dye selective for blue-sensitive and some postreceptoral cells continues. The objective is to trace at the light microscopic level the anatomical relationship between selectively stained blue cones, H1 horizontal cells, and blue-cone (BC) bipolar cells of the area centralis of macaque retina. Data obtained so far provide evidence that, on the central macaque retina, each blue cone contacts one H1 horizontal cell and an average of at least one BC bipolar cell. Because the latter neurons almost always show a significant lateral displacement from the contacting cones, it is difficult to trace their contacts in thin sections.

Additional studies on whole mounted retinas, viewed en face, provide further evidence of a nearly unitary blue cone/H1/BC-bipolar cell distribution. Observations of more peripheral retina, however, support the view that with increasing retinal eccentricity, each BC bipolar cell contacts more than one blue cone. Among our achievements is the use of Lucifer yellow VS dye to increase staining consistency for inner cells of the peripheral retina. Stained bipolar cells, which have anatomical properties similar to those of BC bipolar cells described in Golgi-stained retinas, appear to have weakly stained projections on two and sometimes three stained blue cones.

Significance to Biomedical Research and the Program of the Institute

Information on the anatomical properties of blue-sensitive cones is important not only to understanding the functional properties of these cones by investigators in different basic disciplines. These experimental results also aid clinical research and the diagnosis of acquired retinal disease. Preliminary data obtained from the eyes of diabetic human donors are particularly promising in this respect.

Proposed Course

These studies will be continued.

NEI Research Program

Retinal and Choroidal Diseases—Fundamental Processes and Retinal Disorders (Retinal Organization, Neurotransmission, and Adaptation)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00135-20 OSD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemistry of Retina and Pigmented Epithelium in Health and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Helen H. Hess M.D. Medical Officer (Research) OSD, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of nutrition, oxidation, and other environmental factors (light intensity or darkness) on incidence and progress of posterior subcapsular opacities (PSO) associated with genetically influenced retinal degeneration are being studied in pink-eyed Royal College of Surgeons (RCS) rats, in which rod photoreceptor outer segment debris accumulates secondary to a phagocytic defect in retinal pigmented epithelium. Evidence was obtained that oxidative changes in polyunsaturated fatty acids in debris led to water-soluble toxic aldehydes, detectable in the vitreous and toxic to lens cells and membranes. Dystrophic rats fed a natural-ingredient diet (NIH-07) were highly sensitive to retina light damage, beginning at 10- to 40-lux intensity, and 27% of the rats developed mature cataracts by 7-12 months. Increased light intensity (cyclic or constant) increased the percentage of rats with mature cataracts, while dark-rearing from birth prevented PSO and mature cataracts. Rhodopsin bleaching appears essential for retina light damage and PSO. In vitro, free retinaldehyde can act as a photosensitizer to generate singlet oxygen, an extremely energetic oxidant. Our results suggest a similar effect in vivo, with damage to both lipids and proteins.

Antioxidants may slow or prevent cataracts in some diseases of the human retina. A purified diet (AIN-76A) fortified with antioxidants (0.4% β-carotene + 0.01% BHT) prevented PSO and mature cataracts in RCS rats. After a diet containing additional antioxidants (vitamin C, 1,000 mg/kg, and vitamin E, 150 mg/kg) was fed to dystrophic rats, histopathological examination showed retinal degeneration was retarded during the time the cataracts would have had their onset (23-53 postnatal days) if NIH-07 had been fed. Future studies are being directed to exploring whether retinal degeneration can be delayed further by increasing the vitamin E by a large (7-fold) factor.

Project Description

Additional Personnel

J. Samuel Zigler, Jr.	Ph.D.	Chief, LMOD, NEI
Joseph J. Knapka	Ph.D.	Chief, Scientific Services Branch (SSB), Veterinary Research Program (VRP), National Center for Research Resources (NCRR)
Dennis Bernard	M.S.	Nutritionist, SSB, VRP, NCRR

Objectives

This project is designed to study the biochemical relationships between lens, retinal photoreceptors, retina, retinal pigment epithelium (RPE), and biological fluids in health and disease. It also involves exploring the possibilities for slowing the rate of retinal degeneration and preventing the lens opacities and mature cataracts often associated with retinal degeneration in rats and humans. Diseases in which the RPE may be involved are of particular interest.

Methods

The Royal College of Surgeons (RCS) rat is being studied as an animal model of both hereditary retinal degeneration that results from a defect in the RPE and a type of cataract that is secondary to retinal degeneration. Nutritional biochemistry is being used as a tool to combat lipid peroxidation in the RCS rat retina and prevent water-soluble toxic aldehyde byproducts from reaching and damaging the lens.

Defined diets are prepared and fed to congenic affected and unaffected RCS rats in controlled experiments. The diets are fed to young breeding pairs prior to producing their first offspring and to their offspring after weaning, so that the experimental animals will have received their diets from conception to date of observation. Clinical findings are recorded after indirect ophthalmoscopic and biomicroscopic slit-lamp examination. Post-mortem examinations of the eye include dissecting microscopy and light microscopy of stained specimens. At appropriate times, photography is used to record data in vitro or in vivo. Analytical methods include flameless atomic absorption, standard biochemical assays by spectrophotometry and fluorometry, and separation procedures. Special environmental lighting conditions are employed to determine their histopathological effects on the retina and the lens.

Major Findings

Antioxidant diets that prevent cataracts in pink-eyed RCS dystrophic rats have the effect of retarding retinal degeneration. None of the diets we have tried stops the degeneration, but when a certain degree of retardation is achieved, the lens is protected. The most effective diet we have studied so far is a combination of AIN-76A purified diet containing twice the normal concentration of all minerals in the AIN mineral mix, plus 0.4% beta-carotene and 0.01% BHT, as well as 1,000 mg/kg vitamin C and 150 mg/kg vitamin E. When the rats consumed this diet, histopathological examination showed retarded retinal degeneration during the time the cataracts would have had their onset (23-53 postnatal days) if NIH-07 diet had been fed. Future studies are being directed to exploring whether retinal degeneration can be delayed further by increasing the vitamin E sevenfold. Extreme sensitivity of the dystrophic retina to light damage and to peroxidation of its lipids may be as fundamental as the defect of the RPE; thus it may provide a clue to the underlying metabolic disease.

Significance to Biomedical Research and the Program of the Institute

Retinal deteriorations are the major cause of untreatable blindness in the United States and probably in the world. The retinal pigment epithelium is increasingly implicated as the primary site of many of these disease processes. Posterior subcapsular cataracts occur in humans with various types of hereditary retinal degenerations and in RCS rats, as well as in older persons and in some persons treated with steroids or exposed to various types of short-wave radiation. Nutritional and genetic factors thought to play key roles in many human diseases can often be studied in detail in animal models, giving an opportunity to develop ways to slow, prevent, or cure the diseases. Environmental light intensity as a factor in retinal and lenticular diseases deserves more attention.

Information gained from these studies should contribute to our understanding of human disease and to initiating and conducting trials of possible therapeutic measures. More than 1 million cataract

operations are performed per year, and with an increase in the aging sector of the population, this number will increase. Any measures (eg, protective eye and headwear as well as dietary antioxidants) that may prevent or slow retinal deterioration and prevent or delay development of cataracts would increase the quality of daily life for millions of older people and decrease the costs of national medical care.

Proposed Course

The project will be continued. Further emphasis will be placed on controlled trials of nutritional regimens in RCS rats, toward the objective of preventing or delaying retinal degeneration and preventing cataracts. Studies will continue on the effects of darkness and light and antioxidant factors in retinal degeneration and cataracts in RCS dystrophic rats as well as RCS congenic control rats. Collaborations will continue to investigate biochemical factors in cataractogenesis and retinal degeneration.

NEI Research Program

Cataract—Pathogenetic Mechanisms
Retina—Retinitis Pigmentosa and Other Inherited Disorders

Publications

Hess HH: Effect of nutritional factors on retinal degeneration and cataracts in pink-eyed tan-hooded Royal College of Surgeons (RCS) rats. *Invest Ophthalmol Vis Sci* 33(4):1186, 1992.

Laboratory of Immunology

Report of the Chief, Laboratory of Immunology

Robert B. Nussenblatt, M.D.

In its 6th year, the Laboratory of Immunology has undergone some change. The section head position in the Section on Clinical Immunology, which was vacant a year ago, was filled recently by Dr. Marc de Smet, the acting chief. The other sections are the Section on Immunology and Virology, headed by Dr. John J. Hooks; the Section on Experimental Immunology, headed by Dr. Igal Gery; and the Section on Immunoregulation, headed by the acting chief, Dr. Rachel Caspi. Dr. Gery continues as deputy chief of the Laboratory; his major role in the development of new areas of investigation for the Laboratory was recognized this past year when he received the Director's Award from Dr. Carl Kupfer.

Two new sections recently have been formed. The first is the Section on Experimental Immunopathology, with Dr. Chi-Chao Chan as chief. The newly created Section on Molecular Biology, of which I am acting chief, includes Dr. Moncef Jendoubi and his group. The Fiscal Year (FY) 1992 work of these two sections will be mentioned within their previous section groups because they were created very recently, and the work was done prior to the new Sections' establishment.

Section on Clinical Immunology

The Section on Clinical Immunology has continued to focus on major questions of clinical relevance. In one area, the treatment of secondary glaucoma due to uveitis, the studies are centered around evaluation of the use of the Molteno glaucoma implant and 5-fluorouracil (5FU) combined with trabeculectomy. Our observations continue to make us rather optimistic about the long-term efficacy of the Molteno implant; however, no definitive data are yet available. As this randomized study to evaluate both the efficacy and complications of these two modalities continues at the NEI, we are still recruiting patients with secondary glaucoma due to uveitis to reach numbers that will provide some idea as to the comparability of these two modalities of treatment.

The Section also has been studying patients with AIDS. Because of its time-consuming nature, this major endeavor has included clinicians from various Laboratory Sections. Since completing a Phase 2 randomized control study to evaluate the efficacy of foscarnet in 24 patients with cytomegalovirus (CMV) retinitis, we have continued to evaluate the ability of foscarnet as well as gancyclovir to prevent progression of CMV retinitis.

In collaboration with the National Institute of Allergy and Infectious Diseases, we have begun to evaluate the safety of administering anti-CMV hyperimmunoglobulin to patients at risk for CMV retinitis. To date, this treatment, given monthly to a small number of patients, has had no adverse effects. We also have continued to follow patients who have diagnosed CMV retinitis.

Patients with advanced AIDS and a severe state of immunosuppression are often unable to tolerate gancyclovir, even with the use of G-CSF; some also are unable to tolerate the renal toxicity of foscarnet. Therefore, we have had to revert to the use of intravitreal injections. In the course of clinical therapy of two patients who had silicone oil placed in the eye because of retinal detachment (a complication of CMV retinitis), we showed that intravitrial gancyclovir injections can be given safely as an effective means of arresting the progression of CMV retinitis.

Other infections also can decrease vision in patients with AIDS. The second most common pathogen in most parts of the world is toxoplasmosis. We have evaluated and demonstrated the efficacy of a new agent, 566C80, in the treatment of ocular toxoplasmosis.

Recently a device that slowly releases gancyclovir directly in the eye has been described. It may be an important alternative to systemic anti-CMV therapy for patients who cannot tolerate the intravenous infusions or who possibly do not have a specific

indication for systemic therapy. We have developed a protocol that should allow us to evaluate the safety of the device. This protocol is now in effect, and the study will begin shortly.

Other areas of interest to the Section concern the development of new aspects of immunosuppression. The group has worked through this year in developing rapamycin as a potential therapeutic aid in the treatment of uveitis. Rapamycin, which has a mode of action different from that of cyclosporine and FK506, appears to be very effective in preventing the expression of experimentally induced uveitis.

Section on Immunology and Virology

Work of the Section on Immunology and Virology has continued to emphasize several specific areas. One area has been the study of various virologic and immunopathologic processes that occur when viruses replicate in the ocular microenvironment. This project's coronavirus model for retinal degeneration is capable of inducing an acute infection in the presence of a mild inflammation but without the presence of a virus. The retinal destruction occurred during the ensuing months in spite of the fact no virus was present. The work has shown that while the initial viral infection may induce some degree of degeneration, a potentially autoimmune response may be playing a role in this disorder as well.

The group continues to be actively involved in the study of the retinal pigment epithelial (RPE) cell. The group has developed and characterized primary cell lines of human RPE from donor eyes obtained from eye banks. Using these human RPE cell cultures as an in vitro model, the investigators have conducted a set of experiments to examine the various roles of the RPE in the pathophysiology of retinal disorders. Cultures of these cells exposed to bacterial lipopolysaccharide (LPS), tumor necrosis factor, and interleukin (IL-1α and IL-1β) secreted large amounts of interleukin 6 (IL-6). This response, which is dose dependent, is sustained in the presence of stimulants. It is interesting that growth factors did not induce IL-6.

In addition, the group has developed the ability to produce large numbers of RPE cells that have been used in ongoing RPE transplantation experiments. Utilizing a microinjection technique, they have succeeded in injecting RPE cells into the proper space beneath the retina. The injection of RPE cells obtained from a non-major histocompatibility complex identical source induces a rejection phenomenon. This development has provided the whole laboratory with the model to study ways of altering the immune response to these important cells.

Achieving priority status among the Section on Immunology and Virology has been the use of the molecular diagnosis and pathogenesis of CMV infections in man. In addition, the group has worked over the past year in the development of an animal ocular model for toxoplasmosis.

Section on Experimental Immunology

The Section on Experimental Immunology has maintained its long-term interest in the investigation of the pathogenesis of inflammatory eye diseases grouped under the term "uveitis." This group's work has centered around the evaluation of experimental autoimmune uveitis (EAU) with interphotoreceptor retinoid-binding protein (IRBP). They found that the substitution of certain residues of the bovine IRB peptide 1181-1191 eliminated its high uveitogenicity. It is interesting that the corresponding sequence of the rat IRBP differs from the bovine sequence by two residues and is completely nonuveitogenic. The group has developed an in vitro system to investigate mechanisms of specific unresponsiveness toward uveitogenic peptides. In this system, lymphocytes sensitized toward peptide 1181-1191 were rendered unresponsive.

The group also has examined several approaches aimed at inhibiting the immunopathogenic process of EAU. Injecting peptides that compete with the uveitogenic molecule, they have found that EAU induced by the highly uveitogenic IRB peptide 1181-1191 is completely inhibited in rats coimmunized with peptide A183, an analogue of the peptide derived from a *Mycobacterium tuberculosis* protein. These investigators also have demonstrated that the procedure of oral tolerance, another approach to effectively inhibiting S-antigen (S-Ag)-induced EAU, is modulated by the small peptide 1181-1191. New immunosuppressive agents investigated by the group include mycophenolate mofetil, which is effective in

preventing the expression of this animal model for a human disease.

Section on Immunoregulation

The Section on Immunoregulation's involvement in investigating many aspects of the immune response in the eye ranges from clinical to basic studies. The effect of the oral administration of various antigens on the ocular immune response has been tested in the animal model for severe intraocular inflammatory disease (EAU) induced by both S-Ag and IRBP. Previous work showed that a CDA-positive putative suppressor cell could be isolated from the spleen of such animals. Additional work this year has shown that the role of the spleen is imperative in the induction of this toleragenic state.

In a pilot study, two patients with evidence of in vitro proliferative responses to S-Ag began a S-Ag feeding protocol. A tolerant immune state appeared to be induced in both of these patients after feeding and a program to take them off their inflammatory disease medication was successful. We have now begun a randomized masked trial to evaluate more fully the usefulness of S-Ag feeding in patients with intraocular inflammatory disease.

This Section has demonstrated the expression of endothelial leukocyte adhesion molecule 1 (ELAM-1) on the corneal endothelium in Lewis rats with induced uveitis. They have shown that treatment with the monoclonal antibody against Mac-1 inhibits the development of endotoxin-induced uveitis (EIU) in mice. Furthermore, they have demonstrated that monoclonal antibodies blocking intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen (LFA-1) inhibit the development of both EIU in rats and EAU in mice. These antibodies against ICAM-1 and LFA-1 also inhibited lymphocyte stimulation in vitro. The expression of cell adhesion molecules in animal models of uveitis such as ICAM-1 are expressed in the eye before the infiltration of inflammatory cells. Work performed to evaluate human biopsy tissue from the eye has revealed the expression of ICAM-1, E-selectin, and vascular cell adhesion molecule (VCAM-1) in corneal graft tissue.

Over the past year the Section has devoted itself to an NEI priority, gene therapy. Work has centered on the development of a construct of ornithine aminotransferase that can be used for gene therapy in patients who have gyrate atrophy. The expression of this gene now can be effected in cells that have been transfected with the appropriate construct. Also under way are plans to develop an animal model of gyrate atrophy, using homologous recombination techniques that originated in the Laboratory. These efforts already have yielded a model mouse deficient in the invariant chain of the major histocompatibility complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00279-01 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Immunosuppressants for the Treatment of Uveitis in Animal Models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	François G. Roberge	M.D.	Visiting Scientist	LI, NEI
Others:	Chi-Chao Chan	M.D.	Chief, Immunopathology	LI, NEI
	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Dan Martin	M.D.	Senior Staff Fellow	LI, NEI
	Raymond DeBarge	M.D.	Senior Staff Fellow	LI, NEI
	Alexander Kozhich	Ph.D.	Visiting Fellow	LI, NEI

COOPERATING UNITS *(if any)*

Laboratory of Experimental Surgery, Notre Dame Hospital, Montreal, Quebec, Canada (Dasheng Xu, M.D.; Huifang Chen, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of the project is to develop alternative treatment for noninfectious intraocular inflammatory diseases (uveoretinitis). During this past year we concentrated on a group of noncytotoxic immunosuppressants comprising rapamycin (RAPA), cyclosporin A (CsA), and FK506. The work specifically focused on (1) RAPA treatment of experimental autoimmune uveoretinitis (EAU) and (2) combination therapy using RAPA with dexamethasone (Dex) or CsA.

The noncytotoxic immunosuppressants inhibit the immune system function mainly by preventing the proliferation of activated lymphocytes. CsA and FK506 both prevent lymphokine gene expression. RAPA exerts its action at a different level by interfering with the transduction of the proliferative signal of cellular growth factors. In our studies of the efficacy of RAPA in EAU in the Lewis rat, EAU was inhibited when RAPA treatment was instituted either on the day of immunization with S-antigen (S-Ag) or 7 days later (minimal effective dose, 0.1 mg/kg/day). The inhibition of EAU was correlated with reduced lymphocyte proliferation and antibody production to S-Ag. RAPA also was shown to prevent the adoptive transfer of EAU with a retinal antigen-specific T-cell line, indicating that it could be effective in the treatment of ongoing disease.

Drug combination is commonly used as a strategy to minimize the toxicity of each drug while achieving a full therapeutic effect. Our studies of the combination of RAPA with Dex or CsA in vitro have shown a potent synergistic action of these drugs. In combination, RAPA and CsA could be reduced by 10- and 3-fold, respectively, while Dex with RAPA allowed for a 4.5-fold reduction of both.

Project Description

Clinical Protocol Number

91-191

Objectives

To determine the efficacy of rapamycin (RAPA) to inhibit experimental autoimmune uveitis (EAU), the approach was to test the effect of treatment during the afferent phase of the immune system activation by starting treatment on the day of immunization. Afterward, testing during the efferent phase was enabled by instituting treatment 7 days after immunization. Finally, the fully activated immune system was tested by inducing EAU by T-cell line transfer. In addition, we evaluated the biological correlates of the activity of RAPA by measuring the effect of various doses of treatment on antibody production as well as on the lymphocyte proliferative response to S-antigen (S-Ag).

A major objective of the study of noncytotoxic immunosuppressants is to develop strategies of combination therapy that permit reducing the dose of the individual drugs to lower the toxic side effects while achieving a sufficient level of immunosuppression. To determine the combined effect of RAPA with dexamethasone (Dex) or cyclosporine A (CsA), we planned in vitro inhibition studies of the proliferation of S-Ag-primed lymphocytes with covaried concentrations of the drugs. The type of combination effect was to be determined by submitting the data to a calculation method that distinguishes between additive and synergistic effects.

Methods

EAU was induced either by immunization with S-Ag in Hunter's adjuvant, or by adoptive transfer with an interphotoreceptor retinoid-binding protein (IRBP)-specific T-cell line. Treatment was delivered over 14 days by continuous intravenous infusion by means of miniosmotic pumps implanted in the abdominal cavity and connected to a lumbar vein. The treatment protocol was aimed at the afferent, efferent, and fully differentiated phase of the immune system activation. The combination effects of RAPA with Dex or CsA were evaluated in vitro by the proliferation of S-Ag-primed lymph node cells. The data were evaluated by the medium effect analysis method of Chou et al (in *Synergism and Antagonism in Chemotherapy*, Chou T-C and Rideout DC, eds, Academic Press, San Diego, 1991).

Major Findings

Actively induced autoimmune uveoretinitis could be inhibited with RAPA at a minimal effective dose of 0.1 mg/kg/day. The drug was effective not only when used during the stimulation phase of the immune system, but also when treatment was delayed until the efferent phase, indicating that RAPA could be useful in the treatment of active disease. Because EAU in the rat has a very short evolution course once the eye is inflamed, the treatment cannot be reasonably tested during active disease. To ascertain the efficacy of RAPA in conditions mimicking disease conditions better, we treated animals in which EAU was induced by transfer of fully differentiated T cells. We found that the same 0.1 mg/kg/day dose was completely effective, indicating the RAPA would be applicable in the treatment of ongoing disease.

In vitro we found a strong synergistic effect on the inhibition of proliferation of lymphocytes to retinal S-Ag for the combination of RAPA with Dex or CsA. We calculated a dose-reduction factor for various dose combinations. For example, the combination of Dex with RAPA allowed for a 4.5-fold reduction of both drugs, while the combination of RAPA with CsA yielded reduction factors of 10 for RAPA and 3 for CsA.

Significance to Biomedical Research and the Program of the Institute

The study has demonstrated the efficacy of RAPA in the treatment of a uveitis model, which indicates that, barring unmanageable toxic side effects, the drug could be useful in the treatment of uveitis in humans. Perhaps the most encouraging result is the strong synergistic effect of RAPA combinations with Dex and CsA, which would eliminate most of the toxicity of these drugs while maintaining good therapeutic control of the disease.

Proposed Course

Future work on this project will include (1) evaluation of the toxic side effects of RAPA, (2) conducting in vivo combination treatment to evaluate the dose reduction factors found in the in vitro synergy, (3) evaluation of the effects of RAPA on the compli-

cation of uveitis, particularly subretinal membrane formation and neovascularization, and (3) evaluation of a new noncytotoxic immunosuppressant (SK&F 106610) in an aim to increase the combination's potential to lower the side effects of the various agents.

Publications

Roberge FG, Xu D, Chan C-C: A new effective and non-harmful chemical adjuvant for the induction of experimental autoimmune uveoretinitis. *Curr Eye Res* 4:371-376, 1992.

Roberge FG, Xu D, Chan C-C, de Smet MD, Nussenblatt RB, Chen H: Treatment of experimental autoimmune uveoretinitis in the rat with rapamycin, an inhibitor of lymphocyte growth factor signal transduction. *Curr Eye Res*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00263-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparison of Surgical Treatment in Uveitis Patients With Glaucoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Benjamin I. Rubin	M.D.	Special Volunteer	LI, NEI
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	Marc de Smet	M.D.	Visiting Fellow	LI, NEI
	Chi-Chao Chan	M.D.	Medical Officer	LI, NEI

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SECTION

Section of Clinical Immunology

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NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00248-05 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Magainin Therapy of Infectious Keratitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Juan S. Lopez	M.D.	Visiting Associate	LI, NEI
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	Richard M. Fenton	M.D.	Senior Staff Fellow	LI, NEI

COOPERATING UNITS *(if any)*

Clinical Pathology Department, Clinical Center, NIH (Frida Stock, B.S.)

LAB/BRANCH

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00262-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Selective Accumulation of Vβ8-Positive T Lymphocytes in Experimental Autoimmune Uveoretinitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LI, NEI
	Robert B. Nussenblatt	M.D.	Scientific Director	LI, NEI
	Rachel Caspi	Ph.D.	Visiting Associate	LI, NEI
	Rashid Mahdi		Biologist	LI, NEI

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experimental autoimmune uveoretinitis (EAU) is a T-cell-mediated autoimmune disease that serves as a model of human intraocular inflammatory disease (uveitis). It is initiated in susceptible animals by immunization with retinal antigens, such as interphotoreceptor retinoid-binding protein (IRBP) and S-antigen (SAg). Previous studies of T-cell receptor (TCR) usage by uveitogenic T cells suggested a possible connection between pathogenicity of T lymphocytes and usage of Vβ8 family genes. Here, we have analyzed the T-cell repertoire at the autoimmune site by examining Vβ gene expression in the retinas of animals with SAg- or IRBP-induced uveitis. Our data show a remarkable bias for the use of Vβ8.2 TCR among Vβ8-positive T cells in the retinas of animals with SAg-EAU, while in IRBP-EAU both Vβ8.2-positive and Vβ8.3-positive T cells were found. Similar results were obtained by DNA sequence analysis of 75 Vβ8 cDNA clones from uveitogenic T-cell lines. Twenty clones isolated from SAg-specific T-cell lines were all Vβ8.2 TCRs, whereas, among 55 Vβ8 cDNAs from IRBP-specific lines, 22% were Vβ8.2; 33%, Vβ8.3; and 44%, Vβ8.2 isoforms not previously reported. Vβ8.1-positive T cells were absent from the Vβ8 repertoires of the retina or any of the T-cell lines. In addition, there was marked heterogeneity of the CDR3 sequences utilized by the Vβ8-positive uveitogenic T cells, which contrasts with the reported near homogeneity of encephalitogenic T cells involved in experimental allergic encephalomyelitis (EAE). Taken together, our data show that although there is a bias toward usage of Vβ8-positive cells, the T-cell response in EAU is not oligoclonal. Moreover, distinct Vβ8 subfamilies were differentially activated by the autoantigens SAg and IRBP. In view of these findings, we believe that anti-Vβ therapy might have only limited usefulness in human uveitis because several autoantigens and an unknown number of immunopathogenic epitopes may be involved in the etiology of this diverse group of diseases.

Project Description

Objectives

This project is aimed at gaining knowledge concerning the clonality of the T cells that mediate experimental autoimmune uveitis (EAU). It is noteworthy that similar studies in experimental allergic encephalomyelitis (EAE) found restricted usage of Vβ8 T-cell receptor (TCR) elements. This knowledge was exploited to protect animals against the disease by using antibodies specific to Vβ8.2 or by vaccination against portions of the receptor. Our effort during Fiscal Year 1991 focused on the analyses of the TCRs expressed at the autoimmune site to determine whether there is restricted TCR Vβ usage in EAU.

Methods

Animals were immunized with either S-antigen (S-Ag) or interphotoreceptor retinoid-binding protein (IRBP), and at time points corresponding to the expected onset of EAU, as well as 1 day before and 1 day after expected onset, the animals were sacrificed and RNA was isolated from their retinas. cDNAs generated by reverse transcription were used to amplify all the known Lewis rat Vβ TCR chains. The T-cell lines used in these studies were derived from the lymph nodes of Lewis rats immunized with either S-Ag or peptides derived from IRBP. Conventional recombinant DNA techniques such as Southern blot hybridization, polymerase chain reaction (PCR), cDNA construction, densitometry, and DNA sequencing were used to analyze the resultant PCR products.

Major Findings

Analysis of TCR Vβ8 repertoire induced by S-Ag and IRBP.—We analyzed TCR genes expressed in response to immunopathogenic epitopes of human S-Ag or bovine IRBP by the PCR assay. We found that expression of Vβ8 genes in S-Ag-induced uveitis is restricted to Vβ8.2 TCR, while in IRBP-induced EAU multiple members of the Vβ8 family (Vβ8.2, Vβ8.3, and Vβ8.2 isoforms not previously reported) are expressed.

Analysis of junctional sequences used by uveitogenic T cells.—In contrast to findings among the TCRs of lymphocytes specific against xenogenic antigens such as cytochrome c and sperm whale myoglobin, in which T cells that recognize the same minimal epitope express identical junctional sequences, uveitogenic T cells with the same antigen fine specificity express different junctional sequences. We also found a subpopulation of T cells in IRBP- and S-Ag-specific T-cell lines that expressed similar junctional sequences, suggesting that these TCRs may be specific to a common determinant, possibly a self-superantigen or coligand.

Analysis of the fourth complimentarity-determining region (CDR4).—Forty-five percent of Vβ8 family TCRs analyzed contain amino acid substitutions at positions corresponding to the CDR4 region of the Vβ chain. Amino acid residues within this region (amino acids 67-75) have been shown to be involved in superantigen binding. Conservation of superantigen-binding sequences by a significant proportion of $V\beta8^+$ lymphocytes in these lines may not be coincidental and may suggest that lymphocytes expressing these receptors could have functions other than binding conventional antigens plus major histocompatibility complex.

Significance to Biomedical Research and the Program of the Institute

Taken together, our data show that although there is a bias toward usage of $V\beta8^+$ cells, the T-cell response in EAU is not oligoclonal, and distinct Vβ8 subfamilies were differentially activated by the autoantigens, S-Ag and IRBP. These findings suggest that anti-Vβb therapy might have only limited usefulness in human uveitis since several autoantigens and an unknown number of immunopathogenic epitopes may be involved in the etiology of this diverse group of diseases.

Proposed Course

We intend to induce EAU in nude mice by injection of antigen-activated S-Ag- or IRBP-specific uveitogenic T-cell lines. Because these mice do not normally produce T cells, we expect the intraretinal T-cell repertoire of these mice to be very limited. This finding would allow for a more concise analysis of the identity of autoaggressive T cells involved in uveitis.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Egwuagu CE, Bahmanyar S, Caspi R, Mahdi R, Nussenblatt R, Gery I: Predominant usage of Vβ8.3 T cell receptor in a T cell line that induces experimental autoimmune uveoretinitis. *J Clin Immunol Immunopathol* 65(2):152-160, 1992.

Mahdi RM, Caspi RR, Tsai L, Nussenblatt RB, Gery I, Egwuagu CE: T cell receptor Vβ gene usage in experimental autoimmune uveoretinitis. *Invest Opthalmol Vis Sci* 33(4):1024, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00280-01 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ectopic Expression of Interferon-Gamma in the Eyes of Transgenic Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles E. Egwuagu	M.P.H., Ph.D.	Scientist	LI, NEI
Others:	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Chi-Chao Chan	M.D.	Chief, Section on Immunopathology	LI, NEI
	Ana Chepelinsky	Ph.D.		LMDB, NEI
	Jorge Sztein	D.V.M., Ph.D.	Visiting Associate	VRRS, NEI
	Rashid Mahdi		Biologist	LI, NEI

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Experimental Immunology

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ectopic expression of interferon-gamma (IFN-γ) in the lens provides a model for studying the clinical and biological effects of IFN-γ in the normal development of the eye. In this project, two transgenic mouse lines expressing IFN-γ under the direction of lens-specific αA-crystallin promoter were established using two distinct mouse strains, Balb/c and FVB/N. In both the αACry-IFN-γ/Balb/c and αACry-IFN-γ/FVB/N transgenic mice, the essential histopathological features observed were very similar at all developmental stages studied (12- to 18-day embryos, newborns, adults), suggesting that maldevelopment of ocular tissues of these mice resulted from αACry-IFN-γ expression.

The differentiation of tissues of surface ectodermal, neuroectodermal, and mesodermal origin was adversely affected. In the lens, normal differentiation of the anterior and posterior lens cells was perturbed, resulting in marked disorganization of the entire lens architecture and very little or no lens fiber formation. The retina was very convoluted with retinal detachment, retinal degeneration, partial loss of photoreceptors, and accumulation of intraocular macrophages in the subretinal space. Vitritis, iridocyclitis, and corneal and vitreous neovascularization were commonly observed.

To investigate the molecular basis of IFN-γ-mediated disruption of the normal developmental program of ocular tissues, we analyzed the abundance of mRNA transcripts of several lens genes: αA-crystallin, major intrinsic protein (MIP), αB-crystallin, macrophage inhibitory factor (MIF), transforming growth factor (TGFβ), DNA-binding protein (dbpB/YB-1), and major histocompatibility complex (MHC) class II in αACry-IFN-γ mice and age-matched controls. In the transgenic mice, upregulation of MHC class II gene was observed while MIP gene transcription was silenced. Transcription of the other lens genes was not affected. Although the αA-crystallin promoter is normally active only in lens tissue, its tissue specificity was abrogated in the αACry-IFN-γ/Balb/c and αACry-IFN-γ/FVB/N transgenic mice; expression of αACry-IFN-γ also was found in the brain, kidney, liver, ovary, spleen, testes, and thymus. Furthermore, upregulation of MHC class II also was found in the brain, kidney, liver, ovary, and testes.

Project Description

Objectives

This project was designed to generate transgenic mice that selectively secrete interferon-gamma (IFN-γ) in their eye tissues for use in studying the bioregulatory actions of IFN-γ in nonlymphoid tissues. Because IFN-γ induces high levels of major histocompatibility complex (MHC) class II proteins, the utility of these mice as a model for studying the role of elevated MHC class II in predisposition to autoimmune diseases such as diabetes and uveitis was also explored.

Methods

Transgenic animals were generated according to standard transgenic mouse techniques. The transgenic and wild-type (WT) phenotypes were characterized by histological examinations of representative tissue sections. Conventional recombinant DNA techniques such as Southern blot hybridization, polymerase chain reaction (PCR), cDNA construction, densitometry, and DNA sequencing were used to characterize DNAs and RNAs derived from WT and transgenic mice.

Major Findings

1. Clinical symptoms manifested by the αACry-IFN-γ mice included microphakia, blepharophimosis (decrease in the palpebral aperture), blepharosynechiae (adhesion of the eyelids to the eyeball), exudative retinal detachment, vitritis, iridocyclitis, partial loss of photoreceptors, and corneal neovascularization.

2. IFN-γ gene expression affected normal eye development at the earliest stage studied, 18 days of embryonic development. The anterior chamber was not formed, and the retina was not differentiated into inner and outer neuroblastic layers. In the lens, normal primary lens fibers were replaced by balloon cells.

3. Expression of the αACry-IFN-γ transgene abrogated the characteristic tissue-specificity of the αA-crystallin promoter; we also found expression of the transgene in the brain, kidney, liver, ovary, spleen, testis, and thymus.

4. αA-crystallin and αA^{ins}-crystallin, previously thought to be expressed only in eye tissues, also were detected in normal mouse kidney, spleen, and thymus by PCR.

5. MHC class II gene expression was upregulated while major intrinsic protein (MIP) gene transcription was shut down in the transgenic mice, suggesting that IFN-γ can function as a transcriptional regulator in nonlymphoid cells.

6. Immunoperoxidase antibody staining of γ-crystallin and MIP, both markers of lens fibers, was decreased, while MHC class II staining was increased in the transgenic mice compared with that in normal age-matched controls, indicating that the synthesis of these proteins also was affected by ectopic expression of IFN-γ.

Significance to Biomedical Research and the Program of the Institute

We have generated a transgenic mouse system that can serve as a model for human autoimmune diseases. This mouse is also a useful system for studying the molecular events relating to the normal developmental program of ocular tissues. Because these transgenic mice are MIP-deficient mutants, they afford a useful model to begin characterizing the role of this gap junction protein in the physiology of the eye.

Proposed Course

We will further dissect the molecular basis of IFN-γ actions in the eye, placing particular emphasis on the transcriptional factors that mediate αACry-IFN-γ effects. We also will mate the αACry-IFN-γ mice with transgenic mice with ocular tumors to determine whether constitutive expression of IFN-γ in the eye would lead to tumor regression in the progeny.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Egwuagu CE, Sztein J, Reid W, Chan C-C, Mahdi R, Nussenblatt RB, Chepelinsky C: Gamma interferon gene expression in the lens of transgenic mice directed by the αA-crystallin gene promoter. *Invest Opthalmol Vis Sci* 33(4):846, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00069-15 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immune Responses to Ocular Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LI, NEI
Others:	Yoichi Kawano	M.D.	Visiting Fellow	LI, NEI
	Yoichi Sasamoto	M.D.	Visiting Fellow	LI, NEI
	Alexander Kozhich	Ph.D.	Visiting Fellow	LI, NEI
	Barbara Vistica	B.A.	Microbiologist	LI, NEI
	Belinda Shirkey	B.A.	HHMI-NIH Scholar	LI, NEI
	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Gerald J. Chader	Ph.D.	Chief	LRCMB, NEI
	Barbara Wiggert	Ph.D.	Head, Section on Biochemistry	LRCMB, NEI

COOPERATING UNITS *(if any)*

Metabolism Branch, Division of Cancer Biology, Diagnosis, and Centers, National Cancer Institute, NIH (Jay A. Berzofsky, M.D.); Syntex Research, Palo Alto, CA (Anthony C. Allison, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Experimental Immunology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.8	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Targeted at learning about the pathogenesis of inflammatory eye diseases grouped under the term "uveitis," this project in FY 1992 focused mainly on learning about ocular antigens that induce experimental autoimmune uveoretinitis (EAU), an animal model for uveitis in man, and on procedures that modulate this disease. Major achievements are three: (1) We found that the substitution of certain residues of the bovine IRBP peptide 1181-1191 eliminated its high uveitogenicity. The corresponding sequence of the rat IRBP differs from the bovine sequence by two residues and was found to be completely nonuveitogenic. (2) To investigate mechanisms of specific unresponsiveness toward uveitogenic peptides, we developed an in vitro system in which lymphocytes sensitized toward peptide 1181-1191 are rendered unresponsive. We have defined the process to be highly specific and to involve active metabolic events. (3) We have examined three procedures aimed at inhibiting the immunopathogenic process of EAU: (a) EUA induced in rats by IRBP peptide 1181-1191 was completely inhibited when this peptide was coinjected with a competing peptide, A183, an analog of a peptide derived from a *Mycobacterium tuberculosis* protein. On the other hand, nonuveitogenic analogs of peptide 1181-1191 were inactive in this system. (b) The procedure of oral tolerance, shown in another project to inhibit S-antigen-induced EAU effectively, also was found to modulate EAU induced by the small peptide 1181-1191. Furthermore, using analogs of peptide 1181-1191 we have shown that oral tolerance is highly specific: Only cross-reactive analogs were effective. (c) We have examined a new immunosuppressive drug, mycophenolate mofetil (MM), for inhibition of EAU. MM completely inhibited both actively induced and adoptively transferred EAU when given at doses that had no apparent side effects.

Project Description

Objectives

This project is designed (1) to collect additional information on the immunodominant and highly uveitogenic peptide determinant 1181-1191 of interphotoreceptor retinoid-binding protein (IRBP), with regard to (a) the residues pivotal for the immunological activities of this molecule and (b) the capacities of the rat sequence corresponding to the bovine IRBP 1181-1191; (2) to analyze further the procedure in which lymphocytes sensitized against peptide 1181-1191 are rendered unresponsive ("anergic") to this uveitogenic molecule; and (3) to investigate procedures aimed at suppressing the immunopathogenic processes of experimental autoimmune uveoretinitis (EAU). The procedures included (a) injection of peptides with the capacity to compete with the uveitogenic peptide, (b) induction of specific tolerance by oral administration of the uveitogenic peptide, and (c) treatment with a new immunosuppressive drug, mycophenolate mofetil (MM).

Methods

Synthetic peptides were provided by A.B.I. (Foster City, CA) or synthesized on an Applied Biosystems synthesizer 430A. MM, supplied by Syntex Research (Palo Alto, CA), was administered by gavage. EAU induction and measurements of immune responses were performed as described in our publications. The procedures for induction of anergy in vitro are described in previous NEI annual reports.

Major Findings

Studies concerning IRBP peptide 1181-1191.—In a previous study (Fiscal Year [FY] 1990) we showed that certain residues of peptide 1181-1191 are pivotal for its immunodominance and high uveitogenicity in Lewis rats; substitution of these residues with alanine markedly reduced or even completely abolished the activities of this peptide. We extended this approach by testing analogs in which other amino acids were used for substitution. The data further underscored the pivotal role of residues 1188, 1189, and 1190 and established the role of residue 1183.

The recently published sequence of rat IRBP shows that within determinant 1181-1191 the rat sequence differs from that of the bovine by two residues: Position 1188 is threonine in the rat and valine in the bovine sequence, while position 1190 is asparagine in the rat and aspartic acid in the bovine. Both residues are pivotal for the bovine molecule's activities, and no cross-reactivity was detected between the bovine and rat peptides. Moreover, the rat peptide was nonuveitogenic and even nonimmunogenic in the Lewis rat: Neither EAU nor immune responses were detected in rats immunized with this molecule. We found that a peptide in which only the valine at 1188 was substituted with threonine was weakly uveitogenic and immunogenic; it showed a low-level cross-reactivity with bovine peptide 1181-1191.

Induction of anergy in vitro.—We had previously developed an in vitro system in which specific anergy (ie, unresponsiveness) is induced toward uveitogenic peptides (FY 1991). We further analyzed the system, producing the following findings: (1) Testing additional peptide analogs of bovine IRBP peptide 1181-1191 showed that the system is highly specific: Only analogs with cross-reactivity toward the native peptide were capable of inducing anergy in lymphocytes specific toward this peptide. Accordingly, the rat homologue of peptide 1181-1191 (see above) failed to show any effect in this system. (2) Induction of anergy was accompanied by an increase in inositol phosphate synthesis in the test lymphocytes. The relationship between anergy induction and increased inositol phosphate synthesis was shown by the complete correlation between the two processes when different analogs of peptide 1181-1191 were tested. (3) Antibodies against rat major histocompatibility complex class II antigens had no effect on induction of anergy. On the other hand, the antibodies tested here, OX-3 and OX-6, effectively inhibited the proliferative response of the lymphocytes toward peptide 1181-1191 in the conventional system in which antigen-presenting cells are present.

Modulation of EAU.—We have assessed three procedures for their capacity to modulate the immunopathogenic processes of EAU.

Because published studies have shown that experimental autoimmune diseases can be inhibited by injecting "competing" peptides along with the immunopathogenic antigens (eg, *Proc Natl Acad Sci* 88:9633, 1991), we tested two types of peptides for their capacity to inhibit EAU induced by IRBP determinant 1181-1191: (1) four nonuveitogenic analogs of 1181-1191 with substitution of pivotal

residues (see above), which we found to be inactive in this system, and (2) an unrelated peptide with known competitive capacity. This peptide, designated "A183," an analog of a nonamer determinant of a heat-shock protein of *Mycobacterium tuberculosis*, was found to inhibit the development of adjuvant arthritis and experimental allergic encephalomyelitis in Lewis rats. Peptide A183 completely inhibited the development of EAU induced by peptide 1181-1191 when injected at the molar ratio of 1:500 and caused partial inhibition of the disease at the ratios of 1:40 and 1:100. A183 also inhibited the cellular immune response to 1181-1191 but, unexpectedly, A183 did not competitively inhibit the proliferative response of lymphocytes toward 1181-1191.

Another project (NEI protocol 90-EI-116) had shown that feeding rats uveitogenic proteins or peptides derived from their sequence effectively inhibits the development of EAU induced by these molecules. We tested the fine specificity of this procedure by testing analogs of peptide 1181-1191 for their capacity, when fed, to induce a state of tolerance toward the native peptide. The data showed good correlation between the analogs in inducing such tolerance and in their cross-reactivity toward peptide 1181-1191, which is evident in their recognition by lymphocytes sensitized against the native peptide.

MM, an inhibitor of purine synthesis (Eugui et al, *Scand J Immunol* 33:161, 1991), had been found to efficiently inhibit S-antigen (S-Ag)-induced EAU when given daily at 30 mg/kg/day, on days 0-13 postimmunization. At a higher dose, 60 mg/kg/day, we found MM inhibited EAU, even when administered on days 7-20. In addition, MM at 30 mg/kg/day inhibited the development of EAU adoptively transferred by lymphocytes sensitized against S-Ag. These data thus show that MM can inhibit both the afferent and efferent limbs of the immunopathogenic process of EAU.

Significance to Biomedical Research and the Program of the Institute

1. Peptide 1181-1191 is the immunodominant determinant of bovine IRBP in Lewis rats. It is highly uveitogenic and immunogenic in these animals. Our studies of this molecule provide new information concerning residues that are pivotal for its immunologic activities. Of particular interest are the findings with the rat homologue of peptide 1181-1191. The complete lack of uveitogenicity, immunogenicity, and cross-reactivity with the bovine peptide was unexpected. This finding suggests that a unique mechanism should be used by lymphocytes sensitized against bovine peptide 1181-1191 for the recognition of the target antigen in the rat eye when initiating the immunopathogenic process of EAU.

2. The system of anergy induction in vitro has provided useful new information concerning the mechanisms whereby lymphocytes can be rendered unresponsive toward uveitogenic peptide. Moreover, this information should be applicable for developing novel procedures specifically for suppressing responses toward immunopathogenic antigens.

3. Our EAU modulation studies have yielded several findings of interest: (a) Using peptides to inhibit uveitogenic processes is feasible, but mechanisms other than competitive inhibition also are involved. (b) Oral tolerance can be induced by peptides as small as 1181-1191, and the effect is highly specific. (c) MM inhibits the development of EAU by affecting both the afferent and efferent immunopathogenic processes of disease at doses that have no apparent adverse side effects. Thus, this compound should be considered as a potential new drug for treatment of uveitis.

Proposed Course

Our future efforts will focus on several issues. (1) We will further investigate the enigmatic finding concerning the rat homologue of peptide 1181-1191 (see above) by testing several potential explanations for this observation. (2) We will further analyze the induction of anergy in vitro with regard to the features of the phenomenon and the biochemical processes involved. (3) In particular, we will focus on procedures and agents that modulate the immunopathogenic processes of EAU. More studies will be conducted to understand better the induction of anergy, both in vitro and in vivo. We will test MM and additional immunosuppressive agents, and we will examine the possible synergy between compounds to achieve effective combinations of drugs at significantly lower doses.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Beraud E, Kotake S, Caspi RR, Oddo SM, Chan C-C, Gery I, Nussenblatt RB: Control of experimental autoimmune uveoretinitis by low dose T-cell vaccination. *Cell Immunol* 140:112-122, 1992.

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Fujino Y, Chan C-C, de Smet MD, Hikita N, Gery I, Mochizuki M, Nussenblatt RB: FK506 treatment of experimental autoimmune uveoretinitis in primates. *Transplant Proc* 23:3335-3338, 1991.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00222-07 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathology in the Eyes With Experimental Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chi-Chao Chan	M.D.	Head, Section on Immunopathology	LI, NEI
Others:	Rachel R. Caspi	Ph.D.	Visiting Scientist	LI, NEI
	Igal Gery	Ph.D.	Deputy Chief	LI, NEI
	Qian Li	M.D.	Visiting Associate	LI, NEI
	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Scott M. Whitcup	M.D.	Medical Officer	LI, NEI
	François G. Roberge	M.D.	Visiting Scientist	LI, NEI
	Deborah Luyo		Technician	LI, NEI
	Louis Kasner	M.D.	IRTA	LI, NEI

COOPERATING UNITS *(if any)*

Department of Ophthalmology, Kurume University, Kurume, Japan (Manabu Mochizuki, M.D.); Service Universtaire d'Ophtalmologie, Hôpital Ophtalmique Jules Gonin, Lausanne, Switzerland (Carl P. Herbort, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunopathology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.50	1.25	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The identity and topographic localization of immunocompetent cells and the alteration of surface markers on ocular resident cells in mice and rodents with experimental autoimmune uveoretinitis (EAU) were analyzed by immunohistochemical studies and in situ hybridization. T lymphocytes were the predominantly infiltrating cells in rat EAU, yet both macrophages and T helpers/inducers were the predominantly infiltrating cells in mouse EAU. The migration of inflammatory cells from vessels into target sites is directed by adhesion molecules that can be expressed on vascular endothelium and other resident cells in the eye. T-lymphocyte specificity is directed to small fragments of antigen bound to cell surface major histocompatibility complex (MHC) molecules, which are presented on the surface of specialized antigen-presenting cells. The expression of MHC class II antigens was observed on ocular resident cells such as retinal pigment epithelium (RPE), retinal vascular endothelium, keratocytes, fibroblasts, and ciliary epithelium in rodents. The expression of MHC class II antigens is confined to ocular resident cells immediately at the inflammatory sites in mouse EAU, which is characterized by focal lesions and chronicity. Both the infiltrating cell subpopulation and the expression of class II antigens on ocular resident cells can be altered by various immunomodulating agents.

Experimental endotoxin-induced uveitis (EIU) is a model for anterior uveitis in humans. The mechanism of this inflammation is still unclear, although activation of phospholipase A2 (PLA2) may play a role in the initiation and propagation of this disease. The particular vascular structure of the anterior uvea and the role of adhesion molecules (such as intercellular adhesion molecule and endothelial leukocyte adhesion molecule) and their ligand (lymphocyte function-associated antigen) also were examined in EIU and EAU models. Inhibition of PLA2 (chlorpromazine, antiflammin, etc.) and adhesion molecules can abrogate EIU.

This project will be combined with project numbers Z01 EY 00241-06 LI and Z01 EY 00264-03 LI, entitled "Immunopathology of Experimental and Clinical Ocular Diseases."

Project Description

Additional Personnel

Louis Kasner M.D. LI, NEI

Objectives

This program is designed to evaluate the clinical manifestation, histopathology, and immunopathology of the ocular tissue when experimental autoimmune uveoretinitis (EAU) and endotoxin-induced uveitis (EIU) are induced and/or modulated by various immunosuppressive agents in various animal species. The infiltrating inflammatory cells, ocular resident cells and their products, various lymphokines, and cytokines are examined. The findings will help us understand ocular inflammation.

Methods

Clinical examinations include flashlight, slit-lamp, and fundus examination under the dissecting microscope. Pathological examinations include routine histologic techniques for light and electron microscopy, immunofluorescence, avidin-biotin-peroxidase complex methods, and in situ hybridization techniques.

Major Findings

The kinetics of infiltrating cells inside the eyes with EAU in the rat showed predominantly CD4 cells in the early stage and predominantly CD8 cells in the late stage. In the mouse, both CD4 and CD11c/18 cells predominated in the eye. In the monkey, an influx of CD19 cells among the CD3 cells and subretinal fibrosis coexisted in the late stage. Prior to the infiltration of inflammatory cells, we identified mast cell degranulation, expression of adhesion molecules, and major histocompatibility complex (MHC) class II antigens on ocular resident cells. Immunosuppressive medications could alter the kinetics of the infiltrating cells and the markers of the resident cells.

Mast-cell degranulation, the expression of adhesion molecules, and MHC class II antigens on ocular resident cells also were observed prior to the ocular infiltration of inflammatory cells in EIU.

Significance to Biomedical Research and the Program of the Institute

Different immunopathological findings of EAU can be presented in various species using the same antigen. The pathology of uveitis in human beings may capture a single moment of the changing kinetics and can be influenced by the treatment.

Proposed Course

EAU and EIU will be studied clinically and histopathologically in rodent, murine, and primate models. Various immunosuppressive agents will be used to modulate the disease.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Beraud E, Kotake S, Caspi RR, Oddo SM, Chan C-C, Gery I, Nussenblatt RB: Control of experimental autoimmune uveoretinitis by low dose T cell vaccination. *Cell Immunol* 140:112-122, 1992.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00241-06 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathology of Ocular Diseases in Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chi-Chao Chan	M.D.	Chief, Section on Immunopathology	LI, NEI
Others:	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Qian Li	M.D.	Visiting Fellow	LI, NEI
	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
	Raymond DeBarge	M.D.	Senior Staff Fellow	LI, NEI
	Scott M. Whitcup	M.D.	Staff Medical Officer	LI, NEI
	Juan Lopez	M.D.	Visiting Associate	LI, NEI
	Miguel Burnier	M.D.	Visiting Scientist	LI, NEI
	Richard Fenton	M.D.	Staff Fellow	LI, NEI
	Dev Shah	M.D.	Visiting Associate	LI, NEI

COOPERATING UNITS *(if any)*

Department of Ophthalmology, Armed Forces Institute of Pathology (Ian W. McLean, M.D.); University of Minnesota, Department of Ophthalmology (Edward J. Holland, M.D.); L'Hôpital de la Pitié, Paris, France (Phuc LeHoang, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunopathology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Specimens from human ocular tissues with various diseases such as uveitis, retinal disease, conjunctival and corneal diseases, metabolic genetic diseases, and tumors are studied using immunohistochemical and in situ hybridization techniques as well as light and electron microscopic evaluation. In uveitis, immunocompetent cells and lymphokines are valuable adjuncts to clinical diagnosis, as well as determinants of disease course and prognosis. In nonuveitic conditions, alteration of cellular membrane surface markers and intracytoskeleton of the ocular resident cells may imply damage and abnormalities in these diseases. Elucidating the role of the relationships between infiltrating inflammatory or malignant cells and other resident cells in the clinical behavior of various diseases will increase our understanding of human ocular disorders.

This project will be terminated and combined with Project No. Z01 EY 00222-07 LI next year.

Project Description

Additional Personnel

Debrah Luyo Technician, LI, NEI

Objectives

The program is designed to evaluate the pathology of infiltrating cells and ocular resident cells in various ocular diseases, including inflammatory and non-inflammatory disorders. The study emphasizes the cell surface markers and cell products and brings information on the pathogenesis of each disease.

Methods

Immunohistochemistry and in situ hybridization, in addition to routine light and electron microscopic techniques, are applied.

Major Findings

Infiltrating immunocompetent cells and their products, lymphokines, are vital in the development of ocular inflammation. However, these cells also play a role in noninflammatory diseases, such as intraocular lymphoma (large B-cell lymphoma) and orbital lymphoma. Expression of some cell markers such as adhesion molecules and major histocompatibility complex class II antigens are important for homing the infiltrating cells and immune responses. The handling and processing of human specimens are critical for evaluation and diagnosis, in particular, diagnostic vitrectomy and chorioretinal biopsy.

Significance to Biomedical Research and the Program of the Institute

T lymphocyte-mediated immune response is usually involved in noninfectious inflammation in the eye. The routine use of immunohistochemical techniques and the proper handling of specimens—in particular the biopsies from the vitreous, uvea, and retina—will increase the diagnostic yield and help the therapeutic choice. In addition, they will help us understand the mechanism of the disease.

Proposed Course

The continued evaluation of specimens obtained from patients with different ocular disorders is proposed in the study of their immunopathogenesis.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Burnier MN Jr, Piacentini MA, Shah DN, Chan C-C, Nussenblatt RB, McLean IW: Differentiation between benign and malignant orbital lymphocytic lesions: The use of proliferating cell nuclear antigen. *Invest Ophthalmol Vis Sci* 33(4):1245, 1992.

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Whitcup SM, Chan C-C, Li Q, Nussenblatt RB: Expression of cell adhesion molecules in posterior uveitis. *Arch Ophthalmol* 110:662-666, 1992.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00264-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytokines and Ocular Antigens in the Eye

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chi-Chao Chan	M.D.	Head, Section on Immunopathology	LI, NEI
Others:	Robert B. Nussenblatt	M.D.	Scientific Director	LI, NEI
	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LI, NEI
	Qian Li	M.D.	Visiting Fellow	LI, NEI
	Louis Kasner	M.D.	Fellow	LI, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunopathology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytokines are communication signals between leukocytes and organ resident cells. Interleukin 1 (IL-1), a pleiotropic cytokine produced by many cell types—most notably macrophages, can stimulate a general inflammatory reaction by causing the activation of a variety of cells which then, among their other functions, release a cascade of other cytokines. One of these cytokines is interleukin 8 (IL-8), which takes the more direct action of activating neutrophils and T lymphocytes. CD4 cells can be subtyped to T helper 1 and 2 (Th-1 and Th-2) cells on the basis of the cytokines they release. Th-1 cells produce IL-2, interferon-gamma (IFN-γ), and tumor necrosis factor (TNF), while Th-2 cells produce IL-4, IL-5, IL-6, and IL-10. Th-1 cells are involved in the cell-mediated immune response, while Th-2 cells are involved in allergy and extracellular parasitic infection. Cytokines, lymphokines, and adhesion molecules also are located in ocular inflammatory sites of patients with uveitis and animals with experimental uveitis.

Ocular antigens play an important role in autoimmune diseases. S-antigen (S-Ag), a retinal soluble antigen, can induce experimental autoimmune uveoretinitis (EAU). The expression of S-Ag mRNA of nonretinal tissues in fetuses may suggest its involvement in certain ocular diseases. The expression of S-Ag mRNA was found in the iris of some patients with uveitis after prolongation of corticosteroid therapy. This finding was confirmed by treating EAU rats with long-term steroids.

This project will be terminated and combined with project number Z01 EY 00222-07 LI next year.

Project Description

Additional Personnel

Scott M. Whitcup	M.D.	Staff Medical Officer, LI, NEI
Toshimichi Shinohara	Ph.D.	Head, Section on Molecular Biology, LRCMB, NEI

Objectives

The program is designed to evaluate the presence and localization of various cytokines and their mRNAs as well as ocular antigens and enzymes in normal and diseased eyes from animals and/or humans.

Methods

Immunohistochemistry and in situ hybridization techniques are applied to evaluate the ocular tissues on slides. The amounts of cytokines in the ocular fluids (aqueous humor and vitreous) also are analyzed. Intraocular injection and/or systemic injection of cytokines is used to study their direct effects on the eye.

Major Findings

Interleukin 4 (IL-4) mRNA is found in the conjunctiva of patients with ocular onchocerciasis. This finding suggests that Th-2 helper T cells play an immunopathological role in this parasitic disease.

S-antigen (S-Ag) and its mRNA are found not only in the retina but also in the ocular tissues arising from neurocrest in the fetus. S-Ag mRNA is detected in the iris of some uveitic patients who have undergone prolonged corticosteroid treatment. The observation is confirmed via animal studies.

Significance to Biomedical Research and the Program of the Institute

Cytokine functions are complex, but the analysis of a relatively simple regulatory network suggests that activities determined in vitro are highly relevant to physiological and immunological functions as well as the pathogenesis in vivo. In the future, pharmacologically switching different cytokines, antigens, surface markers (eg, adhesion molecules), and enzymes on and/or off may be useful in the therapeutic intervention of ocular disorders.

The side effects of corticosteroids include cataract formation and glaucoma. These adverse effects may be induced by the aberrant release of signals, such as S-Ag on the trabecular meshwork and lens.

Proposed Course

Ocular tissue and cultured cells will be evaluated.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Chan C-C, Li Q, Kikuchi T, Shinohara T, Nussenblatt RB: Enhancement of S-antigen and its mRNA in the irises from uveitic patients. *J Autoimmunity* 5:719-732, 1992.

Fujino Y, Li Q, Chung H, Hikita N, Gery I, Nussenblatt RB, Chan C-C: Immunopathology of experimental autoimmune uveoretinitis in primates. *Autoimmunity*, in press.

Grossman S, Lopez JS, Shah DN, Rubin B, Altman D, Reznik R, Testa D, Feldman J, Chan C-C, Nussenblatt RB: Inhibition of endotoxin-induced uveitis (EIU) in Lewis rats by AA-313. *Invest Ophthalmol Vis Sci* 33(4):933, 1992.

Kasner L, Chan C-C, Cordella-Miele E, Gery I: The effect of chlorpromazine on endotoxin-induced uveitis in the Lewis rat. *Curr Eye Res*, in press.

Lopez JS, Li Q, Caspi RR, Nussenblatt RB, Kador P, Chan C-C: Use of oral CGS-13080 to suppress the development of experimental autoimmune uveitis in the Lewis rat. *Invest Ophthalmol Vis Sci* 33(4):933, 1992.

Ni M, Yamaki K, Kikuchi T, Ferrick M, Shinohara T, Nussenblatt RB, Chan C-C: Development expression of S-antigen in fetal human and rat eye. *Curr Eye Res* 11:219-229, 1992.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00232-07 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interferon System in Cellular Function and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Hooks	Ph.D.	Head, Section on Immunology and Virology	LI, NEI
Others:	Caroline Percopo	M.S.	Biologist	LI, NEI
	Chandrasekharam Nagineni	Ph.D.	Visiting Scientist	LI, NEI
	M. Cristina Martins	M.D.	Guest Researcher	LI, NEI

COOPERATING UNITS *(if any)*

Department of Pathology, The George Washington University Medical Center (Barbara Detrick, Ph.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunology and Virology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.65	0.40	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytokines, such as interferon-gamma (IFN-γ) and interleukin 2 (IL-2), are a group of specialized hormone-like proteins that exert profound influences on cellular development and on a variety of cellular functions. This project has concentrated on studying the ways in which cytokines interact with cells of the immune system and with cells in the ocular microenvironment. We have shown that IFN-γ and IL-2 are found within the inflamed eye in association with T-cell infiltration and major histocompatibility complex (MHC) class II antigen expression on infiltrating cells and on retinal pigment epithelium (RPE) cells. Furthermore, IFN-γ-activated RPE cells can process and present antigens to helper T lymphocytes.

Experimentally we demonstrated that isolated human RPE cells can be induced to produce another lymphokine, IL-6, following incubation with IFN-γ. IL-6 is a potent inflammatory cytokine capable of enhancing antibody production and cytotoxic T-cell activities. These studies indicate that cytokine-mediated activation of RPE cells may be a basic component of ocular immunity and an important aspect of RPE cell transplantation.

Retinoblastoma cells are an important model for exploring human malignancy and differentiation. Using these cells, we have shown that IFN-γ can regulate MHC class I genes at both transcriptional and posttranscriptional levels. In addition, this modulation is not associated with downregulation of N-myc oncogene expression.

These observations indicate that IFN-γ-induced MHC class I and II antigen expression may serve as a local amplification system in autoimmune and inflammatory eye disease. A better understanding of the role of cytokines in the mechanisms involved in the development of autoimmunity and inflammation may be beneficial in developing treatments for these diseases.

Project Description

Objectives

This project is designed to determine the bioregulatory actions of interferon (IFN) and other cytokines and to evaluate their regulatory actions in the pathogenesis of disease.

Methods

We assayed human IFN using inhibition of vesicular stomatitis virus plaque formation in human amnion (WISH) cells. IFNs were characterized by neutralization of antiviral activity with monoclonal anti-IFN immunoglobulin. Interleukin 2 (IL-2) biological activity was assayed by induction of proliferation of CTL cells. Interleukin 6 (IL-6) activity was assayed by an enzyme-linked immunosorbent assay, immunoblot assays, and Northern blot assays. We used analytical flow cytometry to quantitate retinal proteins. We are using gene transcription techniques, such as Northern blot analysis and nuclear run-off transcription assays, to evaluate IFN-γ modulation of retinal proteins.

Major Findings

IFN activation of retinal pigment epithelial (RPE) cells.—Numerous studies indicate that a variety of autoimmune diseases are associated with the IFN-γ-induced tissue-specific expression of major histocompatibility complex (MHC) class II molecules. During the past 5 years we identified various steps that may be involved in ocular immunopathologic mechanisms. In these studies of retinal degenerations and autoimmune diseases we showed that a critical regulatory cell in the retina, the RPE cell, is capable of expressing MHC class II determinants. We can detect IFN-γ in situ as well as MHC class II-positive RPE cells in retinas from patients with inflammatory eye diseases. In addition, freshly isolated human RPE cells can express these determinants following treatment with IFN-γ. In animal model systems, we found that inoculation of recombinant IFN-γ induces Ia expression of ocular cells and treatment with anti-Ia antibodies can eliminate or inhibit experimental autoimmune uveitis. Most recently we showed that the RPE cell may play an important role in ocular immunity, acting as a resident antigen-presenting cell in the retina.

During the past year we provided the most recent piece of experimental evidence indicating a role for cytokine-activated RPE cells in autoimmune phenomena by showing that the RPE cell is capable of producing the cytokine IL-6. The RPE cell cultures were established from human donor eyes. The isolated RPE cells alone did not produce IL-6. However, IFN-γ induced these cells to produce IL-6 in a dose-dependent manner. Moreover, IFN-γ can synergize with tumor necrosis factor (TNF) to produce IL-6 in human RPE cells. IL-6, a potent cytokine, can act on B lymphocytes to induce growth and antibody production. It also can act on T lymphocytes to induce IL-2 production, IL-2 receptor expression, and cell proliferation. These studies further substantiate the concept that cytokine-mediated activation of RPE cells may be a basic component of ocular immunity. It may have major immunological consequences for RPE cell transplantation studies.

Cytokine-induced modulation of cellular proteins in the retina and retinoblastoma.—Retinoblastoma cells are an important model for exploring human malignancy and differentiation. These multipotent embryonic cells are capable of differentiating into neuronal, glial-like, and RPE-like elements. We have shown that flow cytometric analysis can be used to measure the expression of both cytoplasmic and cell surface proteins in retinoblastoma cells. We used this technique to monitor changes in the expression of selected cellular proteins after exposure to specific cytokines and found that MHC class I molecules were augmented by IFN-α and IFN-γ, but not by TNF. The MHC class II molecules were augmented by IFN-γ, but by neither IFN-α nor TNF. The neuronal markers interphotoreceptor retinoid-binding protein (IRBP) and photoreceptor protein (PR-6), the glial-like marker GFAP, and the RPE cell markers RPE-9 and RPE-15 were not altered by any of the cytokines tested.

The mechanism of induction of MHC class I and II antigens by IFN in retinoblastomas is unknown. We have therefore initiated studies to compare IFN-α, IFN-β, and IFN-γ in their ability to induce the expression of class I antigens and to investigate the role of transcriptional and posttranscriptional mechanisms in this induction. We found that IFN-γ increases HLA-class I antigen expression and induces a 5-fold increase in its transcription rate. Posttrans-

criptionally, IFN-β and IFN-γ increased steady-state mRNA for the HLA-B7 gene. These effects were not associated with downregulation of N-myc oncogene nuclear transcription. Moreover, dexamethasone did not affect the IFN-γ-induced expression of HLA-class I molecules. These studies demonstrate that both transcriptional and posttranscriptional mechanisms are implicated in the modulation of class I molecule expression by IFNs.

Significance to Biomedical Research and the Program of the Institute

These studies highlight the fact that the release of cytokines, such as IFN-γ, within the ocular microenvironment and the subsequent induction of cytokines and MHC class I and class II antigen expression on resident and infiltrating cells may be critical elements in a cascading effect leading to ocular cell destruction. Within the retina, the cell that may play a critical role in autoimmune uveitis is the RPE cell. IFN-γ-induced RPE cell activation may participate in autoimmune disease in the ocular microenvironment.

Cytokines produced and localized in the eye may play a critical role in normal physiology, pathogenic mechanisms, and therapeutic approaches. Because the RPE cell is a pivotal regulatory cell in the retina, an understanding of how cytokines interact with this cell will shed light on avenues for therapeutic intervention in pathogenic states and in transplantation.

Proposed Course

We plan to continue our evaluation of the role of cytokines in autoimmunity and inflammation. We are now developing systems in rat models to monitor directly the effects of altering cytokine production on inflammatory eye diseases. Moreover, we will continue to characterize the antigen-presenting ability of the RPE cell to a variety of antigens and viruses.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Barez S, Boumpas D, Percopo C, Anastassiou ED, Hooks JJ, Detrick B: Modulation of major histocompatibility complex (MHC) class I genes in human retinoblastoma cells by interferons: Evidence for both transcriptional and post-transcriptional regulation. *Invest Ophthalmol Vis Sci*, in press.

Detrick B, Hooks JJ: Autoimmune aspects of ocular disease, in Rose N, MacKay I (eds): *The Autoimmune Diseases - II*. New York, Academic Press, 1992, pp 345-361.

Hooks JJ, Detrick B: Evaluation of the interferon system, in Rose N, DeMacario EC, Fahey J, Friedman H, Penn G (eds): *Manual of Clinical Laboratory Immunology*, ed 4. Washington, DC, ASM Publications, 1992, pp 240-244.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00233-07 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Bioregulatory Aspects of the Retinal Pigment Epithelial Cell

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Hooks	Ph.D.	Head, Section on Immunology and Virology	LI, NEI
Others:	Chandrasekharam Nagineni	Ph.D.	Visiting Scientist	LI, NEI
	Caroline Percopo	M.S.	Biologist	LI, NEI
	Christian Hamel	M.D.		LI, NEI
	T. Michael Redmond	Ph.D.		LI, NEI

COOPERATING UNITS *(if any)*

Hôpital St. Louis, Paris, France (Lawrence Bournsell, M.D.); University of Nice, France (Alain Bernard, M.D.); National Institute of Dental Research, NIH (Reuben Siraganian, M.D.); The Johns Hopkins University (Stanley A. Vinores, Ph.D.; Peter Campochiaro, M.D.); Department of Pathology, The George Washington University Medical Center (Barbara Detrick, Ph.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunology and Virology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.05	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The retinal pigment epithelial (RPE) cell plays a basic role in maintaining the structural and physiological integrity of the neural retina. We have isolated and propagated RPE cells in vitro and have developed monoclonal antibodies directed against human RPE cells. We are using these techniques and reagents to evaluate molecular, biochemical, and biological properties of the RPE cells.

Because the monoclonal antibodies detect epitopes present solely on RPE cells, they provide us with the unique opportunity to evaluate a variety of aspects of RPE cell development and function. Studies on RPE cell development indicate that the epitopes appear only after the cells have begun terminal differentiation. Moreover, studies on RPE migration also demonstrate the value of these antibodies in evaluating epiretinal membrane formation. The RPE epitope is a 67 kD protein that is closely associated with the microsomal membrane. We have isolated a cDNA clone that codes for a protein which matches no other sequence in the data bases.

Studies are also in progress to propagate and transplant RPE cells in various animals. We have propagated human RPE cells in vitro and evaluated their ability to respond to cytokine activation. RPE cells respond to retinal aberrations by dying, proliferating, migrating, losing phagocytic function, expressing major histocompatibility complex (MHC) class II antigens, and presenting antigens to T lymphocytes. The techniques and reagents obtained in these studies allow us to evaluate the mechanisms involved in aberrant RPE cell responses. Moreover, they provide the framework to evaluate RPE cell transplantation.

Project Description

Objectives

The aim of this project is to evaluate the molecular, biochemical, and varied biologic properties of retinal pigment epithelium (RPE) cells in normal and disease states. Moreover, we are evaluating RPE cell transplantation.

Methods

RPE cells were isolated and propagated in vitro. We generated monoclonal antibodies in mice by fusing mouse spleen cells with myeloma cells. Antibodies to RPE cells were evaluated by immunoperoxidase assays and by Western blot assays. The effect of drugs and cytokines are evaluated by cell viability and proliferation assays and by nuclear transcription runoff assays.

Major Findings

Evaluation of epitopes identified by monoclonal antibodies.—We have identified two mouse IgG3 monoclonal antibodies that react with RPE cells from a variety of species ranging from human to frog. Whereas these antibodies detect epitopes present solely on RPE cells, they provide us the unique opportunity to evaluate a variety of aspects of RPE cell development and function.

Electron microscopic immunocytochemistry revealed very similar labeling patterns for the two RPE antibodies. In human eyes, staining was localized to surface and intracellular membranes and the cytoplasm. Staining occurred predominantly on the apical surface of the RPE cells. The RPE protein is a 65-kD protein. We isolated this protein by polyacrylamide gel electrophoresis, transferred it to nitrocellulose blot, and determined the sequence of amino acid residues. The amino acid sequence was used to design a synthetic cDNA probe. A bovine cDNA library was screened, and cDNA clones were isolated and characterized. The cDNA insert is 3,115 base pairs. The open reading frame encodes a protein of 533 amino acids. The protein does not match any other sequence in the data bases. The protein expressed in *Escherichia coli* has a molecular weight similar to that of the native protein. Northern blotting with the cDNA detected protein mRNA in RPE cells.

We have studied the developmental expression of RPE and photoreceptor determinants in the rat retina. We had previously shown that the expression of this determinant in rats is absent the day of birth but detectable at postnatal day 6. Recent studies show that RPE cells express their determinants shortly before the first outer segment formation matches a similar progression of the expression of the RPE determinants. These data indicate that the RPE resumes its maturation during the first postnatal week and that RPE maturation and outer segment growth can be correlated.

RPE cell transplantation.—Recent studies indicate that RPE cell transplantation may be beneficial in restoration of retinal architecture in selected retinal degenerations. It is essential to develop methods for large-scale preparations of RPE cell cultures for somatic cell genetic engineering manipulations. We are evaluating various parameters for human and rat RPE cell culture and transplantation. Preliminary studies show that we can successfully inoculate human RPE cells into the rat retina. Studies are in progress to evaluate the immunologic parameters of this transplantation process.

Significance to Biomedical Research and the Program of the Institute

The monoclonal antibodies developed in this study are the first directed solely at the RPE cell. These antibodies are potentially useful in the identification of RPE cells in situ and in vitro. These antibodies, which can be used to monitor RPE cellular functions, may be used in providing a better understanding of the role of RPE cells in retinal degenerative disorders. RPE cell transplantation to correct retinal degenerative processes is being actively investigated in a number of laboratories. The studies reported here provide the framework to evaluate RPE cell transplantation.

Proposed Course

We will continue to characterize these antibodies as well as the effect of these antibodies on cell function in vivo and in vitro. We will isolate, propagate, and characterize RPE cells for transplantation studies in animals and human. We will design effective ways to maintain the cell in culture and design ways to measure and monitor cell function.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Hamel CP, Tsilou E, Harris E, Pfeffer BA, Hooks JJ, Detrick B, Redmond TM: Characterization of orpenin, a developmentally regulated protein specific for pigment epithelium of the vertebrate retina. *J Neurosci*, in press.

Vinores SA, Orman W, Hooks JJ, Detrick B, Campochiaro PA: Ultrastructural localization of RPE associated epitopes recognized by monoclonal antibodies in human RPE and their induction in human fibroblasts by vitreous. *Graefes Arch Klin Ophthalmol*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00240-06 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Virus Infections in the Eye

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Hooks	Ph.D.	Head, Section on Immunology and Virology	LI, NEI
Others:	Caroline Percopo	M.S.	Biologist	LI, NEI
	Yun Wang	M.D.	Guest Worker	LI, NEI
	Miguel Burnier	M.D.	Visiting Scientist	LI, NEI
	Ingeborg Kirch	M.D.	Guest Worker	LI, NEI

COOPERATING UNITS *(if any)*

Department of Pathology, The George Washington University Medical Center (Barbara Detrick, Ph.D.); Department of Pathology, Uniformed Services University for Health Sciences (Katherine Holmes, Ph.D.); Department of Ophthalmology, Ruprecht-Karl's University, Heidelberg, Germany (Ellen Kraus-Mackiw, M.D.); Laboratory of Biology, NCI, NIH (Charles H. Evans, M.D., Ph.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section of Immunology and Virology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies of various virologic and immunopathologic processes that occur when viruses replicate in the ocular microenvironment comprise three areas: (1) coronavirus infection in ocular and optic nerve cells; (2) the possible roles of viruses in human diseases; and (3) antiviral therapeutic actions of cytokines and drugs. A fourth area of study initiated during the past year is the evaluation of molecular diagnosis and pathogenesis of cytomegalovirus infections in man.

We have established that murine coronavirus can induce ocular disease and may be used as a model system for studying retinal degenerative diseases. This model has many unique features. The virus is capable of inducing an acute infection in the presence of mild retinal vascular inflammation. Initial retinal damage is followed by clearance of the virus and progressive retinal degeneration, even months after the virus is gone. In vitro studies and electron microscopic evaluation of in situ infection indicate that the virus replicates predominantly in Müller cells and can also be detected in retinal pigment epithelium (RPE) and photoreceptor cells. These results demonstrate that the virus can induce biochemical and morphological changes in the retina that persist and progress long after the virus is detectable. This disease may be considered a model for degenerative diseases of the pigment epithelium and photoreceptors in humans.

The need for effective drug treatment and prevention of herpes virus and other viral diseases has assumed growing importance. We found that leukoregulin, a naturally occurring immunologic cytokine, not only increases the antiviral actions of the drug acyclovir but also directly inhibits herpes simplex virus replication. These findings, which demonstrate that combination immunotherapy and chemotherapy can produce substantial inhibition of herpes virus replication, provide a rationale for the application of this approach to the treatment of virus infections.

Project Description

Objectives

This project was designed to determine the various effects of virus infections on the ocular microenvironment and to study modes of antiviral therapy.

Methods

This study involves the propagation and quantitation of viruses, such as herpes simplex virus type 1 (HSV-1), coronaviruses, and cytomegalovirus in vitro and in vivo, as well as immunocytochemical analysis of infected cells and tissues. Techniques used in characterizing virus infection include Western blot analysis, Northern blot analysis of HSV thymidine kinase, and in situ hybridization. Techniques used in the characterization of antivirus antibodies include enzyme-linked immunosorbent assay and neutralization assays.

Major Findings

Coronavirus infection in the eye.—The murine coronavirus, mouse hepatitis virus (MHV), JHM strain, induces a retinal degenerative disease in adult Balb/c mice. The disease consists of an acute phase lasting 2-8 days, during which virus is detected within the retina and initial pathology is noted in the retinal pigment epithelium (RPE) and photoreceptor layers. This is followed by a late phase lasting from 1 to 14 weeks during which virus is not detected but retinal degenerative changes continue with reduction of photoreceptor layer, loss of interphotoreceptor retinoid-binding protein, and retinal detachments. This model provides evidence that viruses can indeed trigger retinal degenerative processes and may provide insight into pathogenic mechanisms in retinal degenerative diseases of man.

Coronaviruses in general and the murine MHV, JHM strain in particular, induce a number of intriguing pathologic processes. Central nervous system involvement following JHM virus infections can consist of acute infections or chronic demyelinating disease. The acute infection is associated with viral replication in selected cells while chronic disease is associated with both virus replication and immune reactivity. Many of the steps involved in the pathogenic processes of these infections have been elucidated by in vitro studies. Some of the studies have shown that virus pathogenesis is associated with infection of specific cell types, such as glial cells, neurons, and oligodendrocytes. However, viral pathogenesis has also been associated with cell-to-cell interactions, release of cytokines, and the state of differentiation of the cells.

The in vivo interactions occurring in the retina are complex and difficult to assess. During the past year we evaluated the cellular basis of JHM virus retinal tropism. We prepared retinal cultures and RPE-retinal mixed cell cultures from eyes obtained from Balb/c mice. We evaluated the ability of JHM virus to infect and replicate in these retinal cultures by light microscopy, immunofluorescent staining, electron microscopy, and virus isolation. Retinal cultures alone failed to support JHM virus replication. No cytopathology was observed and no virus could be detected in supernatant fluid, suggesting that cell-to-cell interactions may be critical because virus particles and virus antigens could be seen in vivo within the neural retina and the RPE.

In contrast to the retinal cultures, RPE-retinal mixed cultures were supportive of JHM virus replication. Syncytial cytopathology was observed for the first 4 days, and virus was isolated from supernatant fluids. By electron microscopy, we found intracellular virus within vacuoles and extracellular virus at the plasma membrane. After day 4, a persistent virus infection was established in which cells produced virus for 5 weeks without cytopathic effects or cell death. Double-labeling immunofluorescent studies of RPE-retinal mixed cultures showed the virus coexpressed with a Müller cell marker, glutamine synthetase. This cell is the most prominent glial element in the retina. These studies demonstrate that JHM virus is capable of establishing a persistent virus infection in RPE-retinal mixed (Müller) cell cultures. Moreover, these data suggest that cell-to-cell interactions influence the establishment of coronavirus infections in the retina.

Preliminary studies indicate that the genetic background of the host can actually determine susceptibility to coronavirus-induced retinal degenerative disease. Moreover, preliminary studies also indicate that the pathogenesis of retinal degeneration may be associated with the development of antiretinal autoantibodies. Thus, both genetic and immunologic factors may contribute to the pathogenic process.

In summary, this model is characterized by the replication of JHM virus in the retina, producing an

acute necrotizing disease of the sensory retina and resulting in only a mild inflammatory response but a longlasting disease (over 14 weeks). These studies indicate that a progressive degenerative disease in the retina may be initiated by an acute virus infection in the absence of major inflammatory response.

Possible role of viruses in human eye disease.— We have initiated studies to evaluate the possible involvement of viruses in the pathogenic processes of a variety of human eye diseases. We are now collecting serum samples and ocular tissue in order to use seroepidemiologic approaches to detect virus and viral antigens via immunocytochemical staining, in situ hybridization, and polymerase chain reaction assays.

Antiviral therapeutic actions of cytokines and drugs.—The need for effective treatment and prevention of herpes virus and other viral diseases has grown in importance during the last 10 years. The development of targeted antiviral agents through combination therapy is becoming an important strategy. One of these strategies consists of the development of cytokines or lymphokines combined with chemotherapy to treat malignancy and infections. Using this approach, we recently showed that the cytokine leukoregulin could enhance the anti-HSV actions of acyclovir (ACV).

Cytokines are a group of specialized, hormone-like proteins that can exert profound influences on cellular development and on a variety of cell functions. Leukoregulin is a lymphokine that performs unique regulatory functions in transformed cells. This molecule, produced by a variety of lymphoid cells, has been shown to be a multifunctional cytokine. It can prevent chemical carcinogen transformation, inhibit neoplastic cell proliferation, and augment target cell sensitivity to natural killer cell cytotoxicity. Furthermore, this cytokine has been shown to increase membrane permeability of tumor cells and to increase drug uptake in these cells. Recently we showed that leukoregulin can selectively increase membrane permeability in HSV-1-infected cells but not in normal (ie, uninfected) cells.

The increase in membrane permeability attributable to leukoregulin is associated with an increase in the antiviral actions of ACV. In these studies, the virus was added to the cell cultures for a 2-hour incubation after which the cytokine and/or antiviral drug was added for 3 hours. The drugs and cytokine were removed, and the production of virus at 24 and 72 hours was evaluated. Under these conditions, the infected cells were exposed to the cytokine or drug for only 3 hours early in the replication cycle. Although short-term exposure of the cells to leukoregulin alone did not alter HSV-1 replication, it dramatically augmented the antiviral actions of ACV.

During the past year we characterized the anti-HSV-1 actions of leukoregulin. The continuous presence of leukoregulin during the replication of HSV-1 significantly decreases the production of new virus and the translation of HSV-1-specific proteins. Human amnion WISH cells were infected with HSV-1 (Wendy and F strains) and vesicular stomatitis virus (VSV). Following a 90-minute incubation period, we washed the cells and treated them with media, leukoregulin, ACV, or leukoregulin plus ACV. Virus replication was evaluated by plaque assays while virus and cellular protein expression was tested by immunoblot assays.

The continuous presence of leukoregulin (0.1 unit/mL) inhibited HSV-1 plaque formation by 50% and 80% in the Wendy and F strains, respectively. In contrast, leukoregulin did not affect VSV replication. Immunoblot analysis revealed that the expression of the 89-kD HSV-1 protein was inhibited by 50%, whereas the cell protein actin was not affected by leukoregulin treatment. Moreover, leukoregulin treatment did not alter the ability of the cells to incorporate tritiated thymidine. These studies show that leukoregulin not only enhances the antiviral actions of ACV but also can act to inhibit HSV-1 replication directly. These findings demonstrate that combination immunotherapy (cytokines) and chemotherapy can produce substantial inhibition of herpes virus replication, thus providing rationale for the application of this approach to the interventive treatment of virus infections.

Significance to Biomedical Research and the Program of the Institute

Elucidating the factors involved in viral spread and pathogenesis will aid better understanding of diseases of viral etiology. We have established a new virus model for retinal degenerative processes in adult animals. This model, which has many unique features, is capable of inducing an acute infection in the presence of mild retinal vascular inflammation. The initial retinal damage is followed by clearance of the virus and progressive retinal destruction, even months after the virus is gone. This model should

assist us in understanding the pathogenesis of selected human diseases of unknown etiology.

We have identified leukoregulin, a cytokine that selectively increases membrane permeability in virus-infected cells. We also have shown that combined cytokine and drug therapy can substantially inhibit herpes simplex virus replication. Moreover, the continuous presence of the cytokine can directly inhibit virus replication. The data from these studies provides rationale for the application of this approach to the interventive treatment of virus infections.

Proposed Course

This project will continue several lines of investigation.

1. We will evaluate coronavirus infections of the eye. Ocular tissue will be evaluated by electron microscopy. We will study the ability of the virus infection to potentiate autoimmune phenomena and correlate the data obtained with what is known about human retinal degenerative disorders.

2. We will initiate studies to determine whether certain viruses can replicate in retinal tissues and cells. Infected cells will be evaluated for the release or expression of uveitogenic proteins.

3. We will continue to collect samples and initiate studies to detect the involvement of viruses in human eye diseases.

4. We will continue to evaluate combinations of leukoregulin and chemotherapeutic agents for the management of virus infections.

5. We will evaluate the molecular diagnosis and pathogenesis of CMV infections in the eye.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Hooks JJ, Detrick B, Evans CH: Leukoregulin, a novel cytokine, enhances the anti-herpesvirus activity of Acyclovir. *Clin Immunol Immunopathol* 60:244-253, 1991.

Robbins SG, Wiggert B, Kutty GA, Chader GJ, Detrick B, Hooks JJ: Redistribution and reduction of IRBP during ocular coronavirus infection. *Invest Ophthalmol Vis Sci* 33:60-67, 1992.

Wang Y, Detrick B, Hooks JJ: Coronavirus replication within the retina: Analysis of cell tropism in muse retinal cell cultures. *Virology*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00277-01 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Retinal Pigment Epithelium in Retinal Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chandrasekharam N. Nagineni	Ph.D.	Visiting Scientist	LI, NEI
Others:	John J. Hooks	Ph.D.	Head, Section on Immunology and Virology	LI, NEI

COOPERATING UNITS *(if any)*

Department of Pathology, The George Washington University, Washington, DC (Barbara Detrick, Ph.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunology and Virology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The retinal pigment epithelium (RPE) plays a critical role in the regulation of retinal and choroidal function in normal and disease states. Due to limited availability of human tissues, an in vitro cell culture system is needed; therefore, we have developed and characterized the primary cell lines of human RPE from donor eyes obtained from eye banks. Using human RPE cell cultures as an in vitro model, we examined various roles of RPE in the pathophysiology of retinal disorders.

Human RPE cultures exposed to bacterial lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF-α), and interleukin 1 alpha and beta (IL-1α and IL-1β) secreted large amounts of interleukin 6 (IL-6). The dose-dependent response to inflammatory mediators was rapid and sustained in the presence of stimulants. Upon withdrawal of stimulants, IL-6 levels dropped to prestimulatory levels within 12 hours, suggesting the reversible response of RPE to inflammation. IL-1α and IL-1β are the most potent inducers of IL-6, followed by TNF-α and LPS. Growth factors did not induce IL-6 production by RPE cells. Western blot analysis of secreted IL-6 revealed multiple molecular forms, indicating posttranslational modifications that involve glycosylation, as was observed in human lymphoid cells. Human IL-6 cDNA probes were prepared to examine the regulation of gene expression. Northern blotting of total RNA prepared from RPE cultures treated with inflammatory mediators correlated with the observed patterns of IL-6 secretion.

The results clearly show that human RPE cells respond to specific inflammatory signals or infections and secrete IL-6. Since IL-6 is a multipotent cytokine, it may in turn perpetuate immune reactions in the pathogenesis of retinal and choroidal diseases.

Project Description

Objectives

Human retinal pigment epithelial (RPE) cell cultures will be established from human donor eyes. Primary cell lines of RPE will be used as an in vitro model to study the effects of growth factors, inflammatory mediators, and toxic compounds on biochemical, cellular, and molecular aspects of RPE structure and function. The usefulness of RPE cultures also will be evaluated for transplantation to restore retinal functions in hereditary and age-related disorders.

Methods

Primary cell cultures are prepared by initial seeding of either freshly isolated RPE cells or RPE-choroid explants. Cells are grown in minimum essential medium supplemented with 10% fetal calf serum, nonessential amino acids, and antibiotics. We are attempting to develop serum-free hormonally defined medium to render cultured cells more suitable for transplantation therapy by minimizing immune-related complications and consequent graft rejection.

Techniques required for routine immunocytochemistry, cytokine assays by enzyme-linked immunosorbent assay, gel electrophoresis, Western and Northern blotting for proteins, and RNA are developed and standardized in our laboratory to conduct these studies.

Major Findings

In the past, age of the donor was considered very critical in preparing human RPE cultures, because eyes from donors over 50 years of age did not yield fruitful cell lines, probably due to senescence-associated loss of viability. In these experiments, RPE cells were first dissociated from the eye cups by digestion with proteolytic enzymes, treatment that might have caused initial contamination with nonepithelial cells, from which it is impossible to purify epithelial cells. Therefore, we modified this method, using RPE-choroid explants, native and without harsh enzyme treatment, to initiate cell growth. Then, by carefully monitoring clusters of cells growing around the explants, we were able (on the basis of morphology combined with experience) to select pure epithelial cells and discard nonepithelial cells at the primary culture stage.

Using this technique, we established primary cell lines of human RPE from eyes obtained from 81- and 87-year-old donors. The epithelial nature of these cell lines was confirmed by immunochemical staining for cytokeratin with monoclonal antibodies. All of the cells expressed cytokeratin at different passages (3 to 10). Immunoblotting analysis of cell proteins indicated cytokeratin-18 as the predominant cytokeratin in these cells. Because RPE is the only epithelial cell in the posterior segment (choroid-RPE-retina), these results establish without doubt that the cell lines developed are, in fact, RPE. The feasibility of using donor eyes from a population over 70 years of age for preparing RPE cultures is demonstrated.

Human RPE cultures produce large quantities of inflammatory cytokine, interleukin 6 (IL-6), when exposed to physiological concentrations of inflammatory mediators—lipopolysaccaride (LPS), tumor necrosis factor α (TNF-α), interleukin-1α and β (IL-1α and IL-1β), and interferon-γ (IFN-γ). More than 98% of the IL-6 produced is promptly secreted into the medium. Western blot analysis revealed post-transcriptionally processed forms of IL-6 in the secreted proteins.

Our observations suggest that in response to the presence of some of the cytokines produced by macrophages and lymphocytes, for example, that infiltrate into the eye during inflammation caused by infection or autoimmune disease, RPE can locally produce IL-6. In turn, IL-6 aids in the proliferation and differentiation of lymphoid cells to regulate immunological phenomena.

Elevated levels of intravitreal IL-6, IL-1, and IFN-γ have been reported in proliferative and other noncomplicated retinal detachments. Moreover, intravitreal injection of IL-1, TNF-α, or IL-6 induced uveitis in experimental animal models. These studies implicate local but not systemic increase in these cytokines as initiating uveitis. Our studies indicate that RPE, possibly in association with other resident cells, reacts to the inflammatory stimuli and participates in the immunopathologic mechanisms.

Significance to Biomedical Research and the Program of the Institute

Primary human RPE cell lines offer an ideal in vitro model for evaluation of several RPE functions and for further elucidation of the mechanisms of RPE

involvement in the pathogenesis of retinal and choroidal diseases. These cells are potentially useful in cell transplant therapy to correct hereditary and age-related macular degeneration defects in humans.

Proposed Course

Two of the major problems associated with human RPE cell cultures are (1) progressive loss of pigmentation upon serial passaging of cells and (2) lack of clear intercellular junctions and in vivo-like morphological appearance. These changes may be due to cytoskeletal reorganization and partial dedifferentiation. Our immediate goal, to examine the mechanisms by which RPE cultures can be induced to resume in vivo characteristics, will be achieved by selecting specific media composition, adding growth and differentiating factors, and/or culturing on suitable extracellular matrix. Development of a fully differentiated RPE cell line is crucial not only to understanding cell functions but also for cell transplant therapy.

Continuing to evaluate the effects of inflammatory cytokines and bacterial endotoxins on RPE cell cultures, we will address three areas: (1) influences of these factors on cellular cytoskeletal organization, intercellular junctions, and adhesion properties; (2) effects on cell functions (eg, membrane permeability and solute transport and phagocytosis); and (3) characterization of proteins such as growth factors, cytokines, and proteolytic enzymes secreted by RPE in response to various stimuli. These studies are likely to shed light on the role of RPE in the pathophysiology of retina and choroid, the tissues that are in close vicinity to and are directly influenced by RPE.

NEI Research Program

Retinal Diseases—Inflammatory Diseases, Macular Degeneration, Photoreceptors and Retinal Pigment Epithelium

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00268-02 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Diagnosis and Treatment of Human Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Scott M. Whitcup	M.D.	Staff Medical Officer	LI, NEI
Others:	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
	Chi-Chao Chan	M.D.	Medical Officer	LI, NEI

COOPERATING UNITS *(if any)*

Department of Medicine, The Johns Hopkins University, Baltimore, MD (David R. Moller, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunopathology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to develop improved methods for diagnosing and treating human uveitis. In the area of diagnosis, biopsy and pathology specimens are examined from patients with uveitis and AIDS to develop improved diagnostic tests for better understanding the pathophysiology of inflammatory eye disease. This laboratory has shown that repeat vitrectomies are required to make the diagnosis of intraocular lymphoma in 30% of cases, and lymphoma can often be diagnosed by examination of the cerebrospinal fluid. To improve methods for diagnosing ocular sarcoidosis, we are testing lacrimal gland and conjunctival biopsies for the presence of interferon-gamma; Kveim antigen; interleukins 2, 3, 4, 6, and 8; and T-cell receptors possibly specific for this disease. Indocyanine green angiography is used to study choroidal blood flow in patients with uveitis. Corticotropin-releasing hormone tests are performed on patients with uveitis to determine whether a defective hypothalamic-pituitary-adrenal axis is associated with increased risk for autoimmune ocular inflammatory disease. We are the first to demonstrate coinfection of the retina and choroid with *Pneumocystis carinii* and *Mycobacteria avium-intracellulare* in a patient with AIDS.

Our studies of animals with endotoxin-induced uveitis have shown that animal strains with increased numbers of mast cells in the anterior uveal tract have more severe disease. We have documented the role of various cytokines in this disease.

In the area of treatment, several studies are in progress: (1) A masked, randomized crossover study comparing acetazolamide to placebo for the treatment of uveitic cystoid macular edema is in progress, and 26 patients have been recruited. (2) Topically applied FK506 is being tested as a treatment for acute anterior uveitis using animals with endotoxin-induced uveitis in the rat. (3) Behçet's disease may cause profound ocular inflammation and blindness. We retrospectively reviewed 19 patients with severe Behçet's uveitis treated with cyclosporine. Followup (mean, 50.9 months) of patients on cyclosporine (initial doses, 2.7-10.9 mg/kg) showed 11 of 19 patients (58%) had stabilization or improvement of visual acuity in each eye. Cyclosporine was discontinued because of side effects in three patients due to renal toxicity, hypertension, and neurologic symptoms. Cyclosporine is an effective treatment for many patients with Behçet's uveitis.

Project Description

Additional Personnel

Richard Fenton	M.D.	Staff Fellow, LI, NEI
Emily Chew	M.D.	Visiting Scientist, BEP, NEI
Frederick Ferris, III	M.D.	Chief, Clinical Trials Branch, BEP, NEI
George P. Chrousos	M.D.	Medical Officer, NICHD, DEB
Daniel Martin	M.D.	Senior Staff Fellow, LI, NEI
George Mastorakos	M.D.	Visiting Scientist, NICHD, DEB
Igal Gery	Ph.D.	Deputy Chief, LI, NEI

Clinical Protocol Numbers

91-EI-30
91-EI-139
92-EI-0070

Objectives

The goal of this study is to develop better methods for the diagnosis and treatment of human uveitis. We also are interested in defining the pathophysiology of inflammatory eye diseases by analyzing human tissue and animal models of uveitis.

Methods

Diagnosis of uveitis.—(1) To improve the diagnostic yield of conjunctival and lacrimal gland biopsies for sarcoidosis, frozen tissue specimens from 10 patients with known sarcoidosis were examined using immunohistochemical staining via an avidin-biotin-peroxidase complex. The application of primary monoclonal antibodies against T-cell markers, T-cell receptors, Kveim antigen, and various interleukins yielded data for comparison with that from biopsies from patients with other uveitic conditions such as Behçet's disease to determine the specificity of these results. (2) Intraocular lymphoma often masquerades as an idiopathic uveitis that delays the start of appropriate therapy. During the past year we retrospectively reviewed the diagnostic features of 12 cases of non-Hodgkin's lymphoma of the central nervous system (NHL-CNS) involving the eye, diagnosed at the NEI between 1984 and 1992. (3) We are performing corticotropin-releasing hormone tests to assess the hypothalamic-pituitary-adrenal axis in patients with autoimmune uveitis. A subnormal cortisol production in response to this hormone may predispose patients to the development of autoimmune disease. (4) Indocyanine green angiography is being used to study the retina and choroid of patients with uveitis. Angiograms are being analyzed to evaluate the choroidal blood flow in patients with active and inactive stages of inflammatory diseases of the retina. (5) The pathophysiology of endotoxin-induced uveitis is being studied using immunohistochemistry, histology, and monoclonal antibodies against various cytokines.

Treatment of uveitis.—(1) The efficacy of acetazolamide for the treatment of uveitis-associated macular edema is being evaluated in a masked, crossover study comparing acetazolamide to placebo. Visual acuity and the height of the macular edema measured on fluorescein angiography are primary endpoints. (2) We are using the animal model of endotoxin-induced uveitis (EIU) in the rat to test the efficacy of topically applied FK506, a new immunosuppressive agent, for the treatment of acute anterior uveitis. We are comparing histologic evidence of intraocular inflammation and aqueous humor protein concentrations in treated and control animals. (3) We studied the safety and efficacy of cyclosporine in the treatment of 19 patients with severe ocular Behçet's disease and demonstrated stabilized or improved visual acuity in the majority of patients.

Major Findings

1. In patients with ocular sarcoidosis, we found a specific γ-δ T-cell receptor in lacrimal gland specimens with active inflammation.

2. In reviewing our 12 cases of non-Hodgkin's lymphoma of the CNS involving the eye, we found that of 11 patients referred with vitritis or retinal infiltrates, 4 had neurologic symptoms at the time of diagnosis; only 4 patients were referred with a diagnosis of possible intraocular lymphoma. A pathological diagnosis was made on vitrectomy specimens in 10 cases, on cerebral spinal fluid in 1 case, and on an enucleation specimen in 1 case.

Initial vitreous biopsy was negative for malignant cells in 3 of 10 patients in whom a repeat vitrectomy specimen revealed intraocular lymphoma. Examination of the cerebrospinal fluid revealed malignant cells in 5 of 11 patients, although malignant cells were identified only after repeated lumbar punctures

in 2 of these patients and after a sample of cerebrospinal fluid from an Omaya reservoir inserted into the ventricles for chemotherapy in 1 patient.

Intraocular lymphoma is a potentially lethal disease that can be difficult to diagnose. Corticosteroids are cytotoxic to NHL-CNS and may contribute to the often low yield of viable, morphologically intact lymphoma cells obtained from vitrectomy and lumbar puncture specimens. Although appropriate handling of specimens may improve diagnostic yield, multiple vitrectomies and lumbar punctures may be necessary for correct diagnosis of intraocular lymphoma.

3. Using histology and electron microscopy, our laboratory demonstrated choroidal infection by both *Pneumocystis carinii* and *Mycobacterium avium-intracellulare* in a patient with AIDS. We also demonstrated that failure of the choroidal lesions of *P. carinii* to resolve with routine therapy may suggest insufficient antimicrobial levels in the blood or raise the possibility of coexistant opportunistic infection.

4. Rat strains with greater numbers of mast cells in the anterior uveal tract were more susceptible to developing EIU. Mast cell degranulation in the induction phase of this disease also suggests that mast cells play an important role in the development of acute anterior uveitis.

5. We studied the efficacy and safety of cyclosporine A in the treatment of severe ocular Behçet's disease in 19 patients. The mean followup on cyclosporine (initial doses, 2.7 to 10.9 mg/kg) was 50.9 months. Eleven of the 19 patients (58%) had stabilization or improvement of visual acuity in each eye. Cyclosporine was discontinued in 3 patients because of side effects, including renal toxicity, hypertension, and neurologic symptoms.

Significance to Biomedical Research and the Program of the Institute

Uveitis accounts for about 10% of visual impairment in the United States. A major goal of the NEI is to improve the methods for diagnosing and treating uveitis in an attempt to preserve useful vision in patients with inflammatory eye disease.

Proposed Course

Patient recruitment continues in these three human clinical protocols, and we will continue using indocyanine green angiography for the study of posterior uveitis. Our studies on the use of topical FK506 for anterior uveitis should be completed during the upcoming year. We will continue to study the role of cytokines, including tumor necrosis factor and interferon-γ in animal models of ocular inflammation.

NEI Research Program

Retinal Diseases—Inflammatory Diseases

Publications

Chan C-C, Li Q, Brezin AP, Whitcup SM, Egwuagu C, Ottesen EA, Nussenblatt RB: Detection of IL-2 and IL-4 mRNA in the conjunctiva of patients with onchocerciasis by in situ hybridization. *Invest Ophthalmol Vis Sci* 33(4):1320, 1992.

Kasner L, Chan C-C, Whitcup SM, Cordella-Miele E, Gery I: The effect of chlorpromazine (CPZ) on endotoxin-induced uveitis. *Invest Ophthalmol Vis Sci* 33(4):934, 1992.

Li Q, Whitcup SM, Fujino Y, Nussenblatt RB, Chan C-C: The role of mast cells in endotoxin-induced uveitis. *Invest Ophthalmol Vis Sci*, in press.

Rubin B, de Smet M, Whitcup SM, Palestine AG, Burnier M, Chan C-C, Nussenblatt RB: Need for repeat vitreous biopsy and lumbar puncture in the diagnosis of intraocular large cell lymphoma. *Ophthalmology* 98(Suppl):111, 1991.

Whitcup SM, Fenton RM, Pluda JM, de Smet MD, Nussenblatt RB, Chan C-C: *Pneumocystis carinii* and *Mycobacterium intracellulare* infection of the choroid. *Retina*, in press.

Whitcup SM, Salvo EC Jr, de Smet MD, Nussenblatt RB: Treatment of severe ocular Behçet's disease with cyclosporine. *Ophthalmology* 99(Suppl):138, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00269-02 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ocular Toxicity of 2′,3′-Dideoxyinosine (ddI)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Scott M. Whitcup	M.D.	Staff Medical Officer	LI, NEI
Others:	Robert B. Nussenblatt	M.D.	Scientific Director	NEI
	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
	Rafael Caruso	M.D.	Visiting Scientist	OGCS, NEI

COOPERATING UNITS *(If any)*

Pediatric Branch, National Cancer Institute, NIH (Karina Butler, M.D.; Philip A. Pizzo, M.D.); Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, NIH (Clifford H. Lane, M.D.); Clinical Oncology Program, National Cancer Institute, NIH (Robert Yarchoan, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunopathology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

2′,3′-Dideoxyinosine (ddI) is a purine analog with antiretroviral activity currently used to treat patients with the acquired immunodeficiency syndrome (AIDS). ddI is being used to treat both adults and children in clinical protocols at NIH. The purpose of this study is to follow patients treated with ddI prospectively and observe the development of ocular complications secondary to drug toxicity. Of 95 children with symptomatic (CDC class P-2) HIV infection enrolled in a phase I-II study to assess the safety and antiretroviral activity of ddI, 5 developed peripheral atrophy of the retinal pigment epithelium during ddI therapy. The two children with the most severe retinal atrophy were enrolled in the study at the highest dose level studied (540 mg/m-squared/day). Electro-oculograms were abnormal in one of three patients with retinal toxicity who could be tested. A group of 75 adults treated with ddI are being followed with periodic fundus examinations and electro-oculograms. In contrast to the results in children, there has been no evidence of retinal toxicity in HIV-infected adults treated with ddI.

The effects of ddI were evaluated on rat retinal pigment epithelial (RPE) cells, the rat lung cell line (CCL 149), and on the human amnion cell line (WISH). In short-term toxicity studies, ddI did not alter cell viability, as determined by trypan blue exclusion, or cell proliferation, assayed by total viable cell counts over time. Future in vitro toxicity studies will be performed on human RPE cells. In addition, radiolabeled ddI will be used to evaluate the concentration of ddI taken up by RPE and other cells.

Project Description

Additional Personnel

Richard Fenton	M.D.	Clinical Fellow, LI, NEI
John J. Hooks	Ph.D.	Head, Section on Immunology and Virology, LI NEI
Caroline Percopo	M.S.	Biologist, LI, NEI
Daniel Martin	M.D.	Senior Staff Fellow

Objectives

The goal of this study is to monitor patients treated with ddI for the development of ocular complications.

Methods

1. Every 3-4 months patients treated with ddI are given complete eye examinations, including dilated ophthalmoscopy and fundus photography of any abnormal retinal findings. Patients treated with the higher dosages of dideoxyinosine (ddI) also receive periodic electrooculograms to assess the electrophysiologic function of the retinal pigment epithelium (RPE).

2. ddI toxicity was evaluated on rat RPE cells, the rat lung cell line (CCL 149), and the human amnion cell line (WISH). Viability was determined by trypan blue exclusion, and cell proliferation was assayed by total viable cell counts over time. ddI was tested at 10, 100, and 1,000 μM concentrations. Cell toxicity was assessed 24 hours after one dose of ddI, 48 hours after one dose of ddI, and 6 days after three doses of ddI.

Major Findings

1. Five of 95 children have now developed peripheral atrophy of the RPE during ddI therapy. The lesions are scalloped areas of RPE atrophy with hyperpigmented borders. They occur predominantly in the midperiphery of the fundus in both eyes. These retinal lesions developed as early as 36 weeks after ddI therapy was initiated. The two children with the most severe retinal atrophy were enrolled in the study at the highest dose level studied (540 mg/m^2/day). Serial electroretinograms performed in the patient with the most severe retinal atrophy demonstrated a diminution in the cone-mediated responses during ddI therapy. Electrooculograms were abnormal in three of five children with retinal toxicity that could be tested. Interestingly, these abnormal electrooculograms returned to normal after ddI was discontinued.

2. In contrast to our findings in children, we have found no evidence of retinal atrophy in adults treated with ddI; no abnormal electrooculograms have been recorded in adults on ddI.

3. At 10, 100, and 1,000 μM concentrations in short-term cell cultures, ddI did not significantly alter viability or proliferation of any of the cells tested.

Significance to Biomedical Research and the Program of the Institute

A drug with in vitro and in vivo activity against HIV infection, ddI is being studied because one of the missions of NEI is to monitor patients for the development of ocular toxicity and to assess the effect of such toxicity on vision.

Proposed Course

We will continue to follow all patients treated with ddI at NIH for signs of ocular manifestations of ddI toxicity or HIV infection. We are performing serial electrooculograms in adults treated with ddI. Further in vitro studies of ddI toxicity are planned on human RPE cells. Radiolabeled ddI will be used to evaluate the concentration of ddI taken up by RPE cells and non-RPE cells.

NEI Research Program

Retinal Diseases—Photoreceptors and Retinal Pigment Epithelium

Publications

Whitcup SM, Butler KM, Caruso R, de Smet MD, Rubin B, Husson RN, Lopez JS, Belfort R Jr, Pizzo PA, Nussenblatt RB: Retinal toxicity in human immunodeficiency virus-infected children treated with 2′,3′-dideoxyinosine. *Am J Ophthalmol* 113:1-7, 1992.

Whitcup SM, Butler KM, Pizzo PA, Nussenblatt RB: Retinal lesions in children treated with dideoxyinosine. *N Engl J Med* 326:1226-1227, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00270-02 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Adhesion Molecules in Ocular Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Scott M. Whitcup	M.D.	Staff Medical Officer	LI, NEI
Others:	Chi-Chao Chan	M.D.	Chief, Section on Immunopathology	LI, NEI
	Rachel Caspi	Ph.D.	Visiting Associate	LI, NEI
	Robert B. Nussenblatt	M.D.	Scientific Director	NEI

COOPERATING UNITS *(if any)*

Biochemical and Molecular Pathology, Merck Sharp & Dohme Research Laboratories (Hugh Rosen, M.D.); Immunology Section, Roberts Pharmaceutical Corporation (Ron Harning, Ph.D.); Department of Ophthalmology, Kurume University School of Medicine, Fukuoka, Japan (Manabu Mochizuki, M.D.)

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell adhesion molecules are surface proteins that mediate the binding of cells to extracellular matrix and to other cells. The expression of cell adhesion molecules, which is important for the migration of leukocytes to sites of inflammation, is partly regulated by the secretion of cytokines. We are studying the expression of cell adhesion molecules in ocular inflammation and investigating the blocking of cell adhesion molecules as a treatment for uveitis and other ocular inflammatory diseases.

Over the past year we have shown that treatment with a monoclonal antibody against Mac-1 inhibits the development of endotoxin-induced uveitis in mice. We later demonstrated that monoclonal antibodies blocking intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1) inhibits the development of both endotoxin-induced uveitis in rats and experimental autoimmune uveitis in mice. These antibodies against ICAM-1 and LFA-1 also inhibited lymphocyte stimulation in vitro. Finally, studies of expression of cell adhesion molecules in animal models of uveitis have shown that ICAM-1 is expressed in the eye before the infiltration of inflammatory cells.

In studies of human biopsy tissue, we demonstrated the expression of ICAM-1, E-selectin, and vascular cell adhesion molecule-1 (VCAM-1) in corneal graft failure. The strongest expression of ICAM-1 occurred in corneal specimens with the greatest inflammatory disease, suggesting that cell adhesion molecule expression plays a role in corneal graft failure. We previously had demonstrated the expression of cell adhesion molecules in human eyes with posterior uveitis and the expression of E-selectin in the anterior segment of animal eyes with endotoxin-induced uveitis.

Project Description

Additional Personnel

Igal Gery	Ph.D.	Deputy Chief LI, NEI
Qian Li	M.D.	Visiting Fellow LI, NEI

Objectives

The goal of this study is to examine the role of cell adhesion molecules in ocular inflammation. Information on the expression of cell adhesion molecules is obtained from animal models of ocular inflammation and from human ocular tissue from patients with uveitis. Studies focus on the importance of cytokines in the regulation of adhesion molecule expression and examination of the use of antibodies against cell adhesion molecules to inhibit the development of ocular inflammation in both animal models of uveitis and in vitro lymphocyte proliferation studies.

Methods

Animal models of ocular inflammation.—Endotoxin-induced uveitis (EIU) by injecting *Salmonella typhimurium* endotoxin, 100 µg into one Lewis rat footpad or 200 µg into one C3H-Hen mouse footpad. Experimental autoimmune uveitis (EAU) in mice is induced by intraperitoneally injecting B10.A mice with 50 µg of interphotoreceptor retinoid-binding protein (IRBP) in complete Freund's adjuvant with pertussis toxin.

Histology and immunohistochemistry of ocular inflammation.—Enucleated animal eyes and human ocular tissue are immediately snap frozen and embedded in O.C.T. compound. The expression of cell adhesion molecules and the presence of cytokines is then assessed with immunohistochemical staining by using an avidin-biotin-peroxidase complex (ABC) on frozen sections of ocular tissue. Eyes also are embedded in methyl methacrylate, and 4-µm sections are examined for histologic evidence of inflammation.

Treatment of ocular inflammation by blocking cell adhesion molecules.—In an attempt to inhibit the development of ocular inflammation, we treat animals with intraperitoneal (ip) injections of monoclonal antibodies against intercellular adhesion molecule 1 (ICAM-1), lymphocyte function-associated antigen (LFA-1), and Mac-1 before the induction of either EIU or EAU.

Effect of blocking cell adhesion molecules on lymphocyte proliferation.—We removed the draining lymph nodes of the mice treated with anti-ICAM-1, anti-LFA-1, and rat IgG antibody at the time of enucleation, 21 days after immunization. We washed and suspended the cells in RPMI 1640 medium containing 25 mM HEPES buffer with L-glutamine, supplemented with CMA 20 mg/mL methyl α-D-mannopyranoside, and 1% sterile-filtered mouse serum at a concentration of 2.5×10^6 cells/mL. Proliferative responses to IRBP at 30 µg/mL, PPD at 20 µg/mL, and lipopolysaccharide at 50 µg/mL were assayed by tritiated thymidine uptake on triplicate 0.2 mL cultures in 96-well culture trays. The cultures were incubated for 48 hours, pulsed with 0.5 µCi of tritiated thymidine, and harvested 18 hours later. Tritiated thymidine uptake was determined by standard liquid scintillation counting, and results were reported as mean counts per minute.

Major Findings

1. In two separate experiments, EIU was produced in a total of 48 mice by injecting *S. typhimurium* endotoxin into one hind footpad. At the time of endotoxin injection, 24 mice received ip injection of anti-Mac-1 antibody and 24 control mice received ip injection of rat IgG. Histopathologic sections of eyes taken 24 hours after endotoxin injection were graded by two masked observers on a scale of 0 to 4. ICAM-1 was first expressed on the ciliary body epithelium 6 hours after endotoxin injection, and Mac-1 and LFA-1 were expressed on infiltrating inflammatory cells 12 hours after endotoxin injection. Treatment with anti-Mac-1 antibody significantly inhibited the development of ocular inflammation compared with control IgG treatment ($p < 0.001$). We concluded that ICAM-1 is expressed in the eye before clinical or histologic signs of inflammation. Furthermore, treatment with antibody against Mac-1 significantly inhibits the development of EIU in mice, suggesting that anti-Mac-1 antibody may be useful for treating acute ocular inflammation.

2. Cell adhesion molecules, which are surface proteins important for cell migration and adhesion, are strongly expressed in eyes with inflammation. We studied the expression of three cell adhesion molecules: ICAM-1 (CD54), LFA-1 (CD11a/CD18), and Mac-1 (CD11b/CD18) in mice with EAU. We immunized B10.A mice with IRBP and, using immunohistochemical staining, examined serial

sections of their eyes for expression of cell adhesion molecules. ICAM-1 was expressed on the vascular endothelium of the ciliary body and retina by 7 days after immunization, and LFA-1 was first expressed on some infiltrating inflammatory cells 9 days after immunization. Clear histologic evidence of ocular inflammation did not occur until 11 days after immunization.

We then studied the effect of monoclonal antibodies against ICAM-1 and LFA-1 on the development of EAU. We immunized three groups of mice daily for 21 days and intraperitoneally injected rat monoclonal antibody against murine ICAM-1 or LFA-1 or, as a control, rat IgG. Graded clinically by examination of the fundus 14 and 21 days after immunization, ocular inflammation was significantly decreased in animals treated with anti-ICAM-1 ($p = 0.003$ at day 14; $p = 0.012$, day 21) and with anti-LFA-1 antibody ($p = 0.003$ at days 14 and 21). The histologic grade of inflammation was significantly reduced ($p = 0.33$) in mice treated with anti-ICAM-1 antibody and diminished ($p = 0.093$) in mice treated with anti-LFA-1 antibody, compared with that in control mice. Proliferative responses to lipopolysaccharide, PPD, and IRBP of lymphocytes obtained from the draining lymph nodes of mice treated with the antibodies were lower than those from the control mice, suggesting that cell-cell binding was impaired in treated mice. These data show ICAM-1 expression in the eye before histologic evidence of inflammation and monoclonal antibodies against ICAM-1 and LFA-1 effectively inhibit EAU and lymphocyte reactivity in mice.

3. Corneal graft failure is frequently mediated by uncontrolled inflammatory disease. Using immunohistochemical staining and monoclonal antibodies against ICAM-1 (CD54), LFA-1 (CD11a/CD18), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and major histocompatibility complex class II antigen (HLA-DR), we studied the expression of cell adhesion molecules in seven penetrating keratoplasty specimens with graft failure. ICAM-1 and HLA-DR were expressed on keratocytes and the corneal endothelium in six of the seven specimens. ICAM-1 expression was strongest in the corneas with the most severe inflammation (corneal allograft rejection and severe intraocular inflammation).

LFA-1 is a counterreceptor for ICAM-1, and we found infiltration with leukocytes expressing either the α or β chain of LFA-1 in areas of ICAM-1 expression in four of the seven corneas. In contrast, E-selectin and VCAM-1 were expressed on the vascular endothelium in only two and one specimen, respectively. These data suggest that ICAM-1 expression may play an important role in the development of corneal graft failure. Furthermore, monoclonal antibodies to block ICAM-1 or its ligands may inhibit the development of corneal inflammation.

Significance to Biomedical Research and the Program of the Institute

NEI's mission includes understanding the mechanisms of sight-threatening eye disease so that new and effective therapies can be developed. The expression of cell adhesion molecules appears to be a fundamental mechanism in the development of intraocular inflammation. With this understanding, we hope to develop new anti-inflammatory therapy for ocular inflammation, which accounts for approximately 10% of the visual impairment in the United States.

Proposed Course

We will continue to study the expression of cell adhesion molecules in eyes with ocular inflammatory diseases, including uveitis, corneal disease, and uveitic glaucoma, by both immunohistochemical staining and in situ hybridization. In addition, we are now examining the use of antibodies against cell adhesion molecules to prevent the development of uveitis in animal models of intraocular inflammation and to treat preexisting ocular inflammation.

NEI Research Program

Retinal Diseases—Inflammatory Diseases

Publications

DeBarge LR, Chan C-C, Caspi RR, Harning R, Nussenblatt RB, Whitcup SM: Expression of cell adhesion molecules in mice with experimental autoimmune uveitis. *Invest Ophthalmol Vis Sci* 33(4):796, 1992.

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Whitcup SM, DeBarge LF, Rosen H, Nussenblatt RB, Chan C-C: Monoclonal antibody against Mac-1 (CD11b/CD18) inhibits endotoxin induced uveitis. *Invest Ophthalmol Vis Sci* 33(4):796, 1992.

Whitcup SM, Wakefield D, Li Q, Nussenblatt RB, Chan C-C: Endothelial leukocyte adhesion molecule-1 in endotoxin-induced uveitis. *Invest Ophthalmol Vis Sci* 33:2626-2630, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00184-10 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and Immunogenetic Mechanisms in Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rachel R. Caspi	Ph.D.	Visiting Scientist	LI, NEI
Others:	Shoshana Savion	Ph.D.	Visiting Fellow	LI, NEI
	Phyllis Silver	B.S.	Biologist	LI, NEI
	Sujata Grover	M.S.	Biologist	LI, NEI
	Luiz Rizzo	M.D.	Visiting Associate	LI, NEI
	Chi-Chao Chan	M.D.	Medical Officer	LI, NEI

COOPERATING UNITS *(If any)*

Laboratory of Immunobiology, Rega Instituut, Katholieke Universiteit Leuwen, Belgium (A. Billiau, M.D.); Depts. of Immunology and Microbiology and of Ophthalmology, Univ. of Miami School of Medicine, Miami, FL (J. Wayne Streilein, M.D.); Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases (Ronald L. Wilder, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	3.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cellular mechanisms in ocular immunologically mediated disease are being studied in animal models of experimental autoimmune uveoretinitis (EAU). Rats and mice are immunized with retinal-derived antigens or synthetic peptides representing fragments of these antigens to induce EAU. Susceptibility to disease induction is being evaluated in various strains of known genetic makeup, in the hope of delineating the hereditary mechanisms that predispose to uveitis. EAU in rats and mice serves as a template for the evaluation of new drugs and compounds, as well as for the study and characterization of the participating cells and their factors. In vivo-functional long-term T-cell lines and T-cell clones are developed from lymphoid organs of rats and mice immunized with uveitogenic ocular proteins. The functional properties and antigen receptors of these cells are studied to develop strategies for in vivo targeting of the autoimmune cells. The goals of these studies are to identify the immunogenetic factors predisposing to uveitic disease, to learn about the pathogenic mechanisms involved, to characterize the immunoreactive cells and their mediators, and finally to use this knowledge for designing rational approaches to immunotherapy.

Project Description

Additional Personnel

Robert B. Nussenblatt	M.D.	Clinical Director NEI
Charles E. Egwuagu	Ph.D.	Staff Fellow, NEI
Igal Gery	Ph.D.	Head, Section on Experimental Immunology, NEI

Objectives

Development and study of animal models of experimental ocular autoimmune disease permits the study of cellular and genetic factors which may be involved in ocular autoimmunity in a general sense. Experimental autoimmune uveitis (EAU) in rats and mice serves as a template for the evaluation of new drugs and compounds, as well as for the study and characterization of the participating cells and their factors. Long-term maintenance of T cells in vitro permits the investigators to separate and selectively grow various T-cell subsets. The goals are (1) to continue to establish and characterize the murine EAU model because the mouse offers some important advantages over other rodents as a model of EAU; (2) to use the EAU model in rodents for the study of cellular mechanisms in ocular autoimmunity (This is done in large part by establishing and using retinal antigen-specific T-cell lines and clones that will permit us to identify and characterize cells capable of ocular immunomodulation, to learn about migration and localization of autoimmune lymphocytes, and to study their interactions with other lymphoid and nonlymphoid cells in eliciting effector mechanisms.); (3) to use the EAU model as a template for the development of immunotherapeutic approaches designed to target autoimmune lymphocytes directly, or to disrupt specific stages in the autoimmune inflammatory cascade; and (4) to use the EAU model in the mouse for the study of the various genetic mechanisms controlling susceptibility to ocular autoimmune disease. The study and understanding of these parameters will help not only in the development of new therapies but possibly in the prevention of ocular disease.

Methods

Rats and mice of various strains are immunized with purified S-antigen (S-Ag) or interphotoreceptor retinoid-binding protein (IRBP) in complete Freund's adjuvant, or with various pathogenic peptides derived from these proteins. After the development of disease, eyes are processed for histopathology and examined for disease, and lymphoid cells from the blood, lymph nodes, or eyes are taken. Cells thus obtained are placed in culture with either mitogen or the retinal antigen with which the donor animal was immunized. Responses of the immune cell are recorded.

Cells also are expanded in culture and used in attempts to transfer EAU to nonimmune animals to determine the cell population responsible for disease induction. Long-term cell lines are developed and in some cases cloned by either the use of the soft agar bilayer or the limiting dilution technique. These lines or clones are then tested for functional characteristics, such as the ability to induce ocular disease, production of soluble mediators, expression of various cell surface molecules, response to therapeutic agents, and interactions with other cells in culture.

Major Findings

Uveitogenic T-helper lymphocyte lines specific to the major pathogenic epitopes of the S-Ag or the IRBP have been developed in the Lewis rat, and a series of oligoclonal sublines was established from the LR16 line, specific to the major pathogenic epitope of IRBP. In vivo testing of these sublines revealed that only some were able to induce disease in recipient animals. This finding opens the possibility of studying the differences between pathogenic and nonpathogenic lymphocytes that recognize a defined antigenic epitope.

The lymphokine profiles and expression of functional adhesion molecules were studied in four T-cell lines in which the degrees of pathogenicity ranged from highly pathogenic to nonpathogenic. The results showed that upon antigenic stimulation all lines expressed similar levels of adhesion molecules, as assessed by adherence to cultured rat endothelial cells. All lines produced interleukin 2 (IL-2) and interferon-γ (IFN-γ), whereas none of the lines produced interleukin 4 (IL-4), confirming the T helper 1-like nature of the lines. In addition, all lines produced tumor necrosis factor (TNF), transforming growth factor-β (TGF-β), interleukin 3 (IL-3), and interleukin 6 (IL-6). The different lymphokines were produced by all lines in similar amounts, irrespective of their pathogenicity. Thus, in these lines, patho-

genicity did not correlate with production of IFN-γ, TNF, TGF-β, IL-2, IL-3, IL-4, or IL-6, with one exception: the nonpathogenic subline that produced relatively less IL-2 was subsequently found to have largely lost the expression of the CD4 molecule. The connection between this line's loss of CD4 antigen and its lack of pathogenicity is being investigated.

In collaboration with Dr. Egwuagu, we are studying the T-cell receptor (TCR) genes of these lines and clones on the molecular level. The data indicate that TCR variable region gene usage in uveitis differs from that reported for other autoimmune diseases and may be more heterogeneous. On the basis of in vitro and in vivo results, TCR Vβ8.2 appears to be a pathogenic clonotype in S-Ag-EAU, whereas TCR Vβ8.3 may be one of the pathogenic clonotypes in IRBP-EAU. These findings impact on the development of therapeutic strategies that would specifically target the pathogenic cells via their TCR.

We studied the importance of "nonspecific" T-cell recruitment in the immunopathogenesis of uveitis, using congenitally athymic Lewis rats (LEW.rnu/rnu) deficient in functional endogenous T cells, but otherwise syngeneic with the euthymic Lewis rats that develop characteristically severe EAU. The uveitogenic stimulus was delivered in the form of phenotypically and functionally homogeneous pathogenic T-cell lines, specific to the major pathogenic epitope of either the intracellular photoreceptor protein, S-Ag, or the extracellular photoreceptor matrix protein, IRBP. Depending on the T-cell line used, EAU in athymic rats was either drastically reduced in severity or absent. Susceptibility was restored when the athymic animals were reconstituted with immunocompetent T cells from syngeneic euthymic donors.

Our results indicate that recruited nonspecific T cells play a major role in the pathogenesis of disease and suggest that the extent of dependence on this phenomenon may be influenced by the antigenic specificity of the T-cell line. This is the first direct demonstration that recruitable nonspecific T lymphocytes are necessary for the expression of the disease itself. This finding impacts upon the development of immunotherapeutic strategies, which must take into account the magnitude of the recruitment phenomenon.

In collaboration with Drs. Chi-Chao Chan and Scott Whitcup, we have shown that adhesion molecules are expressed on the ocular vasculature before the onset and during the active phase of EAU in the mouse model. Moreover, these molecules have a functional significance, because anti-adhesion molecule therapy with monoclonal antibodies to ICAM-1 and LFA-1 is able to suppress induction of EAU. Anti-adhesion molecule therapy is one of the means by which recruitment of nonspecific cells from the circulation can be controlled. This finding has potential clinical significance, because the same adhesion molecules are expressed in the human disease as well.

Studies of genetic control of susceptibility and resistance to uveitis, using various mouse strains, were continued and extended. In experiments using congenic and intra-H-2 recombinant strains, we showed that expression of EAU requires both a genetically susceptible major histocompatibility complex class II region, and a "permissive" genetic background. One of the genetic factors determining the "permissiveness" or "nonpermissiveness" of the genetic background was shown to be connected to the regulation of the lymphokine IFN-γ. Neutralization of endogenously produced IFN-γ abrogated EAU resistance in one low-responder strain of mice. Additional resistance mechanisms, such as the influence of the genetically determined T-cell repertoire, which affects recognition of pathogenic epitopes, are now being studied.

In collaboration with Dr. Wayne Streilein (University of Miami, FL), we are exploiting the mouse EAU model for the study of immunologically induced suppression of ocular autoimmunity by a phenomenon known as anterior chamber-mediated immune deviation (ACAID). We induced protection against EAU by pretreating the animals with the uveitogen IRBP, injecting it in the anterior chamber of the eye before the uveitogenic challenge. The protective effect was found to reside in a splenic population of T cells that could be isolated and could transfer the protection to unmanipulated hosts. Adoptive transfer of these ACAID-induced suppressor cells aborted disease in a previously immunized host, even when pathological signs were already present. We also showed that ACAID-like protection from EAU can be induced not only by injection of IRBP into the anterior chamber, but also by infusion of monocyte/macrophages of the F4/80 phenotype that had been pretreated in vitro with IRBP in the presence of supernatants from immune-privileged sites (amniotic fluid, aqueous humor). These studies,

which contribute to the understanding of the natural mechanisms that protect from ocular autoimmunity, might be exploited in the future for the development of clinically relevant strategies for immunomodulation.

Significance to Biomedical Research and the Program of the Institute

It has become increasingly clear that the cellular mechanisms and possibly the genetic mechanisms observed in animal models of uveitis reflect the mechanisms that operate in ocular immune-mediated disease in humans. The identification and characterization of the cells involved in ocular autoimmunity, and of their functions, will provide new understanding of inflammatory ocular diseases. Successful immunomodulation of EAU in animal models has thus far usually served as a good predictor of the clinical success of a given therapeutic modality. The continued study of basic mechanisms involved in the immunopathogenesis of uveitis in animal models will aid in the development of novel immunotherapeutic approaches for the control of uveitis in humans.

Proposed Course

This project will continue so that more information about the basic mechanisms in experimental uveitis may be obtained.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00258-04 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Autoimmune Uveitis in the Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rachel R. Caspi	Ph.D.	Visiting Scientist	LI, NEI
Others:	Sina Bahmanyar	M.D.	Staff Fellow	LI, NEI
	Chi-Chao Chan	M.D.	Medical Officer	LI, NEI
	Shlomo Grossman	Ph.D.	Special Volunteer	LI, NEI
	Sujata Grover	M.S.	Biologist	LI, NEI
	Barry Grubbs	B.S.	Biologist	LI, NEI
	Martha Kirby	M.S.	Biologist	LI, NEI
	Reza Mozayeni	B.S.	Biologist	LI, NEI
	Benjamin Rubin	M.D.	Staff Fellow	LI, NEI
	Phyllis Silver	B.S.	Biologist	LI, NEI

COOPERATING UNITS *(if any)*

Laboratory of Immunobiology, Rega Instituut, Katholieke Universiteit Leuwen, Belgium (A. Billiau, M.D.); Department of Microbiology and Immunology, University of Miami School of Medicine (Wayne Streilein, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated as a separate entity. Further work in the mouse EAU model has been folded into project number Z01 EY 00184-10 LI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00271-02 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Evaluation of the Antiflammins in the Anterior Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chi-Chao Chan	M.D.	Medical Officer	LI, NEI
Others:	Robert B. Nussenblatt	M.D.	Clinical Director	NEI
	Juan S. Lopez	M.D.	Visiting Fellow	LI, NEI
	Susan Mellow	R.N.	Nurse Specialist	LI, NEI

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Immunology

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Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00218-07 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ocular Manifestations of the Acquired Immune Deficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Scott Whitcup	M.D.	Senior Staff Fellow	LI, NEI
	Margaret Cheung	M.D.	Senior Staff Fellow	LI, NEI
	David Parks	M.D.	Senior Staff Fellow	LI, NEI
	Richard Fenton	M.D.	Senior Staff Fellow	LI, NEI
	Dan Martin	M.D.	Senior Staff Fellow	LI, NEI
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	Miguel Burnier	M.D.	Visiting Scientist	LI, NEI
	François Roberge	M.D.	Visiting Scientist	LI, NEI

COOPERATING UNITS *(If any)*

Department of Critical Care Medicine, Clinical Center, NIH (Henry Masur, M.D.); Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, NIH (H. Clifford Lane, M.D.); Pediatric Branch, National Cancer Institute, NIH (Phil A. Pizzo, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients suffering from AIDS are at risk of developing significant ocular problems either as a result of the human immunodeficiency virus (HIV) itself or as a result of opportunistic infections. Some of these problems can lead to blindness if left untreated. Among the many pathogens that can lead to blindness, cytomegalovirus (CMV) is by far the most common. In FY 1992 several approaches have been taken to investigate this disease. Some CMV retinitis studies focused on prevention. Early initiation of therapy is important for the preservation of useful vision. However, all the drugs available are virostatic, not virocidal; patients are required to take medication for life if they want to preserve their sight. If one could prevent the development of CMV retinitis, it would be considered a major advance. In this regard, we have participated in a phase I trial of immune globulin in patients at risk for development of CMV retinitis.

Other CMV retinitis studies focused on therapy. We have continued to evaluate the ability of foscarnet and ganciclovir to prevent progression of CMV retinitis. Two particular questions evaluated were (1) the possible options in the treatment of patients with end-stage CMV retinitis who have silicone oil in their eyes following retinal detachment and (2) the possibility of using a drug delivery system for the eye, which would obviate the need for systemic therapy.

In studies of pediatric AIDS during FY 1992, we continued to evaluate the incidence of ocular infection in about 220 children with AIDS. The incidence of complications is lower in children than in adults. However, CMV retinitis in children is more difficult to treat. In a subpopulation of pediatric patients treated with 2′,3′-dideoxyinosine (ddI), peripheral retinal changes suggesting drug toxicity have been observed and reported as our monitoring of these children continues.

Other FY 1992 studies on AIDS include evaluation of a new antiviral drug for the treatment of toxoplasmosis in AIDS patients' eyes.

Project Description

Additional Personnel

Susan Mellow R.N.

Clinical Protocol Number

90-EI-208

Objectives

The foremost objective of this project is to evaluate means of identifying and treating known ocular complications of AIDS, using novel approaches. A second, equally important objective is the identification of new manifestations of ocular involvement from the AIDS virus itself and related opportunistic ocular infections. The third objective is to recognize complications related to the therapeutic agents used.

Methods

This project entails the clinical evaluation, diagnosis, and treatment of retinitis in AIDS patients. It also involves the development of novel methods of therapy for the various forms of retinitis observed. Study of pathologic tissue is used to enable better understanding of the nature of infectious processes.

Major Findings

In the past year, our major effort was still centered on the evaluation and treatment of cytomegalovirus (CMV) retinitis. CMV is a major vision-threatening infection found in patients with AIDS. While early detection can lead to preservation of vision, it also commits the patient to lifelong intravenous therapy with anti-CMV drugs. Prevention of CMV retinitis in patients at risk would be a significant advance.

Most AIDS patients develop CMV end-organ disease when their CMV immunoglobulin has dropped to a very low level. Administering exogenous anti-CMV hyperimmunoglobulin thus has the potential to prevent end-organ disease from appearing. In collaboration with the National Institute of Allergy and Infectious Diseases, we have begun to evaluate the safety of administering anti-CMV hyperimmune globulin to patients at risk for CMV retinitis. To date, the treatment, given on a monthly basis, has been administered to eight patients. No adverse effects of this therapy have been noted.

Recently a device that slowly releases gancyclovir directly into the eye has been described. This might be an interesting alternative to systemic therapy for patients who cannot tolerate intravenous infusions. We have developed a protocol that should allow us to evaluate the safety of the device. The protocol will test two dosage regimens: in one, the drug will be released over a 4-month period; in the other, it will be released over 8 months. The protocol, which has demanded considerable effort by several investigators, is now ready for patient enrollment.

We have continued to follow patients with CMV retinitis. Patients with advanced AIDS and a severe state of immunosuppression are often unable to tolerate gancyclovir, even with the use of G-CSF. In some of these patients who are unable to tolerate the renal toxicity of foscarnet, we have had to revert to using intravitreal injections. One particular problem arises in patients with intraocular silicone oil following retinal detachment surgery, which occurs in up to 30% of patients with CMV retinitis. Silicone oil has been considered a contraindication to intravitreal injection. We have shown in two patients with silicone oil that intravitreal gancyclovir injections can be safely given, thus they are an effective means of arresting the progression of CMV retinitis.

Other infections can cause decreased vision in patients with AIDS. The second most common pathogen in many parts of the world is toxoplasmosis, a particularly difficult infection to treat, inasmuch as the most common drug, sulfacetamide, can cause a Stevens-Johnson-like syndrome. We have evaluated and demonstrated the efficacy of a new agent, 566C80, in the treatment of ocular toxoplasmosis. It holds promise as an alternative to the medications now available.

Finally, we have continued to follow about 220 children who developed AIDS by various means. Whereas the overall incidence of CMV is about 1.6%, we have been particularly struck by the lower incidence of CMV in this population. However, in children who have low total T-cell counts (below 100 per cubic millimeter), the risk increases to 16%. Also, children who have acquired HIV in utero or shortly after birth are at increased risk of having strabismus.

Significance to Biomedical Research and the Program of the Institute

The AIDS epidemic is a major public health concern. CMV retinitis remains the number one cause of blindness among patients infected with the AIDS virus. Prophylaxis would offer the best defense against costly and cumbersome lifelong therapy. In addition, an approach that could deliver a drug directly to the eye would be a valuable adjunct in those patients who are unable to tolerate systemic therapy.

The number of infected children with AIDS is on the rise. A good understanding of the epidemiology of AIDS in terms of ocular disease is highly desirable. We are thus continuing to follow these children prospectively to identify the frequency and type of ocular complications which they are likely to develop.

Proposed Course

Further studies on the ocular manifestations of AIDS are planned. We will continue to monitor pediatric patients. We will, in the course of Fiscal Year 1993, evaluate the intraocular device and study further the use of hyperimmune globulin as a means of prophylaxis against CMV retinitis. We also will evaluate new anti-CMV drugs as they become available for clinical trials.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Butler KM, de Smet MD, Husson RN, Mueller B, Manjunath K, Montrella K, Lovato G, Jarolinski P, Nussenblatt RB, Pizzo PA: Treatment of aggressive CMV retinitis with ganciclovir in combination with foscarnet in a child with human immunodeficiency virus infection. *J Pediatr* 120:483-486, 1992.

de Smet MD: Differential diagnosis of retinitis and choroiditis in patients with acquired immunodeficiency syndrome. *Am J Med* 92(Suppl 24):S17-S21, 1992.

de Smet MD, Butler KM, Rubin BI, Whitcup SM, Lopez J, DeBarge R, Martin D, Pizzo PA, Nussenblatt RB: The ocular complications of HIV in the pediatric population. *Proceedings of the 3rd International Symposium on Uveitis*. Brussels, Kluwer, 1992, in press.

de Smet MD, Nussenblatt RB: Ocular manifestations of AIDS. *JAMA* 266:3019-3022, 1992.

Lopez JS, de Smet MD, Masur H, Mueller BU, Pizzo PA, Nussenblatt RB: Orally administered 566C80 for treatment of ocular toxoplasmosis in a patient with the acquired immunodeficiency syndrome. *Am J Ophthalmol* 113:331-333, 1992.

Whitcup SM, Butler KM, Caruso R, de Smet MD, Rubin B, Husson RN, Lopez JS, Belfort R Jr, Pizzo PA, Nussenblatt RB: Retinal toxicity in HIV-infected children treated with 2′,3′-dideoxyinosine (ddI). *Am J Ophthalmol* 113:1-7, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00266-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Immune Responses to S-Antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
Others:	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LI, NEI
	Robert B. Nussenblatt	M.D.	Clinical Director	NEI
	Margaret Cheung	M.D.	Senior Staff Fellow	LI, NEI

COOPERATING UNITS *(if any)*

Department of Ophthalmology, Kurume University, Kurume, Japan (M. Mochizuki, M.D.); INSERM-86, Laboratory of Ocular Immunopathology, Paris, France (J.-P. Faure, Ph.D.); L'Hôpital de la Pitié, Paris, France (P. LeHoang, M.D.); Hadassah Hospital, Jerusalem, Israel (D. Ben Ezra, M.D.).

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the characteristics of soluble retinal antigen (S-Ag) is its ability to induce an intense autoimmune inflammation in the eyes of experimental animals when injected in the presence of an adjuvant. This disease, called experimental autoimmune uveitis (EAU), is critically dependent on T cells and antigen processing by appropriate antigen-presenting cells (APC). In FY 1992, we further characterized the immunogenic and pathogenic sites of human S-Ag in the Lewis rat, using overlapping fragments of S-Ag, thus determining that there are several immunogenic sites in the S-Ag molecule as well as at least three immunopathogenic sites. Of these immunopathogenic sites, only one is immunodominant—a fragment located at sequence 341-360. The immunogenic sites for the Lewis rat do not correspond to the sites identified in the human. While most of the sites recognized by the rat immune system are clustered close to the C-terminal end of the molecule, in the human most of the activity is located in the N-terminal region. We also have tested the response of the immunodominant sequence, as well as the nondominant immunopathogenic sequences in several other rat strains in an attempt to determine whether factors within the major histocompatibility complex (MHC) class II as well as outside MHC class II may play a role in the establishment of an immunogenic and an immunopathogenic response.

In human subjects, we have tested new means of establishing cell lines by seeding multiple wells with S-Ag primed peripheral blood cells and maintaining the cells in culture for 14 days in medium enriched in T-cell growth factors. This approach yields a low number of cell lines which can then be further expanded by additional rounds of stimulation. In addition, this approach allows us to determine the precursor frequency of cells reactive to S-Ag in the peripheral blood of patients and controls. This precursor frequency, which is of the order of 1 in 10 million circulating cells in patients, is lower in controls. The cell lines established using this technique will now be further characterized in terms of their response to fragments of human S-Ag as well as their T-cell receptor usage.

PHS 6040 (Rev. 5/92)

Project Description

Additional Personnel

Sumeet Mainigi		Biologist, LI, NEI
Molly Dayan	B.Sc.	Biologist, LI, NEI
Gerald J. Chader	Ph.D.	Chief, LRCMB, NEI
Barbara Wiggert	Ph.D.	Head, Section on Biochemistry, LRCMB, NEI

Clinical Protocol Numbers

84-EI-214
79-EI-49

Objectives

In Fiscal Year 1992 the study has been aimed at mainly two areas: (1) a determination of the immunogenicity of the fragments of human S-antigen (S-Ag) in a defined experimental animal, the Lewis rat, and in other inbred rat strains that either use the same major histocompatibility complex (MHC) class II antigen or a different antigen, and (2) characterization of the immune response profile to S-Ag in patients with well-defined uveitic conditions, using standard proliferation assays as well as multiple short-term T-cell lines.

Methods

Overlapping synthetic peptides of human S-Ag, each consisting of 20 amino acids, were provided by Applied Biosystems (Foster City, CA). In the animal study, we immunized groups of animals with the various peptides, using conventional methods. The animals were examined daily for the appearance of uveitis. Between days 12 and 14, the cell-mediated immune response was determined in a standard in vitro proliferation assay. Several different rat strains were used for these studies. The strains were chosen on the basis of their class II usage. Certain strains shared the same class II antigen as the Lewis rat while others differed completely in the class II antigens they expressed.

Lymphocytes obtained from the peripheral blood of patients with uveitis in various stages of activity were tested for their response to synthetic peptides in a standard microculture assay. Their response to bovine S-Ag was determined in the same assay. These results were correlated with responses found in normal volunteers provided by the NEI Eye Clinic.

Short-term cell lines were established in a number of patients to determine the precursor frequency of reactive cells and to establish responsive cell lines. Cells were first pulsed with bovine S-Ag, then cultured in standard 96-well microtiter plates. The medium was changed frequently and enriched in T-cell growth factors. In a few patients, this assay was repeated several times to determine the profile of response during and after inflammatory episodes.

Major Findings

Our studies in the Lewis rat have shown that, even in the experimental model, it is possible to generate an immunogenic response to multiple epitopes of the S-Ag sequence. Several of these epitopes are also pathogenic, with the most active located close to the C-terminal end of the molecule and corresponding to sequence 340-360 of human S-Ag. We then tested this fragment as well as some nondominant but pathogenic fragments in different rat strains to determine the degree of restriction to specific MHC class II antigens. The immunodominant epitope was pathogenic in all the strains tested although the level of pathogenicity differed. These strain differences were due not only to differences in class II antigen but also in non-MHC factors; pathogenicity in the Fisher strain, which shares the same MHC class II, was markedly depressed.

For further characterization of the human immune response, it is necessary to establish cell lines and clones. These should help us to determine the exact peptide sequences responsible for the immune reaction. To obtain these cell lines, we have established multiple short-term cell lines. The frequency of positive responses in the controls was around 0-7% for a precursor frequency on the order of 1 in 10 million circulating lymphocytes. In patients, two patterns of response were found. There was either a very high response rate, on the order of 30-40% of wells, or a very low response rate, similar to that of controls. Such a high response rate corresponds to a precursor frequency of about 1 in 1 million circulating mononuclear cells. The highest response rates were observed shortly after attacks of intraocular inflammation, and they developed once the inflammatory episodes had resolved. This suggests that the T cells marginate during periods of intraocular inflammation and that their numbers expand during inflammatory events. We also have noted that the standard proliferation assay becomes very weak after

an inflammatory episode, suggesting that suppressive factors are present in the peripheral blood following episodes of inflammation.

Significance to Biomedical Research and the Program of the Institute

These studies will help to define the parallels between the animal models and human autoimmune ocular diseases. Findings in the rat suggest that an immunodominant epitope may be recognized by more than one MHC class II antigen. This suggests that if one is able to identify such an epitope in man, it may be possible to use it to induce tolerance and prevent rejection from occurring. The establishment of specific human cell lines, which still remains a priority, will help us to characterize the interactions between the human T cells and their antigen-presenting cells.

Proposed Course

In the coming year, the main focus of attention will be on determining the HLA class II restriction of the immune response to fragments of S-Ag. Concentrating mainly on human material, we will try to characterize the T-cell receptor usage as well as the MHC class II being used in various well-defined uveitic entities.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

de Smet MD, Dayan M, Caspi R, Hafler DA, Gery I, Nussenblatt RB: Determination of the precursor frequency to S-Ag in uveitis patients and controls. *Invest Ophthalmol Vis Sci* 33(4):930, 1992.

Dayan M, de Smet MD, Caspi R, Roberge FG, Gery I, Nussenblatt RB: Determination of the response to S-Ag over time in patients with Behçet's disease. *Second International Symposium on Ocular Inflammation.* Jerusalem, Israel, p 230, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00267-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Modulation of Immune Functions Using PE40 Derived Immunotoxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
Others:	Juan Lopez	M.D.	Visiting Fellow	LI, NEI
	Robert B. Nussenblatt	M.D.	Clinical Director	NEI
	Stephan S. Thurau	M.D.	Special Volunteer	LI, NEI

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biology, National Cancer Institute (Ira Pastan, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00276-01 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Surgical Management of Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
Others:	François Roberge	M.D.	Visiting Scientist	LI, NEI
	Margaret Cheung	M.D.	Senior Staff Fellow	LI, NEI
	Scott Whitcup	M.D.	Senior Staff Fellow	LI, NEI
	David Parks	M.D.	Senior Staff Fellow	LI, NEI
	Juan Lopez	M.D.	Visiting Fellow	LI, NEI
	Ray DeBarge	M.D.	Senior Staff Fellow	LI. NEI
	Richard Fenton	M.D.	Senior Staff Fellow	LI, NEI
	Dan Martin	M.D.	Senior Staff Fellow	LI, NEI
	David Callanan	M.D.	Senior Staff Fellow	LI, NEI
	Naofumi Hikita	M.D.	Visiting Associate	LI, NEI

COOPERATING UNITS *(if any)*

Clinical Oncology Program, Medicine Branch, National Cancer Institute, NIH (Robert Wittes, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with uveitis often develop ocular complications that require surgery to prevent permanent loss of vision. Surgery in these patients has been particularly challenging because the surgery itself can induce severe inflammation. The exact timing of the surgery and the postoperative immunosuppressive therapy given to the patient often determine the outcome. Several conditions also exist where there is a need to obtain a tissue diagnosis before proceeding with treatment. We have used vitrectomy and chorioretinal biopsy to help us make an appropriate diagnosis in such cases. Proper handling of the specimen is essential, particularly in cases of intraocular lymphoma in which the lymphoma cells are particularly fragile. Glaucoma remains an important complication in patients with uveitis. Standard trabeculectomies always stop functioning after a few months. We are continuing to compare the uses of 5-FU and Molteno implants in patients with uveitis and glaucoma who require surgery. We now have 10 patients enrolled in the study. The use of intraocular lenses following cataract extraction in patients with uveitis will be addressed in a randomized double-masked study to compare modified intraocular lenses with standard lenses in patients with uveitis that is quiet.

In a rat model, we tested the use of topical FK506 as an immunosuppressant. When heterotopic corneal grafts were performed in Lewis rats to determine the ability of these drops to prevent corneal graft rejection, a 0.3% solution of FK506 was found effective.

Project Description

Additional Personnel

Igal Gery	Ph.D.	Head, Section on Experimental Immunology, NEI, LI
Chi-Chao Chan	M.D.	Head, Section on Immunopathology, NEI, LI
Susan Mellow	R.N.	Nurse, NEI, LI
Susan Whitcher		Psychologist, NEI, LI

Clinical Protocol Numbers

79-EI-49
87-EI-104
92-EI-157

Objectives

One objective of this study is to develop rational surgical approaches for patients with intraocular inflammation. The development of appropriate surgical modalities for patients with uveitis will enable proper management of the complications that arise with chronic intraocular inflammation. A second objective is to devise rational methods for sampling intraocular tissues and to develop the methodology to obtain clinically useful information from limited tissue samples. A third objective is to test new therapeutic modalities for postoperative immunosuppression in animal models.

Methods

Patients with ocular inflammation who have developed ocular complications that require surgery or patients in whom an appropriate diagnosis can be made only with surgery are eligible for one of the protocols. Patients with vitritis and retinitis of unknown etiology in whom a nonspecific trial of immunosuppression is contraindicated may, according to the protocol, undergo vitrectomy and chorioretinal or endoretinal biopsy to obtain a diagnosis. The tissue specimen is partitioned for microbiology, electron microscopy, immunohistochemistry, and polymerase chain reaction.

Patients with a suspected intraocular lymphoma undergo an intraocular lymphoma workup with appropriate computer tomography or magnetic resonance imaging (MRI) scans and lumbar punctures. When these are negative, a vitrectomy is performed and the cells are studied by immunohistochemistry. Patients who have intraocular lymphomas are entered in a central nervous system (CNS) lymphoma protocol and followed prospectively.

Patients with glaucoma and uveitis are entered in a double-masked trial of either trabeculectomy with 5-FU or Molteno implant. They then are followed prospectively to determine the degree of postoperative inflammation and how effective the procedure was over time.

For patients with cataracts and uveitis under good control and with minimal intraocular inflammation, the protocol calls for a cataract extraction and randomization to a standard intraocular lens or a modified lens with a heparin coating. We then monitor patients postoperatively for the appearance of inflammation, using a laser cell flare meter. Patients also are monitored for the appearance of cellular deposits on the lens surface.

In animals, we have tested topical immunosuppression in a corneal transplant model. In this model, rejection of the graft occurs in 100% of animals within 1 week of stopping immunosuppression. Donor corneas are taken from Fisher rats, while the recipients are Lewis rats.

Major Findings

In Fiscal Year 1992, we performed several diagnostic vitrectomies for intraocular lymphoma. This particular lymphoma, a subtype of CNS lymphomas, is on the rise; three times more CNS lymphomas are diagnosed today than 10 years ago. In each case of intraocular lymphoma, the diagnosis was made after a complete workup with MRI brain scans and lumbar puncture. We have found that adding culture medium to the vitrectomy container improves the survival of the lymphoma cells and makes the cytologic diagnosis easier. Enough culture medium is added to give a 10-20% concentration in a full vitrectomy specimen container. One patient who recently died after a recurrence had no signs of systemic disease. There was evidence only of diffuse brain dissemination.

We have found that pretreatment with pulsed methylprednisolone is an effective way of decreasing preoperative inflammation. This pretreatment has been particularly useful in cases in which it has been necessary to perform a vitrectomy while there was still evidence of inflammation. Postoperatively these patients did not develop any significant inflamma-

tion. Data analyses are not yet available on the glaucoma trial or the intraocular lens trial; both trials are still recruiting patients.

In the animal studies, we demonstrated that topical drops of FK506 are an effective means of stopping corneal graft rejection. FK506 predominantly affects T cells, inhibiting both their activation and their recruitment into the transplanted tissue, as demonstrated by immunohistochemistry. It appears to downregulate the expression of both class I and class II antigens in the transplanted cornea. This effect is probably mediated by inhibiting T-cell cytokine secretion. Thus topical FK506 can prevent the onset of inflammation in several pathways and may be effective in the treatment of several immunologically based surface disorders.

Significance to Biomedical Research and the Program of the Institute

Uveitis is the cause of 10% of visual impairment in the United States. Ocular complications that require surgery for correction are common in these patients, despite adequate immunosuppression. Developing appropriate surgical modalities of treatment is an important endeavor. Similarly, conditions exist in which the appropriate therapy can be given only when the proper diagnosis has been made from intraocular tissue. Developing means of obtaining a minimal amount of tissue and properly processing it is thus of major significance.

Proposed Course

This project will continue with study of methods of surgically managing patients with uveitis. Patient enrollment continues. We also will study in animal models new methods to sample intraocular tissue and to test new immunomodulatory agents.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Chan C-C, Palestine AG, Davis JL, de Smet MD, McLean IW, Burnier M, Drouilhet JH, Nussenblatt RB: Role of chorioretinal biopsy in inflammatory eye disease. *Ophthalmology* 98:1281-1286, 1991.

Hikita N, Lopez JS, de Smet MD, Chan C-C, Mochizuki M, Nussenblatt RB: Use of topical FK-506 in the corneal graft rejection model in lewis rats. *Invest Ophthalmol Vis Sci* 33(4):832, 1992.

Whitcup SM, Belfort R Jr, de Smet MD, Palestine AG, Nussenblatt RB, Chan C-C: Immunohistochemistry of the inflammatory response in *Propionibacterium acnes* endophthalmitis. *Arch Ophthalmol* 109:978-979, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00075-14 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immune Functions in Ocular Diseases of Obscure Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Clinical Director	NEI
Others:	Marc de Smet	M.D.	Visiting Scientist	LI, NEI
	Rashid Mahdi		Biologist	LI, NEI
	Scott M. Whitcup	M.D.	Staff Medical Officer	LI, NEI

COOPERATING UNITS *(if any)*

Department of Ophthalmology, University of Kurume, Kurume, Japan (Manabu Mochizuki, M.D.)

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00115-14 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cyclosporine Therapy in Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Clinical Director	NEI
Others:	Marc de Smet	M.D.	Senior Staff Fellow	LI, NEI
	Scott Whitcup	M.D.	Senior Staff Fellow	LI, NEI
	Chi-Chao Chan	M.D.	Head, Section on Immunopathology	LI, NEI
	Richard Fenton	M.D.	Special Volunteer	LI, NEI
	Raymond DeBarge	M.D.	Senior Staff Fellow	LI, NEI
	Dan Martin	M.D.	Senior Staff Fellow	LI, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cyclosporine, an endecapeptide fungal product with specific anti-T-cell characteristics, is being administered to patients with sight-threatening ocular inflammatory disease of noninfectious origin who have failed on either corticosteroid or cytotoxic agent therapy to test its efficacy in the treatment of uveitis. Within the context of these ongoing studies, the combined use of cyclosporine A and ketaconazole has been tested in a randomized masked study of a small group of patients whose uveitis was well controlled with cyclosporine. The combination allowed a significant reduction in the dose of cyclosporine needed to control the disease. In some instances, the dose could be reduced by as much as 90%. No significant increase in side effects was noted. A phase I/II randomized trial using cyclosporine A and cyclosporine G has ended. There is a definite trend showing that combined use of a cyclosporine and low to moderate steroid doses is efficacious in preventing the progression of uveitis. An effective dose of cyclosporine appears to be around 5 mg/kg. At this dosage, toxicity has been reduced for up to 12 months of followup. Cyclosporine G was more effective than cyclosporine A in treating cystoid macular edema.

Project Description

Additional Personnel

Barry Grubbs Biologist, LI, NEI

Clinical Protocol Number

81-EI-33

Objectives

Cyclosporine, an endecapeptide obtained from fungi, has been shown to have specific anti-T-cell activity (*Transplant Proc* 12:234, 1980). We have reported cyclosporine's exceptional effectiveness in preventing the induction of S-antigen autoimmune uveitis in rats, as well as in inhibiting the disease once immunization has occurred (*J Clin Invest* 67:1228, 1981). The goal of this study is to test cyclosporine A (CsA) versus cyclosporine G (CsG) to test their efficacy in treating patients with bilateral sight-threatening posterior uveitis of an autoimmune nature.

Methods

Patients 18 years of age or older, of either sex (females not pregnant), who have not done well on more conventional medical therapy were admitted to this study. All patients must have bilateral sight-threatening uveitis of noninfectious etiology that has not been satisfactorily controlled by either corticosteroid or cytotoxic agent therapy. Lymphocyte cultures are prepared, and the immune cells are tested against various crude ocular extracts, as well as purified human S-antigen, to assess evidence of cellular immune memory, which is considered to be the in vitro equivalent of the anamnestic response in vivo. Patients chosen are treated with CsA or a new analog CsG in a phase I/II trial to evaluate the safety and activity of CsG versus CsA. During this period, the patients' clinical and immunologic course is closely monitored. Specific attention is given to renal function changes, a frequent side effect. Patients who need to continue CsA because of their ocular disease for over 1 year may be asked to undergo renal biopsy to evaluate the reversible and irreversible components to CsA renal toxicity. Some patients who have been entered on previous CsA studies and have continued to be followed in the eye clinic will continue to be monitored for their renal function to determine how and when cyclosporine can safely be tapered.

Major Findings

CsA has been effective in the treatment of some cases of posterior uveitis. An improvement in the inflammatory activity and visual acuity was seen in most patients treated to date. The particular responsiveness of patients with the ocular manifestations of Behçet's disease to this agent has been corroborated by a masked randomized trial performed in Japan. The improvement in the clinical condition was supported by a concomitant improvement in electrophysiologic testing, particularly contrast sensitivity.

Patients treated with CsA had no abnormalities of natural killer cell activity before the initiation of therapy, nor was any noted afterward. CsA significantly decreased skin test responsiveness but did not alter lymphocyte proliferation or antibody production in patients. Renal toxicity has been noted in some patients on long-term therapy, necessitating the addition of systemic corticosteroids and a decrease in CsA dosage. At 3 months, approximately 78% of the patients entering this open study were considered therapeutic successes, while 62% were considered successes at 1 year.

Seventeen patients treated long term with CsA underwent renal biopsy. These biopsy specimens were read in a masked fashion by a group of renal disease specialists who compared these biopsies to those from age-matched controls. An irreversible component of CsA toxicity could be identified, in the main being renal tubular atrophy accompanied by interstitial fibrosis. The majority of these individuals' biopsies had normal serum creatinine values, but a correlation could be made between the alterations noted and previous serum creatinine elevations for some period of time. The cyclosporine A/G trial has shown that the two cyclosporines have overall equal value in treating uveitis. However, CsG was more effective in reducing cystoid macular edema than was CsA, particularly at lower dosages.

Significance to Biomedical Research and the Program of the Institute

Uveitis is one of the most frustrating problems in all of ophthalmology. Present modes of therapy for patients with severe ocular inflammatory disease are

inadequate and nonspecific. CsA appears effective in treating posterior uveitis of noninfectious etiology. This is the first new agent in decades to be found useful in treating the severe form of this condition; therefore, it is important that the optimum therapeutic schedule be developed. Newer therapeutic strategies have already begun.

Proposed Course

Newer studies to look at various cyclosporine combinations will continue.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00278-01 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Administration of Antigen and the Ocular Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Scientific Director	LI, NEI
Others:	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LI, NEI
	Susan Whitcher	M.S.	Clinical Protocol Assistant	LI, NEI
	Marc D. de Smet	M.D.	Visiting Scientist	LI, NEI
	Molly Dayan	M.S.	Special Volunteer	LI, NEI
	Irwin Suh	B.A.	IRTA Fellow	LI, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Section on Immunoregulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	0.50	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of the oral administration of various antigens on the ocular immune response has been tested in the animal model for severe intraocular inflammatory disease experimental autoimmune uveioretinitis induced by both retinal S-antigen and interphotoreceptor retinoid-binding protein (IRBP). Oral tolerance could be induced by repeatedly feeding rats S-antigen. A putative suppressor cell that was CD8 positive could be isolated from the spleen of such animals and transferred to other animals to induce a similar toleragenic effect. In addition, the role of the spleen was confirmed in ongoing animal experiments. A randomized masked trial to evaluate the usefulness of S-antigen feeding in patients with intraocular inflammatory diseases has been put together. A pilot study performed in two patients gave evidence of the induction of such tolerance.

Project Description

Objectives

Exploring means of immunomodulation has been a major role of this laboratory. Although the Laboratory has extensive experience using various immunosuppressive agents, there also has been a major thrust in attempts to use other modes of immunosuppression. The goal of this series of experiments, in animals as well as in humans, is to test the efficacy of oral tolerance with uveitogenic antigens in the treatment of animals induced with experimental autoimmune uveitis and in patients with bilateral sight-threatening posterior and intermediate uveitis of an autoimmune nature.

Methods

The 6- to 10-week-old Lewis rats (either sex) used for these experiments will be fed before and after the induction of experimental uveioretinitis with various antigens. The antigens include whole molecules such as the retinal S-antigen (S-Ag) and interphotoreceptor retinoid-binding protein, as well as their fragments. In a subset of experiments, some animals also will undergo splenectomy before initiation of the experiments; others will be subjected to sham procedures. We will attempt to evaluate the clinical course of their disease and corroborate the clinical observations with histopathology at various points after the initiation of the experiments. The goal is to evaluate the role of the spleen as well as the roles of various fragments in the ability to induce this tolerogenic state.

In these studies, the patients will be individuals, 18 years or older, either sex, who have bilateral uveitis of a noninfectious cause. In addition, their lymphocytes must demonstrate an in vitro proliferative response to the retinal S-Ag. The patients also need to be on systemic immunosuppressive therapy, be it corticosteroids, cytotoxic agents, or cyclosporine. The goal of this study is, in individuals who need high amounts of immunosuppressive therapy to control their disease, to determine whether the addition of oral feeding of retinal antigens will induce a toleragenic state. This study will be performed in a randomized double-masked fashion. Some patients will receive S-Ag; some others, a retinal mixture containing several antigens; still others, placebo. The intent is to reduce the amount of immunosuppressive therapy the patients are taking. It is hoped that a toleragenic state can be induced by feeding these antigens.

Major Findings

In animal work, the spleen appears to play an important role in the induction of oral tolerance of S-Ag. In addition, the spleen is essential for adoptive transfer of tolerance by splenocytes from S-Ag-fed donors. Thus, it would be logical to assume that the spleen acts as a sight for induction and/or amplification of cells with suppressive activity.

The pilot study has demonstrated that—at least in two patients—a toleragenic state can be induced with the feeding of antigen at the dosages in this study. One patient with par planitis and one with Behçet's disease have been able to either come off their medication completely or be reduced to exceptionally low dosages.

Significance to Biomedical Research and the Program of the Institute

Uveitis is one of the most frustrating problems in all of ophthalmology. All the present modes of therapy for patients with severe ocular inflammatory disease have limitations, particularly because of their secondary side effects. In patients identified as having an immune response to the retinal S-Ag, we will be able to induce an immunosuppressive state without the use of pharmacologic agents. Furthermore, the induction of this tolerance will be antigen specific.

Proposed Course

The randomized study will begin shortly.

NEI Research Program

Retinal and Choroidal Diseases—Inflammatory Disorders

Publications

Thurau SR, Chan C-C, Suh E, Nussenblatt RB: Suppression of S-antigen induced experimental autoimmune uveitis by a 20mer peptide. *J Autoimmunity* 4:507-516, 1991.

Weiner HL, Zhang ZH, Khoury SJ, Miller A, Al-Sabbagh A, Brod SA, Lider O, Higgins P, Sobel R, Nussenblatt RB, Haffler DA: Antigen-driven peripheral immune tolerance: Suppression of organ-specific autoimmune diseases by oral administration of autoantigens. *Ann NY Acad Sci* 636:227-232, 1991.

Laboratory of Mechanisms of Ocular Diseases

Report of the Acting Chief, Laboratory of Mechanisms of Ocular Diseases

J. Samuel Zigler, Jr., Ph.D.

Investigators in the Laboratory of Mechanisms of Ocular Diseases (LMOD) have made significant progress during the past year in our mission to determine the molecular mechanisms underlying diseases affecting the eye. As in the past, the primary emphases have been on cataract and the ocular complications of diabetes.

Section on Cataract

Dr. Deborah Carper has continued to investigate the role of the polyol pathway in diabetic complications. Site-directed mutagenesis of specific residues of aldose reductase has identified certain residues important to the enzyme's catalytic function. The primary sequence of human sorbitol dehydrogenase also has been determined, and studies are under way to elucidate how its gene is regulated.

The groups headed by Drs. Paul Russell and Sam Zigler have been involved in the development of an in vitro system for assaying the effectiveness of anti-cataract agents. The successful development of such an organ culture system will allow initial screening of compounds before moving to animal models of cataract for the next phase of testing.

Dr. Donita Garland is investigating various modifications of lens crystallins and their potential role in cataractogenesis. The primary focus has been on metal-catalyzed oxidation; γ-crystallin is used as a model system. A protein that protects against such oxidation in the eyes of many species has been isolated from bovine lens.

Dr. Fielding Hejtmancik and his colleagues have been successful in mapping to human chromosome 11 two genes causing Usher's syndrome type I. The fact that this disease can be caused by more than one gene is a surprising and potentially very important step toward understanding the pathogenesis of this retinal degenerative disease.

Section on Pathophysiology

Dr. W.G. Robison, Jr., head of the Section on Pathophysiology has made significant advances in his studies on diabetic retinopathy. Using the enzyme elastase, he has developed a new procedure for isolation and evaluation of the entire vascular network of the retina. Coupled with the rat model system previously developed, this technique has proven that background diabetic retinopathy can be experimentally produced in rats, thereby facilitating both analysis of the pathogenesis of this disease and testing of potential therapeutic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00201-08 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Expression of Polyol Pathway Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Deborah Carper	Ph.D.	Biologist	LMOD, NEI
Others:	Susan Old	Ph.D.	Staff Fellow	LMOD, NEI
	Takeshi Iwata	Ph.D.	Visiting Associate	LMOD, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Cataracts

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In diabetes, the accumulation of sorbitol is believed to be a key factor in initiating cataract, retinopathy, and neuropathy. We are interested in controlling the accumulation of sorbitol by regulating the action of two enzymes of the sorbitol pathway: Aldose reductase (AR) reduces glucose to sorbitol, and sorbitol dehydrogenase (SDH) oxidizes sorbitol to fructose. Our aim is to design innovative methods to inhibit the action of AR or increase SDH with the purpose of reducing sorbitol accumulation in diabetic tissues.

Site-directed mutagenesis of AR has been a major priority of our laboratory. We have made amino acid substitutions in the rat and human AR and determined that some of these changes affect the kinetics of the protein with its substrate. For example, when cysteine at position 298 is changed to a serine, the kinetics of glyceraldehyde increases 2-fold, while xylose decreases to 15% of normal. These structure/function studies should help define the active site and locate the target areas of the current AR inhibitors.

Another strategy to control sorbitol accumulation is to regulate SDH. We have initiated studies to understand the structure of this protein and its action in vitro. The sequence of human SDH has been determined using cDNA analysis. Northern blots indicate that levels of SDH mRNA are higher in kidney than in other tissues, such as lung, liver, brain, and heart. A major portion of the SDH gene also has been obtained. These tools will allow us to study the regulation of SDH at the gene level.

Project Description

Objectives

The objective of the project is to study the regulation of the enzymes of the polyol pathway.

Methods

The methods employed include molecular biology, protein chemistry, and cell biology techniques.

Major Findings

1. *Structure/function studies.*—Several mutant forms of the aldose reductase (AR) protein were synthesized using the polymerase chain reaction (PCR). Sequencing verified the amino acid substitution. The mutant proteins were expressed in bacteria, purified on three columns using different biochemical characteristics of the protein, then tested for enzyme and inhibitor activity. The change from histidine to glutamine in rat AR at amino acid positions 41 and 187 affected neither the substrate binding rate nor protein inhibition by sorbinil. A change from cysteine to serine at position 298 altered the properties of AR. Glyceraldehyde was a better substrate for the mutant protein than the wild type. In contrast, xylose was a poor substrate for the mutant enzyme. Whereas cysteine 298 is located in the active site, this finding suggests an important role for this amino acid.

2. *Cellular regulation of AR and sodium/potassium ATPase.*—Sodium/potassium ATPase activity changes as sorbitol accumulates. In our studies on the induction of AR and its product, sorbitol, we found that sodium/potassium ATPase activity increased in the early stages of hypertonic stress. We then extended this finding by measuring the mRNA levels of the enzyme under various stress conditions. Sodium/potassium ATPase was synthesized by the reverse transcriptase PCR method. The DNA products were subcloned and verified by sequencing. The DNA products were then used as probes on Northern blots of dog lens epithelial cells grown in hyperglycemic and hypertonic conditions. We found that by 24 hours, medium containing 150 mM NaCl increased the mRNA levels of sodium/potassium ATPase approximately eightfold. In medium containing 300 mM glucose, the mRNA levels also increased, but at a slower rate. These studies show a correlation in the response of AR and sodium/potassium ATPase to stress.

3. *Primary structure of sorbitol dehydrogenase (SDH).*—In determining the complete sequence of human SDH, we employed two different methods: (1) cloning from a human cDNA library, which gave approximately 80% of the structure, and (2) use of the rapid amplification of cDNA ends (RACE) system, which completed the sequence. Using the SDH DNA as a probe, we obtained part of the gene for SDH, which we now are sequencing.

Significance to Biomedical Research and the Program of the Institute

AR has been implicated in diabetic cataracts, retinopathy, and neuropathy. Side effects and lack of efficacy of AR inhibitors in diabetes clinical trials have emphasized the need for innovative approaches to AR inhibition. Our research is a rational approach to designing new types of inhibitors by characterizing the structure/function aspects of the protein and evaluating the signals that regulate this enzyme. In addition, we feel that by understanding the regulation of SDH—the other enzyme of the polyol pathway—we may be able to modulate more fully the accumulation of sorbitol in diabetes.

Proposed Course

The project will continue via site-directed mutagenesis of AR protein to localize the critical amino acids residues in the active and inhibitor binding sites. We will complete the structure of SDH and use these DNA tools for studying the regulation of both AR and SDH in culture.

NEI Research Program

Cataract—Molecular Genetics

Publications

Dasgupta S, Hohman T, Carper D: Hypertonic stress induces αB-crystallin expression. *Exp Eye Res* 54:461-470, 1992.

Graham C, Szpirer C, Levan G, Carper D: Characterization of the aldose reductase-encoding gene family in rat. *Gene* 107:159-267, 1991.

Lin L-R, Carper D, Yokoyama T, Reddy V: Effect of hypertonicity on aldose reductase, αB-crystallin and organic osmolytes in retinal pigment epithelium. *Invest Ophthalmol Vis Sci*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00189-09 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oxidation of Proteins in Cataractogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donita L. Garland	Ph.D.	Research Chemist	LMOD, NEI
Others:	Jose Jimenez	Ph.D.	Visiting Fellow	LMOD, NEI
	Lorenzo Merola	M.S.	Chemist	LMOD, NEI
	Mark Reid	B.A.	Technician	LMOD, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Cataracts

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	3.0	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oxidative changes of lens proteins are thought to occur with aging and to contribute to the development of cataracts. The goals of this project are to determine (1) the extent of oxidative modification of crystallins and metabolic enzymes in both normal and cataractous lenses, (2) the nature of the modifications and mechanisms leading to the changes, and (3) the modifications on the structure and function of lens proteins. Bovine and rat lenses are used. The approach is to study the modifications of lens proteins after treatment in vitro by metal-catalyzed oxidation systems.

The focus of these studies has been on the modification of bovine γ-crystallins as a model system. There are distinct differences in the interaction of metals with each of the purified γ-crystallins. The structural modifications induced by treatment of the crystallins with metal-catalyzed oxidation have been studied. A protein that presumably protects against metal-catalyzed oxidation in cells has been identified in the eye of rats, bovine, primates, and humans; the bovine lens protein has been purified.

We have studied human lens proteins by two-dimensional polyacrylamide gel electrophoresis. These studies have included identification of the human lens proteins, protein distribution within the lens, age-related changes in composition, and identification of the age-related modifications that the proteins undergo.

Project Description

Objectives

Oxidative modifications of lens proteins are thought to occur with aging and to contribute to the development of cataracts. The objectives of these studies are (1) to determine the extent to which oxidation contributes to the modification of lens crystallins and enzymes in normal lenses and cataracts, (2) to determine the nature and the mechanisms of the modifications, (3) to determine the effect of the modifications on the structure and function of the proteins, and (4) to develop methodology to assess levels of oxidation in tissues and cells in culture.

Methods

Bovine, rat, and human tissues were used for these studies. We employed classical methods to purify bovine and rat lens proteins. Other methods used were standard procedures for studying proteins, including amino acid analysis, high-pressure liquid chromatography (HPLC), electrophoresis, isoelectric focusing, circular dichroism, and two-dimensional gel electrophoresis. For metal analyses we used a multi-element atomic absorption spectrometer.

Major Findings

1. A protein that protects enzymes against inactivation by thiol-dependent metal-catalyzed oxidative systems but not other metal-catalyzed oxidation systems is present in rat and bovine lens and in human trabecular meshwork cells. The protein has been purified and now is being sequenced.

2. When lyophilized, γ-crystallins undergo structural changes that we have demonstrated by changes in isoelectric focusing, hydration, and denaturation properties.

3. Continuing studies on the metal-catalyzed oxidation of γ-crystallins, we have focused on demonstrating differences in copper and iron binding to the proteins and on differences in amino acid modification by the copper-ascorbate oxidation system.

4. A great deal of effort has gone into a project done in collaboration with the Ophthalmic Genetics and Clinical Services Branch. We are analyzing proteins of lens samples obtained from normal donors and from extracted cataracts by two-dimensional polyacrylamide electrophoresis. The initial focus has been on setting up equipment, optimizing the technique for processing these samples, and sample preparation. Analyses have primarily been done on samples obtained by aspiration during cataract surgery.

Many proteins have been identified by immunoblotting techniques and protein sequencing. In the analyses of normal donor lenses of various ages, we have identified many proteins and determined the extent of modification of these proteins using immunoblotting techniques.

Significance to Biomedical Research and the Program of the Institute

Oxidative processes have long been considered a major contributing factor in senile cataracts. Treatment of lens proteins in vitro by mixed-function oxidation systems leads to protein modifications that mimic those seen in aging and senile cataracts and in brunescent lenses. The use of these oxidation systems to study the modification in vitro may facilitate our understanding of what role oxidation plays in aging and cataractogenesis. Also, it may be possible to use such an in vitro system to screen for agents that can prevent or protect against oxidative damage in the lens.

Proposed Course

The following studies are proposed for Fiscal Year 1993:

1. The major focus will be continuing the studies on the oxidative modification of lens proteins by metal-catalyzed oxidation systems. The work will include purifying the peptides containing the modified amino acids, identifying the modified amino acid, and studying effects of the modification on structure. These studies will be done on γ-crystallins.

2. The studies on the interaction of copper with γ-crystallins will be continued.

3. The analysis of human lens proteins by two-dimensional electrophoresis will continue. Proteins in various types of cataracts will be compared.

NEI Research Program

Cataract—Lens Development and Aging

Publications

Bettelheim FA, Reid MB, McPhie P, Garland D: On the stability of bovine gamma II crystallin. *Biochem Biophys Res Commun* 187:39-44, 1992.

Datiles MB, Schumer DJ, Zigler JS Jr, Russell P, Anderson L, Garland D: Two-dimensional gel electrophoresis analysis of human lens proteins. *Curr Eye Res* 7:669-677, 1992.

Garland DL: Ascorbic acid and the eye. *Am J Clin Nutr* 54:1198S-1202S, 1991.

Giannessi M, Del Corso A, Cappiello M, Marini I, Barsacchi D, Garland D, Camici M, Mura U: Thiol-dependent metal catalyzed oxidation of bovine lens aldose reductase: I. Studies on the modification process. *Arch Biochem Biophys*, in press.

Russell P, Yamada T, Su G-T, Garland D, Zigler JS Jr: Effects of naphthalene metabolites on cultured cells from eye lens. *Free Rad Biol Med* 10:255-261, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00272-02 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inherited Ocular Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James Fielding Hejtmancik	M.D., Ph.D.	Medical Officer	LMOD, NEI
Others:	John Hope	Ph.D.	Senior Fellow	LMOD, NEI
	Radha Ayyagari	Ph.D.	Special Volunteer	LMOD, NEI
	Ling Lee	M.S.	Chemist	LMOD, NEI
	Anthony Lloyd	M.D.	IRTA Fellow	LMOD, NEI

COOPERATING UNITS *(If any)*

Baylor College of Medicine (J. Towbin, B. Perryman, T. Ashizawa, P. Overbeek); Univ. of Iowa (R. Smith); Univ. of Texas-Houston (S. Daiger); Ocular Genetics and Clinical Services Branch, NEI, NIH (M. Kaiser); Washington Univ. at St. Louis (M. Petrash, R. Hayes); Massachusetts Institute of Technology (G. Benedek, J. Pande); Osmania Univ., Hyderabad, India (J.S. Murty)

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Cataracts

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study of inherited visual diseases provides a means by which both normal and aberrant visual processes might be understood. In addition to directly elucidating the pathophysiology of the inherited disease under investigation, these studies can provide insights into the structure-function relationships of the molecular components of the visual system and their normal physiology. This laboratory is using a number of approaches to study inherited visual diseases affecting the lens and retina.

Lens crystallins, which comprise over 90% of the soluble protein of the lens, are heavily modified in most cataracts. The effects of specific modifications of β- and γ-crystallin structure on crystallin functions, such as stability and formation of macromolecular aggregates, are studied in cultured cells transformed with normal and modified β A3/A1-crystallin genes. Regions of the β-crystallin molecule of special interest include the amino terminal arm and the Greek key motifs of the core domains. The effects of these modifications on lens transparency also are being studied in a transgenic mouse system in which a modified β-A3/A1 gene is driven by an αA-crystallin promoter.

A second approach to understanding inherited visual diseases uses principles of positional cloning to identify genes important in human inherited diseases. Human diseases undergoing linkage analysis, gene isolation, or characterization of mutations include Usher's syndrome, myotonic dystrophy, Duchenne muscular dystrophy, long qt syndrome, and a variety of X-linked syndromes. We are now recruiting families with inherited cataracts in preparation for study of this important group of diseases.

Project Description

Objectives

The long-range objectives of this project include increasing the understanding of inherited visual diseases, with the eventual aims of increasing the diagnostic ability for these diseases and providing a foundation for developing rational therapies based on a thorough knowledge of their molecular pathophysiology. These long-range objectives will be approached by pursuing two specific aims: identifying genes involved in inherited visual diseases and elucidating the mechanisms by which mutations in these genes cause disease.

Methods

Conventional cloning technology utilized in preparing sequences for gene expression studies includes ligation with T4 DNA ligase, screening by NaOH miniprep methodology and ^{32}P-labeled DNA probes, as well as allele-specific oligonucleotide hybridization to screen for specific single-base settings. We introduced sequence changes by site-specific mutagenesis using standard methodology. Gene expression in either Chinese hamster ovary cells (RJK 88) or insect cells (Sf9) uses the baculovirus expression system. Protein expression is monitored by standard two-dimensional gel electrophoresis followed by immunoblotting. Association behavior is assessed by elution volume on seive FPLC.

Isolation of crystallin and other cDNAs and genomic fragments by library screening with cloned genes or oligonucleotides involves routine methods. Sequencing is conducted by cycling or automated fluorescent technology (ABI).

Until recently, linkage analysis has been performed by conventional Southern blotting. Cell lines from patients and other family members are immortalized by Epstein-Barr virus transformation. DNA is isolated by standard methodology and digested by restriction endonucleases. After agarose gel electrophoresis, Southern transfer is performed, and the resulting blot is probed with isolated DNA fragments labeled with ^{32}P by oligonucleotide labeling. Recently, we analyzed short tandem repeat (STR, microsatellite) markers by polymerase chain reaction (PCR) performed in the presence of labeled oligonucleotides on sequencing gels. Linkage data are recorded on a computerized spreadsheet; for both two-point and multipoint analysis, we use the LINKAGE program package.

Major Findings

1. We modified the Greek key motifs of the βA3/A1-crystallin and then expressed them in transgenic mice. The highly conserved glycine at position 23 assumes torsional angles impossible for any other amino acid; it is required to maintain the secondary structure of the Greek key motif. We changed this glycine to a proline residue and created additional transgenic mice, using a βA3/A1 peptide with a stop codon inserted just before the fourth Greek key motif, essentially creating β-crystallin with only three motifs. Although we have obtained animals containing the genomic insertion by Southern blot and PCR analysis, they do not have cataracts. The levels of expression of the transgenes currently are being characterized.

2. The β-crystallins, their structure, and the mechanisms by which heterogeneity arises among this family of proteins are under investigation. The βA3-crystallin is identical to βA1 except for an additional 17-amino-acid N-terminal extension. The same gene is believed to encode and express both polypeptides. We ligated the βA3/A1 coding sequences behind the RSV promoter, and RJK 88 fibroblast cells were transfected stably with this construction. In addition, the βA3/A1 coding sequences were inserted into the Bluebac expression vector (Stratagene) and expressed in SF9 cells.

A single 26-kD protein, the predicted size of βA1-crystallin, was detected on Western blots of soluble extracts of stable clones by antibodies raised to crystallin peptides. However, when the RJK 88 cells are transformed with cDNA that is the same except that codons gln7 and leu10 have mutated in vitro to stop codons, these cells express a protein of only 24 kD, the predicted size of the βA1-crystallin. Thus it appears that the upstream (βA3) start codon is used preferentially in cell lines, although the downstream (βA1) start codon is capable of being utilized.

In SF9 cells, a protein with the same amino terminal sequence as that of the βA1-crystallin is produced when baculovirus-infected cells are grown past their prime. This is temporally correlated with disappearance of the βA3-crystallin band, suggesting that the smaller band is created by processing

or degradation of the larger in this system. In addition, we have isolated clones for the mouse βA2-, βB1-, βB2-, and βB3-crystallins, and we are sequencing them in preparation for characterization of their roles in β-crystallin aggregation.

3. We have constructed an additional crystallin in which the amino terminal arm is deleted and replaced by a glycine residue, an extension identical to that found in γ2-crystallin. Expressed in RJK88 and SF9 cells (Blubac vector), it has an appropriate migration on Laemli gels, as well as CD-spectrum and amino acid sequence. The activity of this β-crystallin in association with the typical 200- to 250-kD aggregates has been tested via FPLC on superdex 75 and superose columns. The normal βA3 polypeptide readily associates into homodimers while the truncated βA3 associates minimally if at all.

We grew SF9 cells expressing the recombinant crystallins in ^{35}S-containing medium, then purified and reassociated them with an excess of lens extract containing normal crystallins (unlabeled), using limited urea denaturation followed by dialysis. Association into β-crystallin aggregates was assessed by FPLC on sizing columns. The recombinant full-length β-crystallin peptide associates into both dimers and tetramers, with the dimer peak migrating slightly before the beta-light peak. However, the truncated βA3-crystallin migrates slightly behind the beta-light peak and does not form obvious tetramers. These data strongly suggest that the amino terminal arm of β-crystallins assists the β-crystallins' association into higher order aggregates.

4. Studies of phase transition properties of the γ-crystallin gene family have begun in collaboration with Drs. Mark Petrash (Washington University, St. Louis) and George Benedek (Massachusetts Institute of Technology, Boston). The bovine γB-crystallin has been modified at two of the four residues proposed to be critical for phase transition behavior.

5. We also studied human genetic diseases that have eye findings as part of complex traits. In addition to elucidating the pathogenesis of visual symptoms in inherited diseases, our efforts have provided reagents and information applicable to genomic analysis in general. Genetic markers in the myotonic dystrophy region have been used to confirm the diagnostic usefulness of bilateral lens opacities in the diagnosis of myotonic dystrophy, data confirmed by examination of the trinucleotide repeat shown to be expanded in persons affected by myotonic dystrophy. The phenomenon of anticipation, long controversial in myotonic dystrophy, was shown to occur with statistical significance in families with the disorder. In addition, we showed that earlier age of onset through anticipation correlates with expansion of the trinucleotide repeat, although this correlation was not perfect. A cDNA clone corresponding to the dystrophin gene product, isolated from a mouse lens library, is being characterized.

6. Ophthalmologic diseases have been studied in humans by linkage analysis of RFLP markers. Diseases that we have mapped within the past year include myotonic dystrophy, X-linked agammaglobulinemia, and Usher's syndrome type I. In addition, we have explored in detail the clinical and genetic heterogeneity of Usher's syndrome in the Acadian population. Genetic analysis confirms the clinical impression that both type I and II Usher's syndrome are found in Acadians, and even within a single extended pedigree. The heterogeneity analysis described above implies that this phenomenon is due to segregation of two different, unlinked genes within this population.

Two genes causing Usher's syndrome type I have been mapped. In the Acadian population of Louisiana, the genetic locus is on chromosome 11p, while in British families in our study, the gene is on chromosome 11q. These findings, which have been subjected to heterogeneity analysis using both the HOMOG2 program and the M-test, are significant at $p < 0.01$ under the most stringent analyses. This surprising finding implies that multiple genes can cause the rather specific clinical findings present in Usher's syndrome.

Significance to Biomedical Research and the Program of the Institute

Elucidation of the genetic defects causing visual disability will have implications far beyond the patient populations suffering from the specific syndromes under study. Inherited diseases provide a means by which the molecular pathophysiology of the visual system may be understood; this knowledge then can be applied to a broad spectrum of diseases. This rationale, which applies to the study of inherited diseases such as visual dystrophy, have already resulted in improved diagnostic capabilities. The mechanism by which cataracts occur in this disease will provide insight into cataractogenesis in other

hereditary syndromes as well as in age-related and nonspecific cataracts.

Proposed Course

1. We will continue studies on the structure-function relationships of lens crystallins, concentrating on the effects modifications of the terminal arms and possibly the interconnecting peptide between the two domains upon aggregation of β-crystallins. We also will continue to explore how modification of the Greek key motifs affects crystallin stability and, where applicable, lens transparency. In addition, we will explore the effects of γ-crystallin sequence modifications on protein phase transitions and their relationship to cold cataract.

2. Sample collection and linkage analysis of a variety of human diseases will continue. The main emphasis will be on inherited visual diseases, especially Usher's syndrome type II. We are initiating a linkage study of autosomal dominant cataracts in families ascertained in collaboration with Dr. Muriel Kaiser-Kupfer (Ophthalmic Genetics and Clinical Services Branch) and of autosomal recessive cataracts ascertained in collaboration with Dr. J.S. Murty (Osmania University, Hyderabad, India). This work will be coordinated with a new project to categorize and map expressed sequences of the human lens and the ongoing mechanistic studies on lens crystallins described above. Together, these projects should elucidate the mechanisms of cataractogenesis in the human lens.

NEI Research Program

Cataract—Molecular Genetics

Publications

Ashizawa T, Dubel JR, Dunne PW, Dunne CJ, Fu Y-H, Pizzuti A, Caskey CT, Boerwinkle E, Perryman MB, Epstein HF, Hejtmancik JF: Anticipation in myotonic dystrophy: Complex relationships between clinical findings and structure of the GCT repeat. *Neurology*, in press.

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E (eds): *Foundations of Clinical Ophthalmology*. Philadelphia, JB Lippincott Co, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00237-07 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of the Lens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul Russell	Ph.D.	Research Chemist	LMOD, NEI
Others:	Geoffrey Kidd	Ph.D.	Senior Staff Fellow	LMOD, NEI
	Santa Tumminia	Ph.D.	Senior Staff Fellow	LMOD, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Cataracts

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have undertaken the development of an in vitro system to check the efficacy of anticataract agents. This work has centered on the organ culture of rat lens. To check the viability of the rat lens prior to experimental manipulation, we developed a method to determine crystallin leakage from the lenses. This method, which provides a simple, yet accurate, indication of the integrity of the lens after dissection from the animal, has been checked by use of a radioactive tracer to show that the organ-cultured lens acts like a lens in vivo. Analysis of the proteins surrounding the lens indicates that a process of protein modification may occur around the lens in vivo.

One way of investigating the lens changes occurring during cataract development is the use of two-dimensional gel electrophoresis of the parts of the lens. We have designed and developed a method to analyze small fractions of the rat lens to determine specific polypeptide changes that occur during cataract development. We have analyzed fractions from the epithelium and found the cell components most influenced by changes that signal cataract development.

The organ culture system has been used to look at mechanisms to protect cells against oxidative damage. Enzymes involved in the one- and two-electron reduction of oxidative components have been investigated not only with whole lenses but also with cultured lens epithelial cells. It appears that the some of the enzymes involved in the detoxification of these oxidative compounds are specifically induced under conditions of oxidative stress. This work represents an integral part of the strategic plan for the Section on Cataracts.

Project Description

Objectives

The purpose of this project is (1) to develop model systems with which to test anticataract agents in vitro, (2) to develop additional model systems to examine how influences such as oxidation alter the tissue in the anterior chamber of the eye, and (3) to develop an in vitro system to explore basic questions concerning the cell and molecular biology of the lens epithelium.

Methods

Numerous biochemical and molecular biology methods used in this research include one- and two-dimensional gel electrophoresis of proteins, Western blot analysis of antigens, mRNA extraction and Northern blot analysis, slot blot analysis and quantitation of proteins, isoelectric focusing, the polymerase chain reaction, and cell culture.

Major Findings

1. A new method for two-dimensional gel electrophoresis allows resolution of samples of less than 2 µg of protein.

2. An in vitro model is being developed to test anticataract drugs. Organ-cultured rat and monkey lenses have been used to study the influence of oxidative stress on the lens.

3. To determine viability of the organ-cultured lenses, we devised a test that can rapidly screen our in vitro system for lenses that may not mimic an in vivo lens.

4. Two-dimensional gel electrophoresis has identified several polypeptides that are modified in the epithelium when the lens is challenged with oxidative stress.

5. In vitro analysis of enzymes involved with detoxification of reactive oxygen species has shown that some of these enzymes are induced under conditions of oxidative stress.

Significance to Biomedical Research and the Program of the Institute

The development of systems to study the lens is vital to understanding the mechanisms involved in cataract formation and to obtaining an in vitro system with which to study anticataract agents. The new protocols developed for two-dimensional gel electrophoresis have enabled us to examine changes in the human epithelium with age and location in the lens. Working under definable and reproducible conditions is an advantage in formulating model systems to study conditions that lead to loss of cell function and to investigate agents that will ameliorate disease states.

Proposed Course

Continued efforts will be directed at obtaining an in vitro model system to test anticataract agents.

NEI Research Program

Cataract—Lens Development and Aging

Publications

Datiles MB, Schumer DJ, Zigler JS Jr, Russell P, Anderson L, Garland D: Two dimensional gel electrophoretic analysis of human lens proteins. *Curr Eye Res* 11:669-677, 1992.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00252-04 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cataract in the Philly Mouse Strain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul Russell	Ph.D.	Research Chemist	LMOD, NEI
Others:	Carolyn Chambers	Ph.D.	Senior Staff Fellow	LMOD, NEI

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Cataracts

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Philly mouse, derived from the Swiss-Webster strain, develops a cataract about 6 weeks after birth. Initial results have shown that in the lenses of these animals the epithelial cells fail to undergo complete differentiation. Biochemically, a 27 kD protein appeared to be missing in the Philly lens. This 27 kD protein is the βB2-crystallin; in the normal lens it is a heat-stable protein. Investigation of the Philly mouse revealed that mRNA with approximately the same size as the normal βB2 mRNA is present in the Philly lens. Furthermore, it was shown that a protein present in the Philly lens is immunologically related to the βB2 protein in the normal lens. This protein shares the same amino terminal as the normal βB2 but lacks part of the carboxyl half of the protein. The altered protein is slightly smaller and has a more acidic isoelectric point than the normal lens βB2-crystallin.

We cloned and sequenced cDNAs for normal and Philly mouse βB2-crystallin. The normal mouse βB2 cDNA is 725 base pairs in length with 618 base pairs of open reading frame. Deduced amino acid sequences suggest that the normal mouse βB2 lacks a C-terminal phosphorylation site that is found in other mammals. The Philly mouse has a deletion of 12 nucleotides in the area encoding its fourth motif. By polymerase chain reaction, we have shown that the deletion exists at the genomic level in the Philly mouse. The properties of the protein encoded with this deletion appear to be consistent with the earlier protein findings and may be responsible for cataract formation.

The work on this project will be terminated to comply with the NEI strategic plan.

Project Description

Objectives

This project was designed (1) to study hereditary cataracts to learn how the alterations in the proteins of the lens affect cataract formation and (2) to map the alteration in the Philly mouse protein and determine whether the alteration of this protein is related to the conformation and heat stability of the native protein.

Methods

Biochemical and molecular biological methods were used in this study. The techniques used included gel electrophoresis, Western blot analysis, mRNA extraction and Northern blot analysis, DNA sequencing, isoelectric focusing, the polymerase chain reaction, and two-dimensional gel electrophoresis.

Major Findings

1. The Philly mouse previously had been thought to lack a protein with a molecular weight of 27,000. That protein now has been shown to be βB2-crystallin, a major heat-stable crystallin in the lens.

2. The Philly mouse has an mRNA of approximately the same size as the βB2-crystallin in the normal lens. It hybridizes with the βB2-crystallin probe.

3. The Philly mouse βB2-crystallin cDNA has a deletion of 12 nucleotides in the region encoding the fourth motif of the crystallin protein.

4. The deletion in the Philly mouse βB2-crystallin exists at the genomic level.

5. Both the normal mouse and the Philly mouse βB2-crystallin appear to lack a phosphorylation site on the C terminal.

Significance to Biomedical Research and the Program of the Institute

To understand how lens proteins are structured to permit the lens to be transparent to visible light, we need to know how a defect in one of these proteins leads to the condition known as cataract. The βB2-crystallin appears to be present in all mammals and is the major heat-stable protein. This developmentally regulated crystallin may be important to the proper orientation of proteins in the inner areas of the lens. The Philly mouse cataract is a useful model because in the Philly mouse strain the βB2-crystallin is altered. The result of the alteration may be the cause of cataract. This finding will facilitate not only our understanding of the structural organization of the lens but also the discovery of properties that make a protein heat stable. This may be the first model to show that alteration of one crystallin can lead to cataract formation.

Proposed Course

This project will be terminated to comply with the NEI Strategic Plan.

NEI Research Program

Cataract—The Molecular Genetics

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00105-13 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Composition of Lens Crystallins with Respect to Cataractogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist	LMOD, NEI
Others:	Vasantha Rao	Ph.D.	Visiting Associate	LMOD, NEI
	Pedro Gonzalez	Ph.D.	Visiting Fellow	LMOD, NEI
	Mohan Rao	Ph.D.	Visiting Associate	LMOD, NEI
	Chuan Qin	M.D.	Visiting Fellow	LMOD, NEI

COOPERATING UNITS *(if any)*

Dept. of Ophthalmology, Univ. of Tennessee (H.M. Jernigan, Jr.); Laboratory of Molecular and Developmental Biology, NEI (G. Wistow, D. Lee); Alcon Laboratories (M. Lou); National Cancer Institute (M. Krishna); Centre for Cellular and Molecular Biology, Hyderabad, India (D. Balasubramanian)

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Cataracts

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	4.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project, directed toward elucidating the molecular mechanisms responsible for cataractogenesis, places special emphasis on the role of the structure and function of the lens crystallins. One system under study involves a guinea pig hereditary congenital cataract that results from a mutation in the gene for a major lens protein, ζ-crystallin. This protein is an enzyme/crystallin, a protein with two distinct functions. We are investigating how the mutation affects both ζ-crystallin's catalytic function as a quinone reductase and its structural role as a lens crystallin. Because this protein is a crystallin in only a few species and is present at just catalytic levels in most species, it is an excellent system to use in analyzing lens-specific protein expression. Studies on the promoters for the ζ gene in several species indicate the presence of a second lens-specific promoter only in a species (i.e., guinea pig) that has high expression of ζ-crystallin in the lens.

A second focus of this project concerns the putative role of α-crystallin as a molecular chaperon. Studies by our group have confirmed that α-crystallin binds denaturing proteins, thus preventing their aggregation. Our current studies involve the nature and stability of the complex produced and the role such complex formation may play in the remarkable ability of the lens to remain transparent for decades, even though it has very limited synthesis and repair capabilities.

A major new initiative of our group concerns utilization of lens organ culture as a means of identifying therapeutic agents with efficacy as anticataract agents. Our studies have involved several cataract-inducing systems and a number of potential anticataract agents, including those with antioxidant activity.

Project Description

Objectives

The primary objectives of this project are (1) to elucidate at the molecular level processes responsible for cataract development, (2) to investigate the structures and functions of the lens crystallins, and (3) to develop model systems suitable for screening potential anticataract agents.

Methods

Conventional protein chemical techniques employed are chromatography, electrophoresis, and isoelectrofocusing. Immunological studies of lens proteins use specific antisera. Physicochemical analyses on the proteins are performed using high-pressure liquid chromatography, fluorescence, and circular dichroism techniques. Lens organ culture experiments use rat or monkey lenses and utilize active transport and membrane permeability parameters to monitor the effects of various stresses on the cultured lenses.

Techniques used in analysis of nucleic acids include RNA and DNA isolation, cDNA and gene cloning, DNA sequencing, various electrophoretic methods, and the polymerase chain reaction.

Major Findings

1. We showed that the susceptibility of lenses from different species to ultraviolet light-induced photodamage is dependent upon the levels of reduced pyridine nucleotide present, supporting the contention that the cellular redox potential may be a parameter which could be modified to increase resistance to cataractogenesis.

2. We have shown that condensates produced from either tobacco or wood smoke are toxic to the lens. Membrane damage has occurred in lenses exposed in organ culture to such smoke condensates.

3. We have measured a variety of biochemical and physiological parameters in cultured lenses exposed to various cataractogenic conditions to determine the best indicators for assessment of the effects of potential anticataract drugs.

4. We have confirmed the "chaperone-like" activity of α-crystallin in protecting other proteins from stress-induced aggregation and have demonstrated by several methods the formation of complexes between α-crystallin and other proteins during thermal stress.

5. We completely characterized the guinea pig ζ-crystallin gene and showed it contains two separate promoters. One promoter accounts for high lens-specific expression while the other appears to direct low-level expression in many tissues.

6. The cDNAs for human and mouse ζ-crystallin have been sequenced and show high homology with the guinea pig sequence. The genes for these species, which are expressed only at low levels in the lens, lack the lens-specific promoter.

7. The human ζ-crystallin gene has been localized to chromosome 1.

8. The ζ-crystallin present in guinea pig lens has been shown to be catalytically functional. This we proved by demonstrating stimulation of the hexose monophosphate shunt in organ-cultured lenses exposed to quinone substrates of ζ-crystallin.

Significance to Biomedical Research and the Program of the Institute

Cataract is a major public health problem worldwide. Better understanding of the biochemistry of the normal lens and of the molecular changes that occur during aging and cataract development are essential if this disease is to be controlled.

Our studies are aimed primarily at elucidating the roles of the lens crystallins, the primary structural elements of the normally transparent lens matrix, in the processes leading to opacification. Such knowledge should contribute to the development of means of intervention that can prevent or delay the process of cataract development.

Proposed Course

We will continue to (1) work to establish viable model systems for testing anticataract agents and use these systems to assess the efficacy of various types of compounds, including antioxidants; (2) complete analysis of the molecular basis underlying the high lens-specific expression of an enzyme/crystallin (ζ-crystallin); and (3) further investigate the chaperone-like function of α-crystallin and determine its physiological significance in the normal lens and in cataract.

NEI Research Program

Cataract—Pathogenetic Mechanisms

Publications

Datiles MB, Schumer DJ, Zigler JS Jr, Russell P, Anderson L, Garland D: Two-dimensional gel electrophoretic analysis of human lens proteins. *Curr Eye Res* 11:669-677, 1992.

Du X-Y, Linser P, Russell P, Zigler JS Jr: Carbonic anhydrase III is expressed in bovine lens. *Curr Eye Res* 11:475-478, 1992.

Krishna CM, Uppuluri S, Riesz P, Zigler JS Jr, Balasubramanian D: A study of photodynamic efficiencies of some eye lens constituents. *Photochem Photobiol* 54:51-58, 1991.

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Roquemore EP, Dell A, Morris HR, Panico M, Reason AJ, Savoy L-A, Wistow GJ, Zigler JS Jr, Earles BJ, Hart GW: Vertebrate lens α-crystallins are modified by O-linked N-acetylglucosamine. *J Biol Chem* 267:555-563, 1992.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00243-06 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ocular Cells Cultured Under Normal and Diabetic Conditions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bruce A. Pfeffer	Ph.D.	Senior Staff Fellow	LMOD, NEI
Others:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Pathophysiology	LMOD, NEI

COOPERATING UNITS *(if any)*

Departments of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO (Christine Blazynski, Ph.D.)

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Pathophysiology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00149-19 LMOD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructure and Function of the Cells and Tissues of the Eye

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Gerald Robison, Jr.	Ph.D	Head, Section on Pathophysiology	LMOD, NEI
Others:	Nora Laver	M.D.	Visiting Associate	LMOD, NEI
	Anne Groome		Histology Technician	LMOD, NEI
	Joe Hackett		Biologist	LMOD, NEI
	Evita Bynum		Microbiologist	LMOD, NEI
	Joel Glover		Biologist	LMOD, NEI

COOPERATING UNITS *(if any)*

Alcon Laboratories, Inc. (Billie M. York, Jr., Ph.D.)

LAB/BRANCH

Laboratory of Mechanisms of Ocular Diseases

SECTION

Section on Pathophysiology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.25	1.75	3.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory has developed a novel procedure which permits, in both animals and humans, isolation of the entire vasculature network of the retina in an intact and essentially undistorted arrangement. Although a trypsin digestion procedure was developed 30 years ago for isolation of human retinal vessels, it has many limitations and has not been as dependable in animals. Systematic tests with rat retinas revealed that elastase, a "contaminant" of the crude trypsin mixture traditionally used, is much more effective than trypsin for the selective preservation of retinal vessels. Digestion with purified elastase permits clear identification of the degeneration of pericytes ("ghosts"), the first lesion of diabetic retinopathy. Elastase-based procedures should apply directly to retinopathy of prematurity and provide new approaches in tissues, such as brain and inner ear. This procedure was the key to obtaining success in our studies on an animal model for the very distinct group of lesions characteristic of human background diabetic retinopathy.

The galactose-fed rat model, first developed in our laboratory, takes advantage of the fact that aldose reductase has a higher affinity for galactose than for glucose, which results in more intracellular polyol accumulation in galactosemia. We fed rats galactose for extended periods in an effort to produce diabetic-like retinal lesions. Galactosemia did indeed induce diabetic-like microangiopathies that were more advanced and more like human diabetic lesions than those which develop in long-term diabetic rats. The galactose-fed rat model has distinct advantages over genetic or chemically induced models of diabetes for intervention studies. Intervention studies are under way to determine appropriate times for intervention via different aldose reductase inhibitors. We plan to attempt, by dietary manipulation, to produce rat models that develop the diabetic-like retinal angiopathies sooner. Also, using cell culture, we will investigate possible mechanisms of endothelial cell proliferation and subsequent pathologies.

Project Description

Objectives

The use of special diets in vivo as well as cell culture of ocular tissues in various controlled media to mimic the diabetic state is designed to determine whether diabetic-like tissue changes can be prevented by inhibitors of aldose reductase (AR).

Methods

Sprague-Dawley rats were fed a normal or a 50% galactose diet with or without an AR inhibitor (tolrestat or sorbinil, 0.05% w/w) for 6 to 28 months. Mild enzyme digestion was used on the retina of one of the eyes of each rat to remove all the retinal tissues except the vessels. This digestion provided a whole mount of the retinal vasculature, thus permitting recognition of degenerated pericytes ("ghosts") and all of the more advanced angiopathies by light microscopy. The retina of the other eye of each pair was sectioned and examined by electron microscopy. Tissue culture of human, bovine, and canine retinal capillary pericytes and lens epithelial cells was used to investigate the mechanism underlying the diabetic angiopathies.

Major Findings

Elastase, not trypsin, is the most effective enzyme of the crude trypsin mixture traditionally used for preparation of retina vessels. Vascular whole mounts prepared by this new enzyme digestion procedure demonstrated multiple retinal angiopathies identical to those typical of human background diabetic retinopathy in the capillaries of rats fed galactose for 24 months. These angiopathies did not occur in the retinas of rats fed a galactose diet with an AR inhibitor. AR was shown to be present in cultured retinal pericytes (1) by immunohistochemistry involving use of antibody against human placental AR, (2) by its activity as indicated by measurements of xylitol production in cells grown in a medium supplemented with xylose, and (3) by the detection of messenger RNA for AR. There was a compromised proliferation rate in pericytes compared with that of endothelial cells with incubation in high (30 mM) sugar concentrations, suggesting toxicity of polyol at the cellular level. AR appears to be involved in all the retinal complications of diabetes, from pericyte degeneration to microaneurysm formation.

Significance to Biomedical Research and the Program of the Institute

Diabetic retinopathy is mainly a disease of retinal capillaries. Recently, potentially beneficial treatments and animal models have become available. However, demonstration of the earliest vessel lesions has relied on the 30-year-old trypsin digestion method for the isolation of retinal vessels. Until now, basic experimental studies and drug testing on diabetic retinopathy have been limited by the lack of reliable and convenient animal models. Now, besides the alloxan diabetic dog and the galactosemic dog, there is a galactosemic rat model. All this has been possible because AR is involved in diabetic retinopathy.

AR has been implicated in sugar cataracts, certain corneal healing defects, and peripheral neuropathy of diabetic and galactosemic animals. It now appears to be involved in all lesions of background diabetic retinopathy as well. While the normal physiological role of this enzyme in most tissues remains unknown, AR under the conditions of high plasma sugar concentrations encountered in diabetes and galactosemia converts these sugars to their respective sugar alcohols (polyols). These polyols are not readily metabolized, nor do they penetrate cell membranes easily. Once formed at significant rates, they may accumulate to very high levels in cells, leading to hypertonicity, alteration of ion permeability, and eventual cell death with consequent tissue changes such as cataract formation. Treatment of diabetic or galactosemic rats with potent AR inhibitors (eg, such as sorbinil or tolrestat) decreases polyol accumulation, which in turn appears to prevent the formation of cataracts in lenses, defective healing in scraped corneas, thickening of basement membranes in retinal capillaries, and decreased conduction velocity in nerves.

We have shown for the first time that the rat can be a good model for human diabetic retinopathy and that demonstration of early lesions can be improved by using a novel vessel preparation method. Pericyte loss, endothelial cell proliferation, microaneurysms, shunts, occlusions, dilations, and all the other microangiopathies that we found in the galactose-fed rat are identical to the histopathologies that characterize human background diabetic retinopathy. Until now,

the only other experimental animal model has been the diabetic or galactosemic dog. We have shown for the first time that diabetic-like retinopathy in galactosemic rats can be prevented with an AR inhibitor.

Proposed Course

The following studies are proposed for Fiscal Year 1993. We will extend the intervention studies to determine how late one can interrupt the disease process and still obtain beneficial results by treatment with various AR inhibitors. We also will examine the early formation of intracellular vacuoles, cell transport systems, the mechanism of basement membrane synthesis, and the relationship of these changes to AR in isolated retinal cells grown under diabetic conditions. We will manipulate the rat diets to shorten the time required for diabetic-like retinal angiopathies to appear, thus improving the rat as a model for diabetic retinopathy.

NEI Research Program

Retinal Diseases—Diabetic Retinopathy

Publications

Katz ML, Robison WG Jr: Senescent alterations in the retina and retinal pigment epithelium: Evidence for mechanisms based on nutritional studies, in Armstrong DA, Marmor MF, Ordy JM (eds): *The Effects of Aging and Environment on Vision*. New York, Plenum Press, 1991, pp 195-208.

Laver N, Robison WG Jr: Diabetic retinal vascular abnormalities compared to the rat model. *Proceedings of the Sixth Congress, US-Japan Cooperative Cataract Research Group, Kona, HI (Nov 30-Dec 5, 1991)*, p 205.

Laver N, Robison WG Jr, Calvin HI, Fu S-CJ: Early lesions in cataracts of GSH-depleted mouse pups retarded by ascorbate. *Invest Ophthalmol Vis Sci* 33(4):1041, 1992.

Laver N, Robison WG Jr, Calvin HI, Fu S-CJ: Early ultrastructural lesions in cataracts of GSH-depleted mouse pups. *Proceedings of the Sixth Congress, US-Japan Cooperative Cataract Research Group, Kona, HI (Nov 30-Dec 5, 1991)*, p 111.

Laver NM, Robison WG Jr, Pfeffer BA: Novel procedures for isolating intact retinal vascular beds from diabetic humans and animal models. *Invest Ophthalmol Vis Sci*, in press.

McCaleb ML, McKean ML, Hohman TC, Laver N, Robison WG Jr: Intervention with the aldose reductase inhibitor, tolrestat, in renal and retinal lesions of streptozotocin-diabetic rats. *Diabetologia* 34:695-701, 1991.

Robison WG Jr, Laver N: Ocular lesions in animal models of human diabetes, in Shafrir E (ed): *Frontiers in Diabetes Research, Lessons from Animal Diabetes IV*. London, Smith-Gordon and Company Limited, 1992, pp 145-163.

Robison WG Jr, Laver N, Kador PF: Meager delay of sugar cataracts and pre-proliferative retinopathy in galactose-fed rats by the ARI ponalrestat. *Invest Ophthalmol Vis Sci* 33(4):878, 1992.

Robison WG Jr, Laver N, Kinoshita JH: Galactosemic rat model for diabetic retinopathy: Prevention of capillary dilations, microaneurysms, acellularity, and shunt meshworks with aldose reductase inhibitors. *Workshop on Aldose Reductase Inhibitors*. NIH Pub No 91-3114, 1991, pp 89-100.

Robison WG Jr, Laver N, Kinoshita JH: Similar retinal vascular lesions in diabetic humans and rats with elevated tissue polyol. *Proceedings of the Sixth Congress, US-Japan Cooperative Cataract Research Group, Kona, HI (Nov 30-Dec 5, 1991)*, p 183.

Zadunaisky JA, Spring K, Sellers J, Robison WG: Contraction and drug sensitivity of cultured human trabecular meshwork cells in normal and glaucoma. *Invest Ophthalmol Vis Sci* 33(4):1163, 1992.

Laboratory of Molecular and Developmental Biology

Report of the Chief, Laboratory of Molecular and Developmental Biology

Joram Piatigorsky, Ph.D.

The Laboratory of Molecular and Developmental Biology (LMDB), completing its 11th year, comprises three sections: the Section on Molecular Genetics (headed by Joram Piatigorsky, Ph.D.), the Section on Cellular Differentiation (headed by Peggy S. Zelenka, Ph.D.), and the Section on Molecular Structure and Function (headed by Graeme J. Wistow, Ph.D.). The laboratory has grown this year, both in numbers of investigators and space. Renovations throughout Building 6 have resulted in an approximate 30% increase in laboratory space. The result is the acquisition of a cold room, a second common equipment room, another small darkroom (for autoradiography and immunofluorescence), and four additional modules for laboratory work. In addition to relieving congestion, the renovations have allowed the three sections to be collated and grouped together in a more logical and productive manner. Thus, the NEI continues to be supportive and generous to us, and we are grateful.

The focus of LMDB research remains to understand the fundamentals of gene expression and cellular differentiation in the eye, with great emphasis on the lens. Our studies occasionally include examination of nonocular tissues, especially since the recent discovery that most crystallins have nonrefractive functions in many tissues. We have also made contributions on the retina, and we are continuing last year's initiative on the cornea. We hope that, in addition to basic research, the accessibility and particular biological attributes of the cornea will lead to applied contributions in the area of gene therapy. Much of our work continues to be placed in a context of evolution. This year has been very productive, as described in the detailed annual reports of the individual sections; some highlights are given below.

The distinctions received by LMDB this year are numerous. Dr. Piatigorsky received a Special Recognition Award from ARVO, delivered the fourth Donald Abbott Lecture at Hopkins Marine Laboratory of Stanford University, and was a Wellcome Visiting Professor in the Basic Medical Sciences at the University of Louisville. Dr. Zelenka received the NEI Director's Award for her outstanding contributions on proto-oncogene expression during lens differentiation; she was also elected to serve on the Visual Sciences A1 Study Section. Drs. Piatigorsky, Zelenka, Wistow, and Chepelinsky all organized symposia in their respective fields at the International Congress of Eye Research in Stresa, Italy, indicative of their leadership roles in eye research. Finally, in the LMDB review by the Board of Scientific Counselors, each group was praised for its research contributions, and the LMDB was considered the leader among research laboratories on the molecular biology of the eye.

Section on Molecular Genetics

The Section on Molecular Genetics has ventured into the complex area of protein:DNA interactions involved in the expression of crystallin genes. These experiments have opened exciting new areas of investigation. For example, recent findings indicate that although *cis*-regulatory elements of the mouse αA-crystallin gene are occupied by nuclear proteins, regardless of whether the gene is active, the interactions with these proteins appear subtly different, depending on whether the gene is being expressed. We established that one of the key regulatory elements (the αA-CRYBP1 site) binds the cloned factor αA-CRYBP1, but that multiple forms of this putative transcription factor exist. In addition, different cell types contain different forms of αA-CRYBP1.

Another intriguing finding is that the αA-CRYBP1 site appears to bind αB-crystallin, raising the possibility that this crystallin may act to control transcription. More *cis*-regulatory elements have been discovered in different crystallin genes. Especially interesting is the finding that the αB-crystallin

gene has a strong muscle/weak lens enhancer upstream of a site which may, according to footprinting analysis, be a lens-specific regulatory element. The muscle-preferred enhancer has a regulatory element which functions only in muscle cells. Other putative regulatory regions appear to be involved in the heat shock response of this gene and in expression in lung and brain, both of which use different promoters than that used in lens and other tissues. Thus, this year has clearly delineated the complexity of crystallin gene expression and has focused our attention on certain key aspects of it.

The Section on Molecular Genetics has continued to examine the crystallins of invertebrates with cellular lenses. We hope that these models of convergent evolution will elucidate basic aspects of crystallins and their genes required for the optical properties of the transparent lens. In addition to cloning and analysis of new crystallins in cephalopods (squid and octopus) and jellyfish, we determined that the S-crystallins, which are related to glutathione S-transferase, are recruited by the cephalopods as crystallins via gene duplication, without recruitment of the parental gene encoding the protein with enzymatic activity. This is an atypical situation in vertebrates: Although variations on the theme exist, the genes encoding the enzyme-crystallins usually are identical to those encoding the active metabolic enzymes, hence we coined the term "gene-sharing." Crystallin recruitment is a fascinating portrait of molecular evolution that continues to kindle our curiosity.

Dr. Chepelinsky's group in the Section on Molecular Genetics has advanced in its characterization of *cis*-acting elements involved in the regulated expression of the lens-specific membrane protein (major intrinsic protein). In addition, the group is continuing to study in transgenic mice the effect of directing growth factors and other biologically interesting proteins to the lens. Of particular interest this year was its finding that ectopic expression of interferon-gamma (IFN-γ) in the lens induced major histocompatibility complex class II gene expression in the eye and disrupted the developmental program of the lens and retina. We hope this transgenic mouse will become a useful model for the study of ocular autoimmune disease. It promises to provide insight into the tight control of gene expression and cell differentiation in the eye.

Section on Molecular Structure and Function

The Section on Molecular Structure and Function is primarily interested in the molecular basis for crystallin gene recruitment and in growth factors or lymphokines with roles in lens differentiation. The putative lens-promoter of ζ-crystallin has been analyzed, showing that discrete regions confer lens preference while upstream positi ⌄ and negative elements confer improved lens specificity. In contrast to the dual promoters of ζ-crystallin, this group demonstrated that δ1, δ2 and ε-crystallin genes use the same promoters for lens and nonlens expression. The complete duck τ-crystallin gene has been introduced into transgenic mice to study the consequences of overexpression of an enzyme in the lens.

The cDNA of μ-crystallin, a mammalian taxon-specific crystallin and homologue of bacterial ornithine cyclodeaminases, has been cloned. Outside the lens, μ-crystallin is expressed in the retina and brain. Human retina μ has been cloned. In the developing chick retina, μ is found preferentially in photoreceptors. This may indicate the presence of an unusual pathway for ornithine metabolism in the retina which could be related to the problems of gyrate atrophy. The expression of lens 10K/MIF (migratory inhibitory factor), previously identified as a lymphokine, has been shown to be coordinated with cellular differentiation. Apart from any role in differentiation, this protein could play a role in lens-induced eye inflammations because mouse lens extract possesses MIF activity.

Section on Cellular Differentiation

Members of the Section on Cellular Differentiation have continued their investigations on the study of proto-oncogenes in the lens. Recent studies have revealed unexpected patterns that provide insights into the mechanisms of cell cycle arrest during differentiation. Extension of this study to include the tumor suppressor genes, p53 and Rb, and the cell cycle regulatory protein, cyclin B, has shown that all three are expressed in embryonic chicken lens fiber cells. Whereas fiber cells are generally

believed to be arrested in the early G1 or G0 portion of the cell cycle, these proteins are usually expressed in cells in late G1, S, or G2 phase. Expression of cyclin B in lens-fiber cells has been confirmed by sequencing of the polymerase chain reaction product and by immunoblotting with an antibody specific for cyclin B.

Another insight into cell cycle regulation stems from analysis of proto-oncogene expression in the synchronized cell population of the lens epithelium, as previously described by this Laboratory. Recent experiments demonstrated that these cells periodically accumulate very high levels of c-fos mRNA. The possibility that high c-fos mRNA levels may be related to the mechanism that leads to synchronization of these cells is under investigation.

The past year also has seen significant progress in determining the functional role of proto-oncogenes in the lens, with the identification of the τ-crystallin/α-enolase gene as a target for transcriptional regulation by c-myc. The function of c-jun in the embryonic chicken lens has been explored using wild-type and mutated cDNAs for chicken c-jun, cloned into an avian retroviral vector. Use of this vector permits transfer of the c-jun constructs to cultured cells; efficiencies approaching 100% make it possible to test for effects of proto-oncogene expression on DNA synthesis, differentiation, and expression of endogenous genes. Preliminary results indicate that a negative dominant mutation of c-jun has a positive effect on transcription of the endogenous αA-crystallin gene.

Finally, insights also have been gained into the cellular mechanisms that regulate proto-oncogene expression in the lens. A collaborative study with Dr. Thomas Lysz (University of Medicine and Dentistry of New Jersey) has demonstrated that c-myc mRNA expression in the neonatal rat lens requires endogenous production of 12-hydroxyeicosatetraenoic acid (19-HETE), a lipoxygenase pathway metabolite of arachidonic acid. Inhibition of the 12-lipoxygenase activity of the lens blocks both c-myc expression and DNA synthesis, suggesting that this may represent a control point in the cell cycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00238-07 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Proto-Oncogene Expression During Lens Differentiation and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peggy S. Zelenka	Ph.D.	Head, Section on Cellular Differentiation	LMDB, NEI
Others:	Barbara Brewitt	Ph.D.	Staff Fellow	LMDB, NEI
	Jo Ann Rinaudo	Ph.D.	Staff Fellow	LMDB, NEI
	John Talian	Ph.D.	Staff Fellow	LMDB, NEI
	Ronald Warwar	M.D.	Staff Fellow	LMDB, NEI
	Chun Yun Gao	M.D., Ph.D	Staff Fellow	LMDB, NEI
	Emmanuel Vacchiano	Ph.D.	Staff Fellow	LMDB, NEI
	Anuradha Rampalli	Ph.D.	Visiting Fellow	LMDB, NEI
	Jaspreet Arora	Ph.D.	Visiting Fellow	LMDB, NEI

(Additional personnel listed under Program Description.)

COOPERATING UNITS *(if any)*

Department of Surgery, New Jersey Medical and Dental College (Thomas Lysz, Ph.D.)

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Cellular Differentiation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.15	4.23	0.92

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the expression of proto-oncogenes in the embryonic chicken lens and their relationship to cell growth, quiescence, and differentiation. The normal developmental profiles of four nuclear proto-oncogene mRNAs (c-*myc*, N-*myc*, c-*fos*, and c-*jun*) have been completed, and the study is now being extended to include the tumor suppressors, p53 and Rb, and cell-cycle regulatory proteins such as the cyclins. Functional studies have demonstrated that c-*myc* is involved in the transcriptional regulation of the τ-crystallin/α-enolase gene, and preliminary results implicate c-*jun* in the transcriptional regulation of α-crystallin. Further analysis of cell-cycle synchrony in the lens epithelium demonstrates that many synchronized cells are present in the central lens epithelium in the 14-day-old chicken embryo and that synchronized cells periodically express high levels of c-*fos* mRNA.

Project Description

Additional Personnel

Sambath Chung	B.A.	Technician, LMDB, NEI
Vu Bui		Summer Student, LMDB, NEI
Graeme Wistow	Ph.D.	Section Head, LMDB, NEI

Objectives

In this project we seek to determine whether the expression of specific proto-oncogenes is altered during lens cell differentiation and to determine the function of the corresponding proto-oncogene products in the developing lens.

Methods

Techniques of molecular biology are used in conjunction with traditional cell biology techniques. Conventional methods employed for protein and nucleic acid analysis include polyacrylamide gel electrophoresis, RNA and DNA isolation, polymerase chain reaction (PCR), nucleic acid hybridization, in vitro transfection, in situ hybridization, immunocytochemistry, and immunoblotting. DNA-protein interactions are studied using DNAseI footprinting, electrophoretic mobility shift assays, and ultraviolet (UV)-cross-linking.

Studies employ lens epithelia and lens fibers of embryonic chickens, explants of embryonic chicken lens epithelia, and primary cultures of embryonic chicken lens epithelial cells.

Major Findings

In the past year we expanded our study of proto-oncogenes in the embryonic chicken lens to include the tumor suppressor genes p53 and Rb and the cell-cycle regulatory protein cyclin B. Development of a PCR assay for Rb mRNA is in progress. PCR assays have been developed for p53 and cyclin B, and DNA internal standards have been constructed to permit quantitation of PCR products.

Expression of these proteins is known to be cell-cycle dependent in proliferating cells, p53 appearing at the G1/S boundary and cyclin B in the S and G2 phases of the cell cycle. Interestingly, our PCR data indicate that both are expressed in embryonic chicken lens fiber cells although these cells are generally believed to be arrested in the early G1 or G0 portion of the cell cycle.

Expression of cyclin B in lens-fiber cells has been confirmed by sequencing the PCR product and by immunoblotting with an antibody specific for cyclin B. Immunocytochemical studies are in progress. These studies may help to define the mechanisms of cell-cycle arrest employed by differentiating lens fiber cells.

A major goal of this project continues to be the biological function of the proto-oncogenes expressed in the lens. The past year has seen significant progress in this area with the identification of the τ-crystallin/α-enolase gene as a target for transcriptional regulation by c-myc. Cotransfection studies involving a c-myc cDNA expression vector and a plasmid construct consisting of 800 bp of 5′-flanking sequence from the duck τ-crystallin gene coupled to the reporter gene, chloramphenicol acetyltransferase (CAT), indicate that expression of c-myc increases transcription from the τ-crystallin promoter. This effect required the putative c-myc binding site at -624/-619.

Nuclear extracts from both HeLa cells and embryonic chicken lenses contained proteins which protected this site from DNAseI digestion and retarded the migration of an oligonucleotide containing this site in electrophoretic mobility shift assays (EMSAs). DNAseI protection and retardation in the EMSA were abolished by mutations in the putative c-myc site. Finally, UV coupling of proteins from HeLa or lens nuclear extracts to an oligonucleotide containing this site, followed by immunoblotting with an antibody specific for c-myc protein, confirmed that c-myc protein itself participates in binding to this site. These findings identify the τ-crystallin gene as a target for transcriptional regulation by c-myc and help explain both the regional and developmental patterns of τ-crystallin expression.

We have explored the function of c-jun in the embryonic chicken lens using wild-type and mutated cDNAs for chicken c-jun, cloned into the avian retroviral vector RCAS. Use of this vector permits transfer of the c-jun constructs to cultured cells with efficiencies approaching 100%, making it possible to test for effects of c-jun on DNA synthesis, differentiation, and expression of endogenous genes. Preliminary results indicate that a negative dominant muta-

tion of c-jun has a positive effect on transcription of the endogenous αA-crystallin gene. The mechanism of this effect and the possible effects on transcription of other crystallin genes are being investigated.

With progress in determining how certain proto-oncogenes may play roles in regulating expression of lens crystallins, we also have gained insights into the cellular mechanisms that regulate proto-oncogene expression in the lens. In collaboration with Dr. Thomas Lysz (University of Medicine and Dentistry of New Jersey), we recently have found that c-myc mRNA expression in the neonatal rat lens involves endogenous production of 12-hydroxyeicosatetraenoic acid (12-HETE), a lipoxygenase pathway metabolite of arachidonic acid. Dr. Lysz has further shown that inhibition of the 12-lipoxygenase activity of the lens prevents DNA synthesis. These studies indicate that 12-HETE synthesis is necessary at an early step in the cell cycle, prior to expression of c-myc. Together with Dr. Lysz, we now are investigating the relationship of 12-HETE to c-fos and c-jun expression and the possibility that production of this eicosanoid may be a control point in the lens epithelial cell cycle.

Previous studies in this laboratory have demonstrated that the lens epithelium of chicken embryos at 13-16 days' development contains a population of cells that are synchronized with respect to their division cycles. We have furthered our investigation of this population using autoradiography of epithelia labeled with tritiated thymidine to localize the synchronized cells. The results indicate that the synchronized population includes nearly all the proliferating cells of the central epithelium and extends into the "proliferative zone" of the lens, where it is intermixed with a large number of asynchronously dividing cells.

It is likely that the large proportion of synchronized cells in the central epithelium caused earlier investigators to underestimate the number of proliferating cells in this region. Analysis of proto-oncogene expression in the synchronized cell population demonstrated that these cells periodically accumulate very high levels of c-fos mRNA. We currently are investigating the possibility that high c-fos mRNA levels may be related to the mechanism that leads to synchronization of these cells.

Significance to Biomedical Research and the Program of the Institute

The proto-oncogenes are normal cellular homologs of retroviral oncogenes. Whereas retroviral transformation disrupts cell growth and differentiation, it is likely that the proto-oncogenes are involved in the normal regulation of these processes. Therefore, a study of proto-oncogene expression during lens cell differentiation may elucidate basic regulatory processes underlying lens cell growth and differentiation. Many types of cataract are associated with abnormal lens epithelial cell growth and the inhibition of lens fiber cell differentiation. In addition, a number of other diseases of the eye involve loss of normal controls on cell proliferation. An understanding of the basic controls of cell growth and differentiation would further our understanding of these disease states.

Proposed Course

Several studies are in progress or proposed for Fiscal Year 1993:

1. We will determine the pattern of expression of p53 and Rb mRNAs at different developmental stages and in different regions of the lens.

2. We will explore further the unexpected expression of cyclin B in differentiating lens fiber cells. If initial findings are confirmed, we will conduct experiments to determine whether cyclin B in lens fibers is associated with the product of the cdc2 gene, p34, and whether the complex has mitosis-promoting factor activity.

3. We will explore the possible role(s) of the c-jun proto-oncogene in proliferation and differentiation using an avian retroviral vector to introduce wild-type and mutant versions of the c-jun cDNA into primary cultures of lens epithelial cells.

4. We will examine the effect of the N-myc proto-oncogene on transcription directed by the τ-crystallin/α-enolase gene, using the techniques used to study the role of c-myc.

5. We will study the possibility that glucocorticoids are involved in regulating the lens epithelial cell cycle, using glucocorticoid analogs and cultured lens epithelial cells.

6. We will investigate the synchronization of the lens epithelial cell cycle that we observed in 13- to 16-day-old chicken embryos in younger and older embryos.

7. We will pursue the collaborative effort with Dr. Lysz to define the role of 12-HETE in regulating c-myc expression and DNA synthesis in lens epithelial cells.

NEI Research Program

Cataract—The Normal Lens

Publications

Brewitt B, Talian JC, Zelenka PS: Cell cycle synchrony in the developing chicken lens epithelium. *Dev Biol* 152:315-322, 1992.

Harris LL, Talian JC, Zelenka PS: Contrasting patterns of c-myc and N-myc expression in proliferating, quiescent, and differentiating cells of the embryonic chicken lens. *Development* 115:813-820, 1992.

Rinaudo JAS, Zelenka PS: Expression of c-fos and c-jun mRNA in the developing chicken lens: Relationship to cell proliferation, quiescence, and differentiation. *Exp Cell Res* 199:147-153, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00251-05 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetically Engineering the Eye with the αA-Crystallin Promoter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ana B. Chepelinsky	Ph.D.	Research Biologist	LMDB, NEI
Others:	Devonne Parker	B.S.	Biologist	LMDB, NEI
	Charles Egwuagu	Ph.D.	Scientist, PHS	LI, NEI
	Chi-Chao Chan	M.D.	Chief, Section on Immunopathology	LI, NEI
	Robert B. Nussenblatt	M.D.	Clinical Director	LI, NEI
	Jorge Sztein	D.V.M.	Visiting Associate	VRRS, NEI

COOPERATING UNITS *(If any)*

Department of Cell Biology, Baylor College of Medicine, Howard Hughes Medical Institute (Paul Overbeek, Ph.D.); Imperial Cancer Research Fund, London, England (Clive Dickson, Ph.D.); Gerontological Research Unit, National Institute of Health and Medical Research, Paris, France (Yves Courtois, Ph.D.; Maryvonne Laurent, Ph.D.)

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interferon-gamma (INF-γ) gene expression was directed to the lens of transgenic mice with the murine αA-crystallin promoter. The transgenic mice obtained exhibited severe microphthalmia and microphakia; the lens architecture was disorganized, and lens-fiber cells were replaced by balloon cells. Differentiation of the neuroretina into inner and outer neuroblastic layers was arrested at the embryonic stage. Retinal detachment and the presence of macrophages in the subretinal space was observed in the adult mouse eye. The ectopic expression of IFN-γ in the transgenic mouse eye induced major histocompatibility complex (MHC) class II gene expression in the eye and arrested differentiation of lens and retina cell lineages.

Our data suggest that IFN-γ can also regulate gene expression in nonlymphoid tissues and provide an animal model for studying the roles of IFN-γ and MHC class II in ocular autoimmune diseases.

Project Description

Objectives

The objective of this project is to understand how aberrant genetic expression of interferon-gamma (IFN-γ), int-2, or acidic fibroblast growth factor (aFGF), under the control of the αA-crystallin promoter, perturbs normal eye development in transgenic mice.

Methods

Recombinant DNA techniques used in this study include plasmid construction, Southern and Northern hybridizations, DNA sequencing, primer extension, polymerase chain reaction (PCR), reversed transcription PCR (RT-PCR), and immunohistochemistry. The project involves the production and analysis of transgenic mice.

Major Findings

IFN-γ.—This project is conducted in collaboration with the NEI Laboratory of Immunology. IFN-γ is specifically expressed by T lymphocytes and natural killer cells in response to viral or bacterial infections. The aberrant expression of IFN-γ in the lens of transgenic mice allowed us to study the effect of IFN-γ on the normal development of the eye and the regulation of major histocompatibility complex (MHC) class II gene expression by IFN-γ in a nonlymphoid tissue such as the lens.

We generated transgenic mice containing as a transgene the murine αA-crystallin promoter (-366/+46) fused to the murine IFN-γ coding sequence. We obtained one FVB/N and two Balb/c transgenic lines to study the effect of different genetic backgrounds on the expression of the transgene. The transgenic lines presented a particular phenotype that segregates with the presence of the transgene: closed eyes and microphthalmia. Histological findings include serous retinal detachment, appearance of macrophages in the subretinal space, and loss of photoreceptor outer segments in adult Balb/c transgenic mice.

Developmental studies indicated that the differentiation of the lens was affected during embryonic development, with the replacement of normal primary lens fibers by balloon cells. Abnormal differentiation of the retina also was observed, particularly in the neural retina. The retinal detachment observed in the adult due to the lack of appropriate differentiation of the retina and the activation of expression of MHC class II gene that is involved in antigen presentation render this an animal model for ocular autoimmune diseases.

int-2.—In collaboration with Dr. Clive Dickson from the Imperial Cancer Research Fund (England) and Dr. Paul Overbeek from the Baylor College of Medicine, we injected the αA-crystallin promoter (-366/+46) fused to int-2 cDNA into mouse embryos. We obtained three transgenic lines that exhibited severe microphthalmia and the unusual presence of putative secretory epithelia and "dermoids" inside their eyes. The characterization of the eye pathology and the abnormal eye development produced by the aberrant expression of int-2 in the lens may shed some light on the function of this proto-oncogene. It may provide an animal model for some human ocular diseases.

aFGF.—In collaboration with Dr. Overbeek and Dr. Yves Courtois from the Institute for Gerontological Research, INSERM (France), we injected into mouse embryos a recombinant DNA containing the αA-crystallin promoter (-366/+46) fused to the bovine aFGF cDNA. We do not yet know whether the transgene is expressed in two founders that were obtained.

Significance to Biomedical Research and the Program of the Institute

The aberrant expression of IFN-γ, int-2, or aFGF will allow us to elucidate the mechanisms underlying eye development. At the same time, it will open new avenues in the development of animal models for the study of eye pathologies and gene regulation in the eye.

Proposed Course

Studies will continue during Fiscal Year 1993 with further characterization of the transgenic mice already obtained. The effect of the expression of these transgenes on lens differentiation and eye phenotype will be studied further.

NEI Research Program

Cataract—Molecular Genetics

Publications

Donovan DM, Sax CM, Klement JF, Li X, Chepelinsky AB, Piatigorsky J: Conservation of mouse αA-crystallin promoter activity in chicken lens epithelial cells. *J Mol Evol* 35:337-345, 1992.

Egwuagu CF, Sztein J, Reid W, Chan C-C, Mahdi R, Nussenblatt RB, Chepelinsky AB: Gamma interferon gene expression in the lens of transgenic mice directed by the αA-crystallin gene promoter. *Invest Ophthalmol Vis Sci* 33(4):846, 1992.

Jaworski CJ, Chepelinsky AB, Piatigorsky J: The αA-crystallin gene: Conserved features of the 5′-flanking regions in human, mouse, and chicken. *J Mol Evol* 33:495-505, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00253-04 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Expression of Lens Fiber Membrane Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ana B. Chepelinsky	Ph.D.	Research Biologist	LMDB, NEI
Others:	Chiaki Ohtaka-Maruyama	Ph.D.	Visiting Fellow	LMDB, NEI
	LaShawn R. Drew	B.S.	Chemist	LMDB, NEI
	Devonne M. Parker	B.S.	Biologist	LMDB, NEI
	Kristen Ault		Summer Student	LMDB, NEI

COOPERATING UNITS *(if any)*

University of Southern California (Margaret McFall-Ngai, Ph.D.; Virginia Weis, Ph.D.)

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.87	0.69	1.18

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns regulation of expression of genes encoding lens fiber membrane proteins involved in cell-cell communication. We are presently studying the regulation of expression of the gene encoding the major intrinsic protein (MIP) of the lens fiber membrane, which belongs to an ancient superfamily of putative transmembrane channel proteins. We cloned 2,840 bp of 5′ flanking sequence of the human MIP gene to study the *cis* regulatory elements responsible for the tissue specificity and developmental regulation of the MIP gene. We found that a DNA fragment containing 253 bp of 5′ flanking sequence and 42 bp of exon 1 of the human MIP gene fused to the reporter chloramphenicol acetyltransferase (CAT) gene is able to express the CAT gene in lens cells in transient assays and transgenic mice. We are presently studying the effect of several transcription factors on the in vitro transcription of the MIP gene in *Drosophila* and HeLa nuclear extracts. Purified human Sp1 activates the in vitro transcription of the MIP promoter, suggesting its involvement in the transcriptional regulation of the MIP gene. These studies will further our understanding of the role of general transcription factors on the tissue-specific expression of the MIP gene.

Project Description

Objectives

The objective of this project is to elucidate the mechanisms involved in the regulation of expression of fiber membrane genes involved in cell-cell communication in the lens. The identification of the *cis* regulatory elements of these genes and their interaction with *trans*-acting factors are essential for understanding the regulation of gene expression in the lens.

Methods

Recombinant DNA techniques used in this study include screening genomic libraries, subcloning, plasmid construction, Southern and Northern hybridizations, DNA sequencing, primer extension, polymerase chain reaction (PCR), reversed transcription PCR (RT-PCR), in vitro transcription, and tissue culture techniques, including transfection of primary lens explants and cell lines, as well as analysis of transgenic mice.

Major Findings

Cis *regulatory sequences of the human lens major intrinsic protein (MIP) gene.*—In order to study the regulation of expression of the MIP gene and identify the regulatory elements underlying its tissue specificity, we isolated and characterized 2,840 bp of the human MIP gene 5′-flanking sequence. Three complete Alu repetitive elements are found in tandem at approximately 2,000 bp upstream from the initiation site of transcription, while a single complete Alu sequence is present in the third intron of the gene. We focused on the 5′-flanking sequence proximal to the initiation site of transcription for the mapping of promoter regulatory elements of the MIP gene. We found in transfection experiments that a DNA fragment containing 253 bp of human MIP 5′-flanking sequence and 42 bp of exon 1 fused to the bacterial chloramphenicol acetyltransferase (CAT) gene activates CAT gene expression in primary chicken lens explants and in a mouse lens cell line, but not in several nonlens cell lines. Therefore, the -253/+42 sequence of the human MIP gene contains information for lens cell expression, suggesting that *cis* regulatory elements responsible for the lens-specific expression of the MIP gene are localized within this domain.

Several motifs, known to bind transcription factors in other genes, are present in the 5′-flanking sequence of the MIP gene. These include CCAAT and CACCC boxes, NF-1, Sp1, and glucocorticoid receptor binding sites, which may function synergistically in binding to the glucocorticoid receptor. To elucidate whether those motifs are involved in the regulation of MIP gene expression we are studying the effect of several transcription factors on the in vitro transcription of the MIP gene using *Drosophila* and *Hela* nuclear extracts. Purified human Sp1 activates the in vitro transcription of the MIP promoter, suggesting its involvement in the regulation of transcription of the MIP gene.

We generated several lines of transgenic mice containing 253 bp of the human MIP gene 5′-flanking sequence and 42 bp of exon 1 fused to the CAT gene as a transgene. In one transgenic line, the CAT gene is expressed specifically in the ocular lens, consistent with the DNA sequence -253/+42 of the human MIP gene containing regulatory elements responsible for gene expression in the lens. In two additional transgenic lines, expression of the CAT gene is observed only in the ovaries of females; no expression is observed in any tissue of males. No MIP transcripts were detected by PCR of reverse-transcribed mouse ovary mRNA. The possibility that species-specific regulatory elements might be responsible for the aberrant expression of the transgene containing the human promoter cannot be excluded. Cloning the murine MIP gene will allow us to study the mouse MIP promoter in its homologous in vivo environment.

3′-Untranslated sequence of the MIP gene.—The 3′ end of the human MIP gene was determined by comparing the nucleotide sequence of the 3′-untranslated region of the gene with the bovine cDNA sequence. As neither contains a classical polyadenylation signal, we are continuing the sequencing of the 3′-flanking region of the human MIP gene to characterize more precisely the polyadenylation site(s) utilized during the processing of the MIP transcripts.

MIP and connexins in the light organ lens and the ocular lens of Euprymna scolopes.—In collaboration with Drs. Margaret McFall-Ngai and Virginia Weis from the University of Southern California, we started investigating the possible expression of MIP, other members of the same transmembrane channel superfamily, and connexins in the ocular lens and the light organ lens of the squid, *Euprymna scolopes.*

The light organ lens is induced during its symbiosis with the luminous bacteria *Vibrio fischeri.*

Significance to Biomedical Research and the Program of the Institute

The differentiation of lens epithelial cells into fiber cells entails extensive cell elongation that results in a dramatic increase in the elaboration of new plasma membrane by elongating cells. Proper membrane biosynthesis and physiology are therefore of utmost importance in maintaining the transparent state of the lens. Membrane protein synthesis is regulated in a temporal and spatial manner in the lens. Lens membrane alterations, particularly those involving MIP, have been observed during cataractogenesis and aging. Studies on MIP gene expression should further our understanding of not only the mechanisms involved in the regulation of gene expression in the normal lens but also its disruption during disease.

Proposed Course

The following studies will continue during Fiscal Year 1993: (1) characterization of the *cis* regulatory elements of the human MIP gene in transient assays and in transgenic mice, (2) investigation of the interaction of the human MIP gene *cis* regulatory elements with *trans*-acting factors, (3) sequencing and characterization of the 3′-flanking region of the human MIP gene, and (4) isolation and characterization of the murine MIP gene promoter.

NEI Research Program

Cataract—Molecular Genetics

Publications

Chepelinsky AB, Drew LR, Pisano MM: Regulation of human MIP gene expression. *Exp Eye Res* 55(suppl 1):S25, 1992.

Drew LR, Pisano MM, Chepelinsky AB: CAT gene expression in transgenic mice directed by the human MIP gene promoter. *Invest Ophthalmol Vis Sci* 33(4):1043, 1992.

Pisano MM, Chepelinsky AB: Genomic cloning, complete nucleotide sequence, and structure of the human gene encoding the major intrinsic protein (MIP) of the lens. *Genomics* 11:981-990, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00273-02 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Expression of the Retinoblastoma-Associated Gene During Lens Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Andrew Cuthbertson	M.D., Ph.D.	Visiting Associate	LMDB, NEI
Others:	Joram Piatigorsky	Ph.D.	Chief	LMDB, NEI

COOPERATING UNITS *(If any)*

Howard Florey Institute for Experimental Physiology and Medicine, University of Melbourne, Australia (Robb Fernley, Ph.D.)

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00126-11 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Crystallin Genes: Structure, Organization, Expression, and Evolution

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joram Piatigorsky	Ph.D.	Chief	LMDB, NEI
Others:	James Brady	Ph.D.	NRC Fellow	LMDB, NEI
	Ana B. Chepelinsky	Ph.D.	Research Biologist	LMDB, NEI
	Sambath Chung	B.A.	Technician	LMDB, NEI
	Ales Cvekl	Ph.D.	Visiting Fellow	LMDB, NEI
	Peter Frederikse	Ph.D.	Senior Staff Fellow	LMDB, NEI
	Cynthia J. Jaworski	Ph.D.	Chemist	LMDB, NEI
	Marc Kantorow	Ph.D.	Staff Fellow	LMDB, NEI
	Xuan Li	Ph.D.	Visiting Fellow	LMDB, NEI
	Joan B. McDermott	M.S.	Biologist	LMDB, NEI

(Additional personnel listed under Program Description.)

COOPERATING UNITS *(if any)*

Jules Stein Eye Institute, UCLA (J. Horwitz, Ph.D.); National Institute on Child Health and Human Development, NIH (Kevin Becker, Ph.D.; K. Ozato, Ph.D.); University of Southern California (Virginia Weis, Ph.D.; M. McFall-Ngai, Ph.D.); Whitney Laboratory, University of Florida (Russel Buono, Ph.D.; Paul Linser, Ph.D.)

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Laboratory of Molecular and Developmental Biology

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Section on Molecular Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
13.70	11.45	2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure, expression, and evolution of crystallin genes of vertebrates and invertebrates are being studied. *Cis*-acting control elements for lens expression have been identified in α- and β-crystallin genes. Unexpectedly, the αA-crystallin elements are occupied in both expressing and nonexpressing cultured cells. Footprint analyses have shown that αB-crystallin gene expression in lens and muscle involves a combination of shared and tissue-specific *cis*-elements.

Two promoters are used in a tissue-preferred fashion for expression of the αB-crystallin gene; a strong muscle/weak lens enhancer is present between these promoters. This enhancer uses a muscle regulatory factor (MRF) site exclusively for muscle expression and at least two other sites for lens and muscle expression. Transgenic mouse and transfection experiments have shown that the αA-crystallin *cis*-acting elements are functionally redundant. A *trans*-acting factor (αA-CRYBP1) important for expression of the mouse αA-crystallin gene was shown unequivocally to bind to its cognate *cis*-element. Immunoblotting experiments indicated that this factor exists in different overlapping sizes in different tissues, and the promoter for its gene has been cloned and expressed in transfected lens cells.

The two δ-crystallin genes were shown to be under tissue-specific and developmental regulation. Crystallin recruitment in the squid was shown to differ from that in vertebrates in that the parental enzyme gene (glutathione S-transferase) gave rise to a related family of crystallins without enhancing its own lens expression. The ALDH-like crystallin cDNA of cephalopods has been cloned and sequenced. Cloning of the three J1-crystallin genes of cubomedusan jellyfish showed that they lack introns, encode novel proteins, and have surprisingly diverse 5′ flanking sequences.

Project Description

Additional Personnel

Rashmi Gopal-Srivastava	Ph.D.	NRC Fellow, LMDB, NEI
Barbara Norman		Chemist, LMDB, NEI
H. John Roth	Ph.D.	Senior Staff Fellow, LMDB, NEI
Christina M. Sax	Ph.D.	Senior Staff Fellow, LMDB, NEI
Stanislav Tomarev	Ph.D.	Visiting Scientist, LMDB, NEI
Peggy S. Zelenka	Ph.D.	Geneticist, LMDB, NEI
Rina Zinovieva	Ph.D.	Research Worker, LMDB, NEI

Objectives

The objectives of this project are to understand the structure, organization, expression, and evolution of the gene families encoding the lens crystallins. Particular attention is given to the regulation of crystallin gene expression in the developing lens and, in the case of multifunctional crystallins and enzyme-crystallins, in nonlens tissues.

Methods

Conventional methods for analysis of proteins and nucleic acids used include polyacrylamide and agarose gel electrophoresis, RNA and DNA isolation, molecular hybridization (Southern and Northern blots), cDNA and gene cloning, DNA sequencing, recombinant DNA construction, in situ hybridization, expression of recombinant DNAs in transfected cells and transgenic mice, polymerase chain reactions (PCRs), site-directed mutagenesis of recombinant DNAs, in vitro and in vivo footprinting, gel mobility shift analysis, and chromatographic purification of proteins.

Major Findings

Vertebrates.—Last year we reported on the identification of numerous *cis*-acting regulatory elements for expression of the mouse and chicken αA-, mouse αB-, and chicken βA3/A1-crystallin genes. Site-specific mutagenesis experiments have established that these are indeed important control elements. DNAse I and in vivo dimethylsulfate interference footprinting experiments have shown that three major control elements in the mouse αA-crystallin promoter (DE1, αA-CRYBP1, and a CAT sequence) are all occupied in αTN4-1 cells that express the gene and in L929 cells that do not express the gene although the details of the footprints indicate subtle differences in binding specificity and affinity. The αA-CRYBP1 site has been investigated in greater detail and shown in immunoblots to bind a cloned factor called αA-CRYBP1. αA-CRYBP1 is a ubiquitous zinc finger protein.

This year we showed that this transcription factor has multiple sizes and that different tissues show different patterns of αA-CRYBP1 sizes. Each of the sizes binds its cognate site in the αA-crystallin promoter. Preliminary immunoblotting experiments also suggest that αB-crystallin joins in the complex formed with the αA-CRYBP1 site. The 5′-flanking sequence for the αA-CRYBP1 gene has been cloned and shown to be active in transfected lens cells; the production of transgenic mice containing this promoter has been initiated.

Taken together, these experiments suggest that tissue-specific processing (either at the level of RNA or protein modifications) of the αA-CRYBP1 factor and its interaction with other proteins play a significant role in transcriptional regulation of the αA-crystallin gene. In addition, these experiments raise the possibility that crystallins themselves contribute to the regulation of crystallin genes. These results have obvious significance for the cascade of crystallin gene expression that occurs during lens development.

Comparison of the activity of bacterial chloramphenicol acetyltransferase (CAT) genes driven by various mutant promoters of the mouse αA-crystallin gene in transfection and transgenic mice have shown that each of the control elements mentioned above functions redundantly, ie, at least two must be inactivated before the promoter loses its function.

Experiments on the expression of the mouse αB-crystallin gene in lens and muscle have continued. We have used cultured C2C12 cells to study muscle expression and the SV40 T-antigen-transformed αTN4-1 cells to study lens expression. The enhancer responsible for strong muscle and weak lens expression identified last year has been studied in detail this year. Site-specific mutagenesis and DNAse I footprinting experiments have demonstrated that at

least two separate sites in the enhancer are functionally important in cultured muscle and lens cells. An additional site, the muscle regulatory function (MRF) site is only used in the muscle cells. Cotransfection experiments with cloned MyoD and myogenin cDNAs showed that the MRF site is capable of activating the enhancer in NIH 3T3 cells, which normally do not express the αB-crystallin gene.

In contrast to the muscle-specific control element, DNAse I footprinting experiments showed that the -148/-118 sequence of the αB-crystallin gene, which is downstream from the -427/-259 enhancer, is occupied only in the lens cells and not in the muscle cells, suggesting that it comprises a lens-specific regulatory element. Thus, the expression of the αB-crystallin gene in lens and muscle appears to be controlled by a combination of shared and tissue-specific control elements.

Experiments performed last year showed that the αB-crystallin gene uses an upstream (-470) transcription initiation site in lung and brain and a downstream (+1) initiation site in lens, muscle, and other tissues. No RNA splicing occurs when the upstream site is used, so the control elements in between the two start sites are represented in the 5′-untranslated sequence of the mRNA. This year both sites were shown to be recognized in a HeLa cell extract. Deletion constructs of the promoter fused to the CAT gene have been integrated into NIH 3T3 cells, forming a set of transformed cells. Preliminary experiments have indicated that the upstream promoter is required for activity of the fusion gene. Moreover, heat shock experiments have been initiated to identify the sequences required for this stress response.

Last year we identified a number of control elements in the chicken βB1-crystallin gene and a minimal functional promoter in the chicken βA3/A1-crystallin gene. This year in vivo dimethylsulfate interference experiments demonstrated that the βB1-crystallin control elements are occupied in the lens, indicating that the in vitro experiments and transfection experiments are directly relevant to the natural situation in chromatin. Function of the βA3/A1 promoter was shown by deletion and site-specific mutagenesis experiments to depend on an AP-1 site and a T-rich tract. This finding fits with our finding that AP-1 sites are prevalent throughout crystallin genes, even in invertebrates. However, further experiments are clearly necessary to identify the basis for lens specificity of this gene.

We have been investigating the expression of the two chicken δ-crystallin genes for many years. These fascinating genes are closely linked, the 5′ δ1 gene being specialized for lens expression and the 3′ δ2 gene encoding the active argininosuccinate lyase (ALS) gene. Thus, δ-crystallin is an enzyme-crystallin. In the past several years we have shown by PCR experiments that both of the chicken δ-crystallin genes are expressed in many tissues, including the heart, brain, and lens. These experiments indicated that the δ1 gene is predominantly expressed in the lens, while the δ2 gene is preferentially expressed in the heart and brain of the chicken embryo. Despite the difference in the ratio of the two δ-crystallin mRNAs, the embryonic lens always has virtually thousands of times more δ2 mRNA than other tissues, consistent with δ2-crystallin having a refractive function in lens and an enzymatic role in non-lens cells.

This year, in collaboration with Dr. Peggy Zelenka (LMDB, NEI), we reinvestigated more quantitatively with internal controls the relative expression of the two δ-crystallin genes. Particular attention was given to δ-crystallin expression in the cornea and retina, which, like the lens, are transparent ocular structures. The results showed that each embryonic tissue examined (cornea, retina, heart, brain, lens) has a characteristic ratio of expression of the two δ-crystallin genes. Moreover, we found unexpectedly that embryonic cornea, retina, and lens all contain greater amounts of δ1 mRNA than δ2 mRNA.

By 1 week after hatching, however, δ2 exceeded δ1 mRNA in the cornea and retina. In contrast to the eye tissues, the embryonic heart had a trace more δ2 than δ1 mRNA and the embryonic brain had much more δ2 than δ1 mRNA. The absolute amount of δ-crystallin mRNAs was lower in the 1-year-old chicken in all tissues examined and, in all tissues, there was more δ2 than δ1 mRNA. Thus, there is clearly a complex tissue-specific and developmental regulation of the two δ-crystallin genes in the chicken. One intriguing possibility under investigation is that although the δ1-crystallin polypeptide lacks ASL activity, it modulates enzymatic activity of the native ASL by interaction with the δ2-crystallin polypeptide in the tetrameric proteins.

This year we completed a project examining the developmental expression of carbonic anhydrase-II (CA-II) in the chicken lens. The results showed that, in contrast to its prevalence in the retina (3% of the protein), CA-II makes up only about 0.1% of the protein of the lens, an amount insufficient to make it an enzyme-crystallin in this species. Transfection of embryonic chicken lens epithelial cells with deletion mutants of the CA-II promoter fused to the CAT gene led to a gradual loss of promoter activity, consistent with an additive effect of putative *cis* regulatory elements found in many crystallin genes.

Invertebrates.—Within the past few years we have studied the crystallins of invertebrates with cellular eye lenses for several reasons. First, while the vertebrate crystallins have been studied extensively, virtually nothing was known of invertebrate crystallins, despite the abundance of invertebrate species with complex eyes. In addition, invertebrate eyes are a model of convergent evolution, and many have eyes and lenses that were apparently derived independently from those of vertebrates. Consequently, we reasoned that the most fundamental characteristics of crystallins for transparency and of their genes for high expression in the lens might be shared.

Our initial studies on squid and octopus showed that their crystallins, called S-crystallins, were derived from the detoxification enzyme glutathione S-transferase and consist of a family of at least 10 genes. We also showed last year that octopus contains a minor crystallin related or identical to aldehyde dehydrogenase (ALDH), called Ω-crystallin. This year we cloned this cDNA and established its structure. Whereas ALDH is the major crystallin of the mammalian elephant shrews, Ω-crystallin is the first crystallin common to both vertebrates and invertebrates. Another common feature of vertebrate and invertebrate lenses is their use of detoxification enzymes for refractive purposes.

This year we cloned the squid gene that encodes the active GST enzyme and showed that it is highly expressed in the digestive gland and barely expressed in other tissues, including lens. Thus, in contrast to vertebrate enzyme-crystallin recruitment, which depends on modification of gene expression to produce high concentrations of protein in the lens (often without gene duplication), the cephalopod S-crystallins recruitment involved multiple gene duplications of the parental enzyme. Furthermore, cephalopod recruitment did not use the ancestral gene for active GST for refractive purposes in the lens. A similar situation might exist for the β/γ-crystallins in vertebrates, although their nonrefractive function has not yet been discovered.

We also have explored noncrystallin proteins of squid and octopus lens by cloning, and those being analyzed include actin, tubulins, and ferritin. Of special interest is the finding that some actins and tubulins appear to be expressed selectively in these invertebrates' lenses.

Collaborating with Dr. Margaret McFall-Ngai (University of Southern California) and Dr. Ana Chepelinsky (LMDB, NEI), we have cloned abundant cDNAs of the squid light organ. The original idea was to clone the ALDH-like crystallin that is extremely abundant in the muscle-derived cellular lens of this unique organ. To our surprise, however, we found a number of myeloperoxidase-encoding cDNAs not derived from the light organ lens. Whereas the light organ is infected with luminescent bacteria, it is possible that the abundant myeloperoxidases are used for this symbiotic relationship.

A little-known fact is that cubomedusan jellyfish have extremely well-developed eyes with cellular lenses. Our previous studies have shown that these lenses contain at least three different crystallins called J1-, J2-, and J3-crystallin. Last year we reported cloning three members of the 35-kD J1-crystallin genes of cubomedusae. Their coding sequences are 85-98% identical and lack introns; by contrast, their 5′- and 3′-untranslated sequences are extremely divergent, consistent with an ancient duplication of their genes.

This year, we established, by primer extension, PCR, and Northern blotting experiments, the initiation site for transcription of the J1-crystallin genes. Their putative promoters each have TATA and putative CCAAT boxes; however, alignment of their 5′-flanking sequences has provided little clue of what they have in common for their shared high expression in the lens. One short sequence was found to be repeated in the J1A-crystallin 5′-flanking sequence and also partially present further 3′ in the 5′-flanking region of the J1B-crystallin gene. Thus, it is possible, as we recognized earlier for the αA-crystallin gene promoter of vertebrates, that regulatory sequence motifs are arranged in a modular fashion in the J1-crystallin genes. Functional studies have been initiated by using J1 promoter/CAT constructs in

transfected lens cells of vertebrates and cell-free transcription of the cloned genes in HeLa extracts. So far we have been unable to obtain clear evidence of promoter activity in these heterologous systems.

Finally, analysis of the deduced sequences for the J1-crystallin polypeptides has not revealed any striking homology with any other proteins in the database. Thus, the jellyfish crystallins fit the taxon-specific character of crystallins among vertebrates and other invertebrates. Heat denaturation studies showed that J1-crystallin is stable at 50°C but precipitates at 60°C, making it unlikely that it is a heat-shock protein. Interestingly, the smaller-molecular-weight J2/J3-crystallins appeared to be much more stable at 60°C than J1-crystallin. They will be studied further next year.

Significance to Biomedical Research and the Program of the Institute

The lens crystallins, which comprise several families of conserved proteins that are differentially expressed in the developing lens, are necessary for lens transparency. Understanding the structure, function, and evolution of these protein families and their genes contributes to our knowledge of embryonic development, eukaryotic gene expression, cell differentiation, molecular evolution, the visual system, and cataract. The finding that many crystallins are multifunctional proteins that are expressed in nonlens tissues gives these genes another dimension of general interest and has implications for metabolism, cell biology, and drug and gene therapy.

Proposed Course

The following are proposed for Fiscal Year 1993:

1. We will continue studies on identified *cis*-acting control elements of the crystallin genes, focusing on both their mechanism of action and interactions and seeking additional elements.

2. We will continue studies on αA-CRYBP1 and will attempt to clone other *trans*-acting factors that regulate the crystallin genes.

3. We will modify the squid's active GST and S-crystallin genes in an effort to identify the active sites for this ubiquitously important detoxification enzyme.

4. We will continue to test the idea that δ1-crystallin modulates enzymatic activity of native ASL.

5. We will clone and characterize the J2- and J3-crystallin cDNAs and their genes and continue functional studies on the jellyfish crystallin gene promoters.

6. Time permitting, we will continue efforts to obtain a cell-free transcription system for studying lens-preferred crystallin gene expression.

NEI Research Program

Molecular Biology

Publications

Barbosa P, Wistow GJ, Cialkowski M, Piatigorsky J, O'Brien WE: Expression of duck lens δ-crystallin cDNAs in yeast and bacterial hosts. *J Biol Chem* 266:22319-22322, 1991.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00259-03 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of the Cornea

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joram Piatigorsky	Ph.D.	Chief	LMDB, NEI
Others:	R. Andrew Cuthberson	M.D., Ph.D.	Visiting Associate	LMDB, NEI
	Xuan Li	Ph.D.	Visiting Associate	LMDB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Last year we initiated a program on the molecular biology of cornea. The major finding was that many of the abundant proteins of the corneal anterior epithelial cells of the mouse, human, kangaroo, chicken, and squid are either related or identical to metabolic enzymes, as are the lens crystallins. Indeed, many of these abundant corneal proteins are the same as those used as lens crystallins. Moreover, as with the lens crystallins, a taxon specificity was observed. In addition, we cloned the cDNA and gene for the most abundant protein in the epithelial cells of the mammalian cornea, class 3 aldehyde dehydrogenase (ALDH).

This year we made transgenic mice that carry the 5′ flanking sequence of the class 3 ALDH gene fused to a reporter gene to test whether this promoter preferentially directs gene expression in the cornea. Ten founder mice have been bred. Five ALDH and two 60 kD protein cDNAs have been cloned from the mouse cornea. The 60 kD protein is an abundant corneal protein that is highly conserved between mouse and human. A chicken corneal cDNA library also has been made in order to clone cyclophilin, an enzyme that last year was shown to comprise approximately 10% of the protein of the epithelial cells of the chicken cornea. Interestingly, this enzyme does not seem especially abundant in the mammalian cornea.

Project Description

Objectives

The objectives of this project are to identify and characterize the genes that are preferentially expressed in the epithelium and endothelium of the cornea and to study the molecular basis for their expression in this transparent tissue.

Methods

Conventional molecular biology methods of cloning, sequencing, recombinant DNA construction, transfection, and transgenic mice production are used.

Major Findings

Last year we reported the cloning of a 6.5 kbp fragment of the mouse class 3 aldehyde dehydrogenase (ALDH) gene. This year we sequenced about 2 kbp of putative 5′-flanking sequence of the class 3 ALDH gene and made recombinant DNAs containing about 1.1 kbp of 5′-flanking sequence of the gene inserted in both orientations into pNASSβ. This promoterless vector contains the β-galactosidase gene as a reporter gene. Transgenic mice have been made to test the functional ability of this putative promoter and to examine whether it may be corneal preferred. In addition to the gene, we have isolated five class 3 ALDH cDNA clones that remain to be sequenced.

We have isolated two cDNAs selected by hybridization to oligodeoxynucleotides that encode two different peptides obtained from an abundant novel 60-kD protein. Our peptide analyses indicate that this protein is highly conserved between mouse and man. One of the cDNAs is 1.5 kbp; the other, approximately 5 kbp. The shorter cDNA has been sequenced and its coding region for the peptide encoded in the oligodeoxynucleotide identified; the longer cDNA is now being sequenced.

Significance to Biomedical Research and the Program of the Institute

The molecular biology of corneal epithelium and endothelium has not advanced to the same extent as that of the collagenous stroma; consequently it needs to be investigated. The cornea is a transparent ectodermally derived tissue like the lens; thus, comparative studies between it and the lens are of special interest with respect to transparency. Moreover, because of our finding that corneal epithelial cells use a similar principle of taxon-specific gene sharing of metabolic enzymes, comparative studies on the cornea and lens, our major tissue of research, are of obvious importance from developmental and evolutionary viewpoints. Finally, the cornea is particularly accessible for gene therapy on account of its exposure to the surface and its association with numerous hereditary diseases.

Proposed Course

Several projects are proposed for Fiscal Year 1993: (1) continued analysis of the class 3 ALDH promoter, to determine its tissue preference and to identify its *cis*-acting regulatory elements; (2) cloning and characterization of the chicken cyclophilin cDNA and gene(s); (3) completion of cDNA analysis for the 60-kD mouse corneal epithelial cell protein; and (4) time permitting, cloning and characterization of the squid corneal cDNAs to determine whether they are identical to those expressed in the lens, as suggested by the protein data obtained last year.

NEI Research Program

Molecular Biology

Publications

Cuthbertson RA, Tomarev SI, Piatigorsky J: Taxon-specific recruitment of enzymes as major soluble proteins in the corneal epithelium of three mammals, chicken and squid. *Proc Natl Acad Sci USA* 89:4004-4008, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00274-02 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression in the Retinal Pigment Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert Y. Kim	M.D.	Senior Staff Fellow	LMDB, NEI
Others:	Graeme J. Wistow	Ph.D.	Visiting Scientist	LMDB, NEI

COOPERATING UNITS *(if any)*

Department of Ophthalmology, University of California, San Francisco (Ge Ming Lui, Ph.D.)

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Molecular Structure and Function

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00255-04 LMDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology and Functions of Lens Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Graeme J. Wistow	Ph.D.	Chief, Section on Molecular Structure and Function	LMDB, NEI
Others:	Robert Kim	M.D.	Staff Fellow	LMDB, NEI
	Douglas Lee	Ph.D.	NRC Fellow	LMDB, NEI
	Michael Shaughnessy	B.S.	Biologist	LMDB, NEI
	Jason Hodin	B.S.	Special Volunteer	LMDB, NEI
	Vishwas Paralkar	Ph.D.	Visiting Associate	LMDB, NEI
	Caroline Graham	B.S.	Biologist	LMDB, NEI
	Lorenzo Segovia	Ph.D.	Visiting Fellow	LMDB, NEI
	Peggy Zelenka	Ph.D.	Chief, Section on Cellular Differentiation	LMDB, NEI

COOPERATING UNITS *(if any)*

The George Washington University (F. Noonan, Ph.D.); Harvard University (S. Bruhn, B.S.; C. Cepko, Ph.D.); University of Melbourne, Australia (R. Gasser, Ph.D.)

LAB/BRANCH

Laboratory of Molecular and Developmental Biology

SECTION

Section on Molecular Structure and Function

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.41	1.00	4.41

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Crystallins, which have been recruited from stress proteins and enzymes, generally maintain dual roles without gene duplication. The gene recruitment of orthoquinone reductase/ζ-crystallin has occurred through the use of alternative promoters. We have analyzed the putative lens promoter of this gene showing that discrete regions confer lens preference, while upstream positive and negative elements confer improved lens specificity.

The rapid amplification of cDNA ends (RACE) polymerase chain reaction (PCR) technique has been used to show that δ1, δ2, and ε-crystallins use the same promoters for lens and nonlens expression. The duck τ-crystallin gene has been introduced into transgenic mice to study expression in lens. The cDNA for μ-crystallin, a mammalian taxon-specific crystallin, has been cloned. μ-Crystallin is a mammalian homologue of bacterial ornithine cyclodeaminases. Outside the lens, μ is expressed in the retina and brain, and we have cloned human retina μ. In the developing chick retina, μ is preferentially associated with photoreceptors.

We have continued to analyze the expression and function of growth factors and other proteins that have roles in lens differentiation. The coordination of lens 10K/major intrinsic factor (MIF) with differentiation has been established. Furthermore, we have found that total mouse lens extract has MIF activity, suggesting that it could play a role in lens-induced eye inflammation. The cloning of the human 10K/MIF gene is under way. We have also found that transforming growth factor beta (TGF-β) may play a role in regulating 10K/MIF expression.

Project Description

Objectives

We are investigating some of the most basic characteristics of the normal lens. In particular, we continue to identify the major structural components of the lens and their functions in both lens and nonlens tissues. We also are identifying protein markers of the differentiation process which is characteristic of the normal lens and which may be disrupted in cataract and in eye inflammation. Apart from its significance for eye research, this work is of considerable importance in the growing field of molecular evolution.

Methods

We use a wide range of modern molecular biology techniques, including RNA analysis, gene and cDNA cloning and sequencing, and expression of genes in bacterial hosts. The polymerase chain reaction (PCR) is becoming increasingly important to our work. We continue to analyze proteins by gel electrophoresis and contract microsequencing. We make extensive use of computers for analysis and molecular modeling. For gene promoter studies, we are using electrophoretic mobility shift analysis, "footprinting," transfection into cultured cells, and construction of transgenic mice.

Major Findings

Gene recruitment: enzyme crystallins.—The putative lens promoter for guinea pig ζ-crystallin has been cloned and expressed. Promoter fragments up to -180 bp from the transcription start site have no activity in lens-derived N1003A cells or in NIH 3T3 cells. However the -380 bp promoter, which contains T-rich stretches and other elements common to other crystallin promoters, is an active promoter in N1003A cells although it is still inactive in fibroblast 3T3 cells. Increasing the promoter to -527 bp causes a drop in activity, revealing a negative element. A further increase to -751 bp causes a very large increase in activity although it is again inactive in 3T3 cells. A -1500 bp construction is somewhat less active. The -380 and -751 bp promoters were tested in transgenic mice. The shorter promoter is highly lens preferred, but it has weak expression in the brain. The longer fragment is apparently lens specific. Thus the ζ-crystallin gene has been recruited through a lens-specific promoter that comprises several positive and negative elements working together.

The lens promoter of ζ-crystallin lies in what otherwise would be the first intron of the gene. This is an appealing model for recruitment because it does not disrupt the existing enzyme promoter; however, it does not appear to be a general model. We used the RACE PCR method to clone the 5′ ends of mRNA for three taxon-specific crystallins, δ1, argininosuccinate lyase/δ2, and lactate dehydrogenase B/ε, from lens and nonlens tissues of embryonic duck. In all three cases, identical 5′ ends were found in lens and in the brain and liver (for δ1 and δ2) or heart (for ε). Thus, these three genes, like τ-crystallin described earlier, use the same promoter for both lens and nonlens expression.

We constructed a line of transgenic mice containing the entire duck α-enolase/τ-crystallin gene with 5′- and 3′-flanking regions and all introns. Full-sized duck α-enolase mRNA was expressed in the transgenic mice with the same pattern as the endogenous mouse α-enolase isozyme. Although there was no evidence of tissue preference, the concentration of enolase increased markedly in transgenic lens as well as in other tissues. Nonetheless, transgenic lenses were transparent, and the animals were normal in appearance.

The increase in enolase levels in the transgenic lens mimics the stepped increase that might occur in the early stages of enzyme-crystallin recruitment. These results demonstrate that lens transparency is sufficiently robust to be refractory to some increase in metabolic enzyme concentration without the need for compensatory adaptation. Circumstantial evidence has linked α-enolase expression with the oncogene c-myc. We found a possible c-myc binding site in the α-enolase/τ-crystallin gene promoter. Collaborating with Dr. Zelenka's group, we showed that c-myc is a positive regulator of the duck τ-crystallin gene, the first example of a target gene for this proto-oncogene.

The major component of the eye lens in several Australian marsupials is μ-crystallin. The complete sequence of kangaroo μ-crystallin has now been obtained by cDNA cloning. The predicted amino-acid sequence shows similarity with ornithine cyclodeaminases (OCD) encoded by the tumor-inducing (Ti) plasmids of *Agrobacterium tumefaciens*. Until now, neither OCD nor any structurally related enzymes have been observed in eukaryotes. Northern

analysis of kangaroo tissues shows that μ-crystallin is abundantly expressed in lens; but outside the lens it is preferentially expressed in neural tissues, retina, and brain. This pattern of expression and relationship to an enzyme involved in unusual amino-acid metabolism raises the possibility that mammalian μ-crystallin may have a role in the metabolism of excitatory amino acids, while an alternative or additional role in lens could lie in osmoregulation. Human retinal μ-crystallin has been cloned and sequenced. In the developing chicken retina, μ-crystallin detected by immunofluorescence is preferentially associated with the photoreceptors, perhaps indicating an unusual pathway for ornithine metabolism in the retina and possibly related to the problems of gyrate atrophy.

Growth factors and lymphokines in the lens.—A discrete 10-kD polypeptide (10K) is expressed from early stages in the embryonic chicken lens. Because of its potential as a marker for lens cell development, we identified chicken 10K and its homologues from mouse and human lenses by protein sequencing and cloning. Surprisingly, the lens 10K protein appears to be identical to a lymphokine, macrophage migration inhibitory factor (MIF), which was originally identified in activated human T cells. Using microdissection and PCR techniques, we found that expression of 10K/MIF is strongly correlated with cell differentiation in the developing chicken lens. This finding suggests that proteins with MIF activity may have roles beyond the immune system, perhaps as intercellular messengers or as part of the machinery of differentiation itself. Indeed, partial sequencing of other small lens proteins has identified another MIF-related protein (MRP8) in calf lens.

The relatively abundant expression of MIF in lens may have clinical significance, with the possibility of involvement in ocular inflammations such as endophthalmitis and uveitis that may follow damage to the lens. Preliminary data suggest that total lens extracts do indeed have MIF activity. Recombinant mouse 10K/MIF is being produced in *Escherichia coli*, and the human gene is being cloned in our Section.

The important growth factor TGF-β1 has been cloned from mouse lens. In cell culture experiments and in isolated rat lens epithelium we have demonstrated that TGF-β1 can regulate the expression of 10K/MIF, which we have shown to be a marker for lens cell differentiation. We are investigating the interplay of these and other growth factors in normal lens and in several transgenic and gene-knockout models, including mice carrying most of the HIV-1 genome and others in which the TGF-β1 gene has been eliminated.

Significance to Biomedical Research and the Program of the Institute

We are discovering fundamental mechanisms in the processes by which complex tissues are organized and develop. Along the way, we have discovered a novel enzyme that has possible significance in neural tissues, particularly retina, and which may be a useful pharmacological target. We also have discovered important markers for cellular differentiation that may have clinical relevance in ocular inflammation.

Proposed Course

Work on this project will continue with (1) examination of the molecular mechanisms for lens preferred expression and for gene recruitment, (2) engineering of crystallins and other lens components in transgenic animals to mimic gene recruitment and constructing specific cataract models, and (3) exploration of the molecular biology and function of lymphokines and growth factors expressed in lens and examination of their role(s) in eye disease.

NEI Research Program

Cataract—Molecular Genetics

Publications

Barbosa P, Wistow GJ, Cialkowski M, Piatigorsky J, O'Brien WE: Expression of duck lens δ-crystallin cDNAs in yeast and bacterial hosts: δ2-crystallin is an active argininosuccinate lyase. *J Biol Chem* 266:22319-22322, 1991.

Doniger J, Landsman D, Gonda MA, Wistow G: The product of unr, the highly conserved gene upstream of N-ras, contains multiple repeats of the cold-shock domain (CSD), a putative DNA-binding motif. *New Biologist* 4:1-7, 1992.

Kim RY, Gasser R, Wistow GJ: μ-crystallin is a mammalian homologue of *Agrobacterium* ornithine cyclodeaminase and is expressed in human retina. *Proc Natl Acad Sci USA* 89:9292-9296, 1992.

Kim RY, Wistow GJ: The cDNA RPE1 and monoclonal antibody HMB-50 define gene products

preferentially expressed in retinal pigment epithelium. *Exp Eye Res* 55:657-662, 1992.

Lee DC, Gonzalez P, Rao PV, Zigler JS Jr, Wistow GJ: Carbonyl-metabolizing enzymes and their relatives recruited as structural proteins in the eye lens, in Weiner H (ed): *Enzymology and Molecular Biology of Carbonyl Metabolism*, Vol 4, in press.

Roquemore EP, Dell A, Morris HR, Panico M, Reason AJ, Savoy L-A, Wistow GJ, Zigler JS Jr, Earles BJ, Hart GW: Vertebrate lens α-crystallins are modified by O-linked N-acetylglucosamine. *J Biol Chem* 267:555-563, 1992.

Shaughnessy M, Wistow G: Absence of MHC gene expression in lens and cloning of dbpB/YB-1, a DNA-binding protein expressed in mouse lens. *Curr Eye Res* 11:175-181, 1992.

Wistow G: Lens crystallins: A model system for gene recruitment. *Methods Enzymol*, Vol 224, in press.

Laboratory of Ocular Therapeutics

Report of the Chief, Laboratory of Ocular Therapeutics

Peter F. Kador, Ph.D.

The Laboratory of Ocular Therapeutics (LOT) focuses on the development, evaluation, and mechanism of action of new ophthalmic drugs to treat eye diseases. The LOT research team is examining aldose reductase inhibitors (ARI's) and anticataract agents. The development of clinical ARI's has evolved primarily around analogs derived from related carboxylic acids and spirohydantoins. Unfortunately, these compounds have demonstrated potentially significant problems, including low biological half-lives, poor tissue penetrations, and nonspecific side effects that range from skin rash to the induction of liver enzymes.

Pursuing more effective and less toxic therapies, LOT scientists discovered an inhibitor unrelated to previously reported ARI's. Studies are being conducted to characterize this inhibitor by biochemical, pharmacological, and computer molecular design techniques. In addition, drug distribution studies in ocular tissues are being conducted using noninvasive magnetic resonance imaging (MRI) techniques. Other studies are designed to elucidate the specific mechanism(s) by which aldose reductase initiates diabetic complications.

In studies utilizing galactose-fed dogs, LOT investigators have established the progression of retinal changes associated with diabetic retinopathy and their dose-dependent arrest with ARI's. Studies are now focused on the observation that long-term galactose-fed dogs develop advanced retinal changes similar to clinical proliferative retinopathy. In addition, investigators are analyzing the specific role of aldose reductase in neuropathy, thyroid changes, and immunological system responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00003-19 LOT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology of Ocular Complications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter F. Kador	Ph.D.	Chief	LOT, NEI
Others:	Julia Derr	B.A.	Biologist	LOT, NEI
	Jun Inoue	M.S.	Pharmacy, Special Volunteer	LOT, NEI
	Yong Lee	Ph.D.	Staff Fellow	LOT, NEI
	Anita Bartoszko-Malik	Ph.D.	Visiting Fellow	LOT, NEI
	Kazuhiko Mori	M.D., Ph.D.	Visiting Fellow	LOT, NEI
	Matteo Schaffhauser	Ph.D.	Visiting Fellow	LOT, NEI
	Yukio Takahashi	M.D., Ph.D.	Visiting Associate	LOT, NEI
	Tomoyuki Terada	Ph.D.	Special Volunteer	LOT, NEI

COOPERATING UNITS *(If any)*

National Heart, Lung and Blood Institute, Bethesda, MD (Robert Balaban, Ph.D.); Ohio State University College of Pharmacy, Columbus, OH (Duane Miller, Ph.D.)

LAB/BRANCH

Laboratory of Ocular Therapeutics

SECTION

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.6	6.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Events leading to the onset of various ocular complications are being investigated. Specifically, the studies focus on the role of the enzymes aldose reductase and aldehyde reductase in the onset and progression of retinopathy, cataract, keratopathy, pupil function changes, and iris and ciliary process structural changes associated with diabetes and galactosemia. In addition, methods for either delaying or preventing the onset and progression of these complications through the pharmacological control of these enzymes are being developed.

Also being studied are events leading to the formation of several types of cataracts as well as methods for controlling the onset of these cataracts through pharmacological intervention.

PHS 6040 (Rev. 5/92)

Project Description

Objectives

This research is designed to gain insight into the mechanisms by which polyol-induced ocular diabetic complications and cataracts are formed and to develop methods for their regulation.

Methods

Diabetes can be experimentally induced in animals through the injection of streptozotocin. Diabetes-related complications linked to the sorbitol pathway can also be induced in animals such as rats and dogs by feeding them a galactose-enriched diet. Cataract formation and clinical retinal changes in experimental animals can be monitored through fundus photography. Biochemical studies used to purify enzymes include column chromatography, polyacrylamide gel electrophoresis (PAGE), isoelectric focusing, chromatofocusing, and high-pressure liquid chromatography (HPLC). Polyol levels were determined by gas-liquid chromatography (GLC). Immunological analyses include the use of enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), Western blots, and immunohistochemical techniques employing the coupled antibody DAB-PAP technique. Computational methods for enzyme analysis, inhibitor structure-activity studies, and pupil-function changes require the use of the NIH PROPHET computer system and Charm and Quanta computer systems from Molecular Design.

Major Findings

Biochemical studies.—Recent studies have demonstrated that the dog is an excellent animal model for investigating ocular diabetic complications. Cataracts in the dog develop in a manner similar to that of cataracts in humans with diabetes. They are characterized by formation of anterior and posterior superficial cortical opacities, with the posterior polar region more advanced. Furthermore, the dog develops advanced retinal changes similar to those observed clinically in human diabetics. Polyol formation initiated by the NADPH-dependent enzyme aldose reductase has been demonstrated to initiate these lens and retinal changes.

Investigations of NADPH-dependent reductases in the dog lens, retina, and thyroid have revealed the presence of a third, labile NADPH-dependent enzyme in dog tissues that is not inhibited by aldose reductase inhibitors. This enzyme has not been observed in either human or rat lens and kidney. Its stability increases with 0.2 to 0.3 M sodium chloride, has a higher molecular weight than either aldose and aldehyde reductases on gel filtration. Kinetic study indicates that it primarily utilizes DL-glyceraldehyde as substrate and that its activity with either D-glucuronate or aldose sugars (D-glucose, D-galactose, and D-xylose) is negligible. It is not inhibited by aldose reductase inhibitors. On the basis of these studies, the enzyme has been assigned the tentative name "glyceraldehyde reductase." Interest in the enzyme stems from the fact that its utilization of NADPH may contribute to changes in cellular redox potentials.

Studies on defining the inhibitor site(s) of aldose reductase and aldehyde reductase continue with the evaluation of a number of Michael addition affinity-labeled aldose reductase inhibitors. In addition, the pharmacophore requirements of the inhibitor site and the location of reactive nucleophilic and electrophilic sites are being refined through molecular modeling. New, potent aldose reductase inhibitors have been uncovered, and patent application has been made.

Lens studies.—Posterior migration of lens epithelial cells, believed to be a common phenomenon in cataracts, has been observed in human lenses from diabetics with posterior subcapsular cortical opacities. However, similar epithelial cell migration has not been reported in the various rodent sugar cataract models that form via a common osmotic mechanism linked to the aldose reductase-associated excess lenticular production of sugar alcohols. Unlike what occurs in human lenses, sugar cataract formation in young rats occurs rapidly; their lens opacities form predominantly in the anterior cortical regions. In addition, rat lenses contain higher levels of aldose reductase. Recent studies in the dog, an animal model whose lenses contain levels of aldose reductase activity more similar to those of humans than of rats, indicate that dog lenses also form aldose reductase-linked sugar cataracts. However, their formation in the dog is slower than in the rat, and they progress to form anterior and posterior superficial cortical opacities, with the posterior polar region more advanced.

Histological studies were conducted on 44 cataractous lenses obtained from young male beagle dogs fed a 30% galactose diet for 19-60 months. Exami-

nation of toluidine blue-stained sections (2.5 μm) of methacrylate-embedded lenses revealed epithelial cell migration to the posterior pole in 14 of 44 lenses. All lenses with migration displayed a separation of the posterior Y suture and liquefaction. In addition, we observed a higher incidence of lens-fiber cell nuclei disarrangement and separation of the anterior Y suture than in lenses from similar dogs not demonstrating migration. These lesions are similar to those observed in humans, supporting the contention that the dog is a better animal model than the rat for investigating human sugar cataracts.

In previous studies we established through in vitro dog lens experiments that aldose reductase activity can be accurately assessed by measuring the conversion of 3-deoxy-3-fluoroglucose to 3-deoxy-3-fluorosorbitol by nuclear magnetic resonance spectroscopy (^{19}F NMR). This work has now been extended to the in vivo evaluation of aldose reductase activity in rabbit lenses. Initial spatial coordinates for lenses were calculated from ^{1}H-images determined on a 2.0 Tesla GE Omega-CSI spectrometer of the eyes of normal rabbits (2.7 kg) anesthetized with halothane. The spectral localization with optimal pointspread function (SLOOP) technique was then used with a proton decoupler to measure the accumulation of sorbitol in the lens. We used a double spin-echo sequence with selective excitation and refocusing pulses and with optimized phase-encoding gradient pulses that had 1 sec repetition times and 25 msec echo times.

Fluorinated glucose was administered to the rabbit either by infusing 40 ml of 150 mM 3-deoxy-3-fluoroglucose into the right carotid artery at a rate of 10 ml per hour or by topical application of soft contact lenses hydrated in saturated 3-deoxy-3-fluoroglucose over a 48-hour time period. In SLOOP experiments, 3-deoxy-3-fluorosorbitol was only observed in spectra of the anterior portion of the lens, not in the nucleus or anterior chamber. These results demonstrate the good localization properties of the SLOOP technique and the usefulness of this method for in vivo determination of aldose reductase activity.

Retinal studies.—We have observed that vascular changes associated with diabetic retinopathy can be produced experimentally in beagle dogs fed a 30% galactose diet. In addition, we have reported in prevention studies in which dogs were fed a galactose diet for up to 36 months that the concomitant administration of the aldose reductase inhibitors can affect the onset and progression of the appearance of pericyte ghosts (19-21 months), acellular capillaries (24 months), microaneurysms (27 months), and intraretinal hemorrhages (33 months) in a dose-dependent manner. These changes, observed in isolated trypsin-digested preparations, are initiated by the aldose reductase-catalyzed reduction of galactose to galactitol.

To determine whether the progression of retinal changes can also be arrested through reduction of galactitol production at early stages of retinal lesion development, we conducted an intervention study using 60 young male beagles. Ten dogs fed a control diet containing 30% non-nutrient filler served as controls while 50 dogs were fed a 30% galactose diet. From the galactose-fed group, 15 dogs were switched to normal diet (Purina chow) after 24 months (a period during which pericyte ghosts and acellular capillaries are present); an additional 15 dogs were switched after 30 months (a period in which microaneurysms are present). Twenty-four months after the initiation of galactose feeding, four to five eyes from each experimental group and two to three eyes from the control group were enucleated at 6-month intervals. Investigations of those eyes' retinal vessels, isolated by trypsin digestion, revealed no apparent reversal of retinal lesions. Nevertheless, differences in the progression of retinal lesions between the galactose-fed and reversed groups became evident 12-18 months after reversal.

Asteroid hyalitis (hyalosis) is characterized by minute particles considered to be liquid crystals of lipids suspended without orderly arrangement in the vitreous. Although rarely causing visual impairment, they have recently been incriminated in hindering visualization of the retina for diagnostic purposes and in predisposing to refractive errors in the selection of intraocular lenses for use following cataract extraction. The etiology of these bodies has not been definitively identified; however, 27-70% of patients with asteroid hyalitis also have diabetes mellitus. Humans with hypercholesterol also have a greater incidence of asteroid hyalitis than an at-risk population. Systemic disease has also been incriminated as a cause of asteroid hyalitis.

In dogs fed a long-term 30% galactose diet, we have observed that the formation of asteroid hyalitis begins 42-48 months after the onset of galactose feeding. Ten dogs fed 30% galactose for 72 months

were more closely examined and compared with five age- and sex-matched dogs fed a control diet containing 30% fiber. Nine of the ten dogs whose lenses allowed visualization of the fundus demonstrated severe asteroid hyalitis, particularly in the dependent part of the vitreous. In the dog whose lens precluded ophthalmoscopic examination, we found severe asteroid hyalitis when the eye was examined after euthanasia. None of the control dogs manifested asteroid hyalitis. All of the dogs on the galactose diet had various degrees of retinopathy identified as those seen in humans with ocular complications of diabetes mellitus.

We believe this model will provide an opportunity to study the etiopathogenesis of asteroid hyalitis and possible methods for its treatment.

Significance to Biomedical Research and the Program of the Institute

Loss of vision from cataract and diabetic retinopathy are significant; therefore, methods for the pharmacological control of these ocular complications are needed. We have developed an animal model that demonstrates advanced retinal vessel changes that are virtually clinically and histologically identical to those observed in advanced diabetic retinopathy. Our present studies in dogs demonstrate for the first time that loss of retinal pericytes, associated with aldose reductase, initiates retinal changes associated with both background and advanced diabetic retinopathy. The results show that administration of aldose reductase inhibitors in prevention studies can ameliorate the loss of pericytes and subsequent microaneurysms and retinal hemorrhages in a dose-dependent manner. The successful development of noninvasive methods for monitoring aldose reductase activity by NMR procedures may have direct impact on ongoing and planned clinical trials in which this procedure could serve as a quantitative indicator of drug efficacy. Cataract is also one of the major causes of blindness in the developing world; loss of vision due to cataract is one of the major health problems of both diabetic and aging populations in the United States.

Proposed Course

These studies will be continued. Recently discovered aldose reductase inhibitors will be pharmacologically evaluated and developed. The inhibitor site will be further probed through the use of affinity labe'ls so that more potent and specific inhibitors may be developed. Studies of the mechanisms through which aldose reductase induces diabetic complications in various tissues will be continued.

NEI Research Program

Retinal Disease—Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00275-01 LOT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of NADPH-Dependent Reductases in Ocular Complications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sanai Sato	M.D.	Visiting Scientist	LOT, NEI
Others:	Shigeru Fukase	M.D.	Special Volunteer	LOT, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Ocular Therapeutics

SECTION

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In diabetes, the incidence and progression of complications associated with hyperglycemia have been linked to the production of sugar alcohols (polyols). The relationship between aldose reductase, other closely related NADPH-dependent reductases, and polyol production is being investigated to define the biochemical mechanism(s) that initiate the onset of these complications and to develop specific pharmacological methods of intervention.

Project Description

Objectives

This project is designed to assess the importance of aldose reductase versus aldehyde reductase in polyol accumulation in various tissues in which diabetic changes occur. The research will provide information important for the development of aldose reductase inhibitors.

Methods

Biochemical techniques include gel filtration and affinity chromatography, electrophoresis, immunoblotting, and isoelectric focusing on high-pressure liquid chromatography (HPLC). Gas chromatography is used to identify and quantitate sugars. In vitro techniques include the culture of retinal capillary pericytes and endothelial cells, leukocytes, and fibroblasts. Results, including enzyme kinetic evaluations, are calculated using the NIH PROPHET computer system.

Major Findings

Kidney.—The production of polyols by both aldose and aldehyde reductases has been confirmed by in vitro experiments in which the newly produced polyol peaks were demonstrated by gas chromatography after the incubation of purified rat kidney enzymes with aldoses in the presence of an NADPH-generating system containing citric acid and citrate dehydrogenase. Polyol production by aldehyde reductase was confirmed in vivo in galactosemic rats by demonstrating that aldose reductase-specific inhibitors reduced the polyol level in the aldehyde reductase-rich kidney cortex less than nonspecific inhibitors. These results strongly suggest that the inhibition of both aldose reductase and aldehyde reductase is required to ameliorate diabetic nephropathy. Comparison of rat and human kidneys has shown a similar aldose reductase versus aldehyde reductase distribution. The kinetic properties of the human enzymes are similar to those of the rat.

Retina.—Evaluation of cultured human retinal pigment epithelial (RPE) cells has revealed that these cells possess both aldose and aldehyde reductases. Aldose reductase predominates in this tissue whereas the amount of aldehyde reductase is negligible. The data indicate that, as in the lens, only aldose reductase contributes to polyol accumulation, which causes several dysfunctions of the RPE associated with diabetes.

Leukocytes.—Both polymorphonuclear cells and mononuclear cells contain aldose and aldehyde reductases. Examination of dog leukocytes revealed that aldehyde reductase is the predominant enzyme in these cells. Although aldose reductase activity was minimal, polyol accumulation was detected in both polynuclear and mononuclear cells. The sorbitol pathway in neutrophils was confirmed through nuclear magnetic resonance (NMR) studies with 3-fluorodeoxyglucose (3FDG) in which 3FDG was converted to 3-fluorodeoxyfructose through 3-fluorodeoxysorbitol. These results suggest that aldehyde reductase contributes to polyol accumulation in these cells under hyperglycemic and/or galactosemic conditions.

Significance to Biomedical Research and the Program of the Institute

Despite the establishment of insulin therapy, significant numbers of persons with diabetes are at risk for debilitating complications associated with this disease. These complications can lead to loss of vision, neuropathy, and kidney failure. Although experimental studies demonstrate that excess polyol production is linked to the onset and progression of many of these complications, understanding the interrelationship of NADPH-reductases and polyol production is essential for the development of specific pharmacological methods of intervention.

Proposed Course

These studies will be continued. The presence of NADPH-dependent enzymes and their ability to produce polyols will be evaluated in target cells at sites of diabetic complications. Targets for investigation will be retinal capillary pericytes and endothelial cells, fibroblasts, key cells associated with proliferative changes, both mesangial and endothelial cells of the kidney glomerulus, and leukocytes.

NEI Research Program

Diabetic Complications and Cataract Research

Publications

Carper D, Sato S, Old S, Chung S, Kador PF: In vitro expression of human placental aldose reductase in Escherichia coli. *Adv Exp Med Biol* 284:129-139, 1991.

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Laboratory of Retinal Cell and Molecular Biology

Report of the Chief, Laboratory of Retinal Cell and Molecular Biology

Gerald J. Chader, Ph.D.

The mission of the Laboratory of Retinal Cell and Molecular Biology (LRCMB) is to uncover new aspects of functioning of the retinal pigment epithelium (RPE) complex in health and disease. The focus is on elucidating new genes and biochemical mechanisms and learning the underlying causes of ocular diseases. Most of the approaches taken are molecular biological and/or candidate gene approaches. Where possible, the LRCMB will seek clinical collaboration to translate the findings from laboratory to clinic.

Most of the work of the lab members is within the following NIH Strategic Initiatives and NEI Priorities: (1) molecular medicine, (2) gene research and gene therapy, and/or (3) research of high clinical relevance.

Within the Laboratory, three areas are emphasized:

- Immunopathology of uveoretinitis
- Establishment of an ocular gene center focusing on genetic research and candidate genes for retinal diseases
- Transgenics and gene therapy for retinal diseases.

Immunopathology of Uveoretinitis

Several LRCMB investigators are involved in immunogenetic and immunopathogenic studies. Most if not all of the work is performed in collaboration with investigators in the Laboratory of Immunology (LI); its focus is on the induction of experimental autoimmune uveitis (EAU). The areas of concentration are several.

Immunopathology.—Collaborative work with Dr. Igal Gery (LI) has established an excellent animal model for studying human uveitis. Continued dissection of the immunopathological site(s) of the interphotoreceptor retinoid-binding protein (IRBP) in the Lewis rat and in the human has the final goal of controlling or preventing the disease process in man.

Immunogenetics.—Collaborative work with Dr. Rachel Caspi (LI) has established the IRBP-mouse model for EAU as very useful for studying the genetics of the disease and its relapsing characteristics, for example.

Antigen presentation.—Collaborative work with Drs. Gery Marc de Smet, and Robert Nussenblatt (LI) has demonstrated the presence of a 70-kD B-cell surface protein that specifically binds the major 23-amino acid immunopathological determinant of IRBP. This cell surface protein may function as a molecular chaperon in antigen presentation.

S-antigen (S-Ag)-responsive element.—A major new discovery is that the S-Ag promoter has a steroid hormone consensus site. This, the first reported visual cycle protein, opens a new aspect of vision research involving the effects of steroid and thyroid hormones on the expression of visual proteins. Furthermore, it has direct clinical implications (eg, glucocorticoid treatment), which will be pursued in collaboration with Drs. Nussenblatt and Chi-Chao Chan.

Genetic Research and Genes for Retinal Diseases

The main focus of most of the LRCMB scientists is the identification of specific genes that cause ocular disease. To accomplish this goal, a consortium of LRCMB investigators has been established to form an NEI Gene Center. Our purpose is to develop a chromosomal map of eye-specific genes involved in ocular diseases. There are four areas of concentration.

Fatty acid and tubulin defects in retinal degeneration.—Fatty acid uptake and metabolism in Bietti's crystalline retinopathy and a tubulin acetylation defect in a form of Usher's syndrome are being investigated in collaboration with Dr. Muriel Kaiser-Kupfer (Ophthalmic Genetics and Clinical Services Branch) in the hope of elucidating the specific defects. Significant progress has been made in pinpointing the metabolic problems.

Candidate genes for hereditary retinal diseases.—Genes expressed predominantly or exclusively in ocular tissues are being identified by subtractive cloning. Retina-specific genes and genes located on

the short arm of the X chromosome are of specific interest. These genes are being chromosomally localized to see whether they are disease linked. Concurrent genomic cloning and sequencing is being done to generate appropriate polymorphisms and/or microsatellite repeats are generated. Using patient material, Dr. Bruce Pfeffer, an expert in tissue culture, will immortalize cell lines to facilitate subsequent laboratory experiments.

RPE-specific genes.—Although the RPE is absolutely essential in the photoreceptive process, very little is known about the specific complement of genes in the RPE. In our major effort to clone genes unique to RPE and its functioning, the highlight is the isolation of a new, specific 65-kD protein from the human RPE. Cloning of the gene for the new protein, which has potential immunologic importance, allows the study of tissue-specific expression. This gene is the first RPE-specific gene to be reported and characterized.

Unique photoreceptor processes.—Diseases unique to vision are probably caused by problems with processes that are unique to the eye. Several Laboratory members have focused on identifying and characterizing proteins and enzymes that seem to be critical in functioning of the retina/RPE complex.

The molecular biology (eg, expression control) of two proteins of the phototransduction cascade, S-Ag and phosducin, is under intensive study. Both proteins are rod outer segment-specific and interact with visual cycle components (eg, opsin), but their actual function remains controversial. cDNAs and genomics for S-Ag and phosducin have been cloned and thoroughly analyzed, allowing for current advances in our understanding of the expression, function, and pathology of the gene products.

IRBP is also a critical link in the chain of enzymes and proteins that make up the visual cycle and visual transduction but, because of the huge size of the gene, it would be very difficult to sequence in potential disease cases. In an attempt to get around this problem, we are cloning the *Drosophila* (fruit fly) homolog of the human IRBP gene. It has now been mapped to an area of the *Drosophila* genome that is rich in ocular mutants. The idea of this work is to see whether the fly IRBP maps to a known eye-disease locus within the *Drosophila* genome. Then, knowing the characteristics (eg, erg) of the disease in the fly, we can pinpoint a specific human population with similar characteristics and examine the gene for defects in specific human families.

Many retinal degenerations are manifest in very early development and thus may involve autocrine or paracrine growth factor defects. We have identified a novel neurotrophic protein, pigment epithelium-derived factor (PEDF), that is synthesized by fetal human RPE cells. PEDF may be critical in the development of retinal neurons. At very low concentration, PEDF causes the extension of elaborate neuronal processes from cultured retinoblastoma cells in culture. An important possibility is that, as with the Royal College of Surgeons rat, a defect in the PEDF of the RPE could cause a retinal degeneration. Also being evaluated is the clinical use of PEDF in retinal transplantation in a rabbit model system with Dr. Manuel del Cerro. The molecular biology of this potentially very important neurotrophic agent is now being studied for application to retinal dysfunctions.

Transgenics and Gene Therapy of Retinal Diseases

A growing focus of the LRCMB is on the establishment of transgenic models of retinal disease and on possible modalities of therapy, such as the use of ribozymes.

Transgenic studies.—The IRBP and S-Ag genes are the best studied retinal genes other than rhodopsin. The *cis*-acting elements that control IRBP and S-Ag promoters, and thus their protein expression, are being studied in transfected human cells and in transgenic mice. Transgenic studies, in particular, will uncover factors controlling gene activation in the embryonic period, specifically in the photoreceptor cell.

Gene analysis systems in transgenic mice and in transient transfections in cultured human retinoblastoma cells using a chloramphenicol acetyltransferase or CAT assay have been established for IRBP. Almost 2 kb of IRBP 5′ flanking region has been thoroughly examined to date; similar progress has been made with the S-Ag. Promoter (+ or -) and enhancer elements necessary for normal and abnormal expression are being defined through target mutagenesis studies. Tissue- and stage-specific elements, including TATA and CAAT boxes, will be precisely defined as to retinal and/or pineal expression. This work is important because specific molecules can be precisely gene-targeted to the retina.

Gene therapy.—Ribozymes are specifically constructed RNA species that can control protein expression within cells. By linking these simplified gene forms to appropriate promoters and utilizing a suitable transfer vector, we can construct new therapeutic modalities. Gene therapy can then be planned for the treatment of now unmanageable autosomal dominant disorders.

Following the design of ribozyme constructs for IRBP, they are being studied in a transfected human retinoblastoma cell system, concurrently with rearing transgenic mice to determine whether an retinitis pigmentosa-like condition can be mimicked through downregulating IRBP synthesis. Once perfected, ribozymes should be useful not only with IRBP-related retinopathies but in conditions such as diabetic retinopathy and retinopathy of prematurity in which the disorders probably involve overexpression of normal proteins such as growth factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00070-15 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Vitamin A and Ocular Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barbara Wiggert	Ph.D.	Head, Section on Biochemistry	LRCMB, NEI
Others:	Kalpana Rengarajan	Ph.D.	Visiting Fellow	LRCMB, NEI
	R. Krishnan Kutty	Ph.D.	Senior Staff Fellow	LRCMB, NEI
	Todd Duncan	M.S.	Biologist	LRCMB, NEI

COOPERATING UNITS *(if any)*

U. Lund, Sweden (T. van Veen, Ph.D.); U. Illinois Coll. of Med., Chicago (D. Pepperberg, Ph.D., T.-I. Okajima, Ph.D., H. Ripps, Ph.D.); U. Penn. Sch. Vet. Med., Philadelphia (G. Aguirre, D.V.M., Ph.D.); Med. U.S.C. (R. Crouch, Ph.D., S. Hazard, Ph.D.); SLU Inst. F. Kir, Sweden (K. Narfstrom, D.V.M., Ph.D.); U. Hosp., Utrecht, The Netherlands (B. Zonnenberg, M.D., Ph.D.); U. Maryland Med. Sch. (M. Rodrigues, M.D., Ph.D.)

LAB/BRANCH

Laboratory of Retinal Cell and Molecular Biology

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	1.1	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on factors regulating interphotoreceptor retinoid-binding protein (IRBP) gene expression in the mouse eye demonstrated that light deprivation markedly downregulated IRBP message in developing and adult mice. However, IRBP protein levels were not decreased, suggesting regulation by some sort of feedback control mechanism.

Studies on retinoid levels in the eyes of the C57vit/vit mouse model of retinal degeneration showed markedly elevated levels of retinyl palmitate as well as some elevation of levels of 11-*cis* retinal, all-*trans* retinal and all-*trans* retinol at 6 weeks of postnatal development, suggesting a malfunction in some aspect of the visual cycle in this mutant.

An effect of IRBP on the process of 11-*cis* retinal formation has been suggested by studies of retinoid processing in the toad retinal pigment epithelium (RPE) eye cup which show that both the molar amount and the specific radioactivity of 11-*cis* retinal increase with increasing IRBP concentration.

Analysis of endogenously bound ligands of *Drosophila* head retinoid-binding protein demonstrated the presence of a possible isomer of retinol and both covalent and noncovalently bound fatty acids. We also characterized the binding affinities of retinol and fatty acid.

Project Description

Additional Personnel

Igal Gery	Ph.D.	Head, Section on Experimental Immunology, LI, NEI
Rachel Caspi	Ph.D.	Visiting Associate, LI, NEI

Cooperating Units

Medical College of Georgia (S. Smith, Ph.D.); Emory Eye Center (J. Nickerson, Ph.D.)

Objectives

The purpose of this research project is to investigate the role of specific retinoid-binding proteins such as interphotoreceptor retinoid-binding protein (IRBP) in mediating the action of retinoids in both normal and diseased ocular tissues.

Methods

Affinity chromatography, high-performance liquid chromatography (HPLC), SDS-polyacrylamide gel electrophoresis, Western blotting, Northern blotting, slot-blotting, and the enzyme-linked immunosorbent assay were used to study retinoid-binding proteins.

Major Findings

In a study investigating the possibility that light or the lack of light stimulation may play a role in regulating gene and/or protein expression of IRBP, we found an 80-90% reduction in IRBP message in the eyes of mice reared from birth to postnatal day 14 (P14) under dim red lighting conditions, compared with normal control animals reared under cyclic light. At the same time, immunochemical quantitation of IRBP showed no reduction in protein levels, and immunocytochemistry demonstrated normal interphotoreceptor matrix of mice reared under dim red lights. When adult mice were placed under dim red light for 14 days, there was also a marked decrease in IRBP message in their eyes and no reduction in IRBP. These results may indicate both a lower IRBP turnover rate when bleaching of rhodopsin does not occur and a regulation of IRBP gene expression by some sort of feedback control mechanism.

In a study of retinoid processing in the toad retinal pigment epithelium (RPE) eyecup as a function of the amount of extracellular IRBP available to receive 11-*cis* retinal from the RPE, both the molar amount and the specific radioactivity of 11-*cis* retinal in the extracellular medium increased with increasing IRBP concentration. These results suggest an effect of IRBP on the process of 11-*cis* retinal formation during the visual cycle.

We studied the C57vit/vit mouse, which has a progressive retinal degeneration in which photoreceptor cells are lost slowly over a year's time and RPE cells are unevenly pigmented. HPLC analysis of its retinoids demonstrated levels of retinyl palmitate approximately 2.5 times greater in the eyes of 6-week affected mice compared with those of congenic controls. Levels of 11-*cis* retinal, all-*trans* retinal, and all-*trans* retinol were elevated to levels more than 1.5 times greater than controls. Whereas in the normal retina, photoreceptor cells and RPE cells function cooperatively to regulate retinoids involved in the visual cycle, one or more aspects of the visual cycle may malfunction, thus contributing to the progressive retinal degeneration in the C57vit/vit mouse.

In studies of ligand binding by a retinoid-binding glycoprotein from *Drosophila melanogaster* heads, analysis of endogenously bound retinoids by HPLC and mass spectrometry identified a component with a molecular ion m/z at 286, indicating a possible isomer of retinol. Analysis of endogenously bound fatty acids demonstrated both covalent and noncovalent binding of several fatty acids (including lauric, myristic, palmitic, oleic, and linolenic acids) characteristic of vertebrate IRBP, which is also a fatty acid-binding protein. Binding affinities of retinol and 16-(9-anthroyloxy) palmitic acid were characterized by fluorometric titrations.

Large-scale purification of IRBP was continued for studies on the production of experimental autoimmune uveitis (EAU) in rats and mice and possible modes of suppression of the disease.

Significance to Biomedical Research and the Program of the Institute

Because of its importance in normal photoreceptor cell physiology, ie, in facilitating the transport of retinoids during the visual cycle as well as transport of fatty acids that are essential to normal function,

abnormalities in IRBP function resulting from changes in concentration, distribution, or affinity for retinoids or fatty acids could be important, either directly or indirectly, in visual cell pathogenesis.

Proposed Course

We will continue to analyze factors controlling IRBP gene expression, using polymerase chain reaction quantitation of IRBP message and comparison with other retinal proteins such as opsin and S-antigen. Further studies will be conducted on the physiological role of IRBP in the visual cycle and how removing IRBP during retinal detachment affects regeneration of visual pigment. Studies on the C57vit/vit mouse model of retinal degeneration will include the quantitation of IRBP and message and further analyses of retinoid levels at different stages of postnatal development. Further studies on *Drosophila* retinoid-binding protein will include peptide analysis and antibody production, toward the goal of cloning and sequencing this protein for comparison to IRBP. Both retinol and fatty acid-binding sites on IRBP will be analyzed further via fluorescence studies. Large-scale purification of IRBP protein for studies on EAU will continue.

NEI Research Program

Retinal Diseases—Retinitis Pigmentosa and Other Inherited Disorders

Publications

Caspi RR, Chan C, Fujino Y, Oddo S, Najafian F, Bahmanyar S, Wilder RL, Wiggert B: Genetic factors in susceptibility and resistance to experimental autoimmune uveoretinitis. *Curr Eye Res* 11(suppl):81-86,1992.

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Kutty G, Hayden B, Osawa Y, Wiggert B, Chader GJ, Kutty RK: Heme oxygenase: Expression in human retina and modulation by stress agents in a human retinoblastoma cell model system. *Curr Eye Res* 11:153-160, 1992.

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Robbins SG, Wiggert B, Kutty G, Chader GJ, Detrick B, Hooks JJ: Redistribution and reduction of interphotoreceptor retinoid-binding protein during ocular coronavirus infection. *Invest Ophthalmol Vis Sci* 33:60-67, 1992.

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Smith SB, Lee L, Nickerson J, Si JS, Chader GJ, Wiggert B: Synthesis and secretion of interphotoreceptor retinoid-binding protein (IRBP) and developmental expression of IRBP mRNA in normal and rd mouse retinas. *Exp Eye Res* 54:957-963, 1992.

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Yamamoto JH, Okajima O, Mochizuki M, Shinohara T, Wiggert B, Chader GJ, Gery I, Nussenblatt RB: Cellular immune responses to retinal antigens in retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol* 230:119-123, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00196-09 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of the Eye and Ocular Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Diane E. Borst	Ph.D.	Staff Fellow	LRCMB, NEI
	Steven Bernstein	Ph.D., M.D.	Senior Staff Fellow	LRCMB, NEI

COOPERATING UNITS *(If any)*

Emory University, Atlanta, GA (J.M. Nickerson, Ph.D., J-S. Si, M.D.); University of Michigan, Ann Arbor (E. Farr, M.D.)

LAB/BRANCH

Laboratory of Retinal Cell and Molecular Biology

SECTION

Section on Gene Regulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interphotoreceptor retinoid-binding protein (IRBP) is an abundant glycolipoprotein expressed in the retina and pineal gland. Photoreceptor cells in the retina contain IRBP mRNA. We are characterizing the *cis*-elements regulating IRBP expression using a transient transfection assay and transgenic mice. There are two conserved areas of sequence in the 5′ flanking regions of the bovine, human, and mouse IRBP genes, one from -1 to -350 and another from -1200 to -1410. The 5′ flanking region is necessary for expression of IRBP in transient transfection assays in Y79 retinoblastoma cell cultures. In transgenic mice, the same region also shows promoter activity in the retina and pineal, demonstrating that tissue specificity is engendered within the tested 5′ flanking regions of the gene.

Project Description

Additional Personnel

Eric Wawrousek	Ph.D.	Research Biologist, OSD, NEI

Objectives

This research is designed to define the *cis*-acting elements and *trans*-acting factors that regulate interphotoreceptor retinoid-binding protein (IRBP) gene expression in a tissue-specific and/or developmentally specific manner and to downregulate eye-specific genes by exogenously derived genetic elements introduced either as antisense/catalytic RNA or as antisense DNA. In addition to the obvious gene therapeutic possibilities, this approach also can be used when total deletion of a target gene would be deleterious to survival of the organism.

Methods

These studies use conventional techniques for cloning and analysis of nucleic acids. Transgenic mice have been made using standard techniques. Chloramphenicol acetyltransferase (CAT) activity is measured by an enzyme-linked immunosorbent assay or the biphase assay.

Catalytic RNA/antisense (ribozyme) constructs are used for permanent transfection of cell lines that actively transcribe the messenger for IRBP. In addition, the transgenic animals produced express high levels of ribozymes targeted against endogenous IRBP mRNA. IRBP mRNA levels are quantitatively measured by polymerase chain reaction (PCR)-based techniques.

Major Findings

Gene expression.—Constructions containing presumptive elements of the IRBP promoter joined to an indicator gene (CAT) were made with both the bovine and mouse IRBP promoters for study of the expression of the IRBP gene. Two sites in the 5′ flanking (promoter) region show significant homologies across species, and we have made constructions containing both conserved blocks, only one of the two blocks, or neither. Tests of these constructions in several systems, including retinoblastoma cells (Y-79 and WERI), frog oocytes, mixed pinealocyte primary cultures, transformed pinealocytes, and normal mouse fibroblasts show promoter activity in Y-79 cells transfected with the IRBP promoter-CAT constructs containing both conserved blocks of sequence.

This is the first report of transfection of any retinoblastoma cell line yielding successful transient expression. In each block there is gel-shift experimental evidence for the binding of *trans*-acting factors confirmed in the proximal upstream area by DNAse footprinting experiments (collaboration with Drs. John Nickerson and Jing-Sheng Si). Southwestern blot analysis reveals a protein of 120,000 MW that binds to the -300 region of the promoter. This binding activity is not unique to the retina, being present in the heart, kidney, and lung; however, it is not ubiquitous.

Downregulation of gene expression.—IRBP-based ribozymes have been developed which exhibit activity in vitro against an IRBP mRNA substrate. These ribozymes have been selected and modified to yield molecules with high activity. Constructions that use ribozymes in tandem with promoters from both human β-actin (Kedes et al., 1987) and mouse phosphoglycerokinase have been made and transfected into human Y-79 retinoblastoma cells. Because of the extremely low level of IRBP mRNA in these cells, even when induced with butyrate, IRBP mRNA levels were not detectable following transfection with the ribozymes, in either the sham-transfected controls or transfected cell lines. Significant levels of the ribozyme construct were detected in the experimental lines. Because of this difficulty, PCR-based detection methods are under development, and we anticipate the methodology will permit mRNA detection at much lower levels, as well as provide quantitative estimation of IRBP mRNA inhibition.

We now are utilizing a mouse retinoblastoma cell line derived from mice transfected with SV-40 large T antigen. This cell line, which we have shown expresses high levels of IRBP mRNA, secretes IRBP in moderately high levels. We expect that transfection of these cells will provide a better model for evaluating IRBP downregulation, as well as correlating directly with the mouse transgenic animals now being developed.

Sequence analysis of the mouse IRBP genome.—Sequencing the genomic clones encoding the mouse IRBP gene has shown that mouse IRBP gene is similar in the coding regions to both human and bovine genes. They differ, however, in that the mouse fourth exon contains a 3′-untranslated region that is intermediate in length (1.0 kb) between bovine

(2.4 kb) and human (0.7 kb) orthologs. We are examining the sequence to determine alternative splice sites that may explain the unique appearance of two IRBP mRNA size classes as well as the difference between the forms of uveitis in rat and mouse species.

Significance to Biomedical Research and the Program of the Institute

Elucidation of the gene sequences of IRBP is fundamental to understanding normal retinal development and function.

Proposed Course

We have finished the major structural studies on the IRBP gene. With this foundation of information and battery of cloned genes, we have begun to study the regulation of IRBP gene expression. Related questions about the consequences of abnormal or absent IRBP function can be investigated in transgenic mice and in vitro systems.

Gene expression.—A deletion series of IRBP-promoter plasmids has been made, and preliminary experiments indicate that both the distal and proximal conserved sequences are important for expression of the IRBP gene in Y-79 cells. However, fewer than 205 bases of the 5′-flanking region are needed for basal promoter activity in the Y-79 cells. Some of these constructions have been injected into fertilized mouse eggs, and offspring are being examined for the expression of the constructions. We will compare the expression of these constructions in transgenic animals and Y-79 cells under different culture conditions. Preliminary studies show that the transgene is active in development as early as embryonic day 9, but high levels of expression coincide with the beginning of outer segment elongation, and steady-state adult levels are not reached until about postnatal day 30. In future studies, we will examine other transgenic mouse lines in the retina and several other tissues during development. We will characterize the *cis*-acting DNA sequences that bind proteins in the promoter region by making alterations to these sequences. We plan to isolate the proteins that bind to these elements by screening retina cDNA expression libraries by our established Southwestern blotting procedure. Preliminary screenings have yielded two potential clones.

NEI Research Program

Retinal Diseases—Photoreceptors and Retinal Pigment Epithelium

Publications

Humayun M, Bernstein SL, Gould HL, Chavis RM: Orbital childhood acute lymphoblastic leukemia as the initial presentation. *J Pediatr Ophthalmol Strabismus* 29:252-255, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00124-12 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolism of the Retina and Pigment Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gerald J. Chader	Ph.D.	Chief	LRCMB, NEI
Others:	Robert Waldbillig	Ph.D.	Expert	LRCMB, NEI
	Bruce Pfeffer	Ph.D.	Senior Staff Fellow	LRCMB, NEI
	Joyce Tombran-Tink	Ph.D.	Staff Fellow	LRCMB, NEI
	Stephen Gaudet	Ph.D.	Staff Fellow	LRCMB, NEI
	S. Patricia Becerra	Ph.D.	Visiting Scientist	LRCMB, NEI
	Timothy Schoen	B.S.	Biologist	LRCMB, NEI

COOPERATING UNITS *(if any)*

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Laboratory of Retinal Cell and Molecular Biology

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Section on Gene Regulation

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NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	5.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The retina and pigment epithelium are neuroepithelial tissues that work in close cooperation, as do other ocular tissues. Specific growth and differentiating factors found in the eye guide the development and interactions of individual ocular tissues to form a functional visual system. For example, ocular tissues synthesize insulin-like growth factor (IGF-I) and its binding proteins (BP), thus may self-regulate activity of this important growth factor in the vitreous and surrounding tissues. In addition to IGFBP, we have identified a unique differentiating protein secreted from fetal human pigment epithelial cells called pigment-epithelial-derived factor that is neurotropic to cultured human retinoblastoma cells and may affect neural retinal development in vivo. This protein maps to chromosome 17p where there is a cluster of cancer-related genes.

Project Description

Objectives

Our objective is to obtain a better understanding of the growth, development, and general metabolism of ocular tissues in health and disease. Study of growth and differentiation factors, be they protein (eg, pigment epithelium-derived factor [PEDF], polypeptide (eg, insulin-like growth factor [IGF]-I), or low-molecular-weight compounds (eg, cyclic AMP), is critical in obtaining a view of the events that control the early development of the eye and also in maintaining normal function in the adult.

Methods

Standard biochemical, molecular biological, and immunocytochemical techniques are used. Tissue culture is performed using various cells, in particular, the human retinoblastoma cell line, Y-79, which is used as a test system for differentiating agents.

Major Findings

PEDF.—Purified from conditioned medium of cultured fetal human pigment epithelial cells, PEDF also appears to be present in normal adult interphotoreceptor matrix (IPM). The protein, which migrates at approximately 540 kD on SDS-polyacrylamide gels, causes marked differentiation of human Y-79 retinoblastoma cells in culture. This differentiation is characterized by an extensive elongation of neurite-like processes and a gathering of cells into "rosette-like" aggregates. Immunocytochemically, the expression of specific neuronal markers is also enhanced. PEDF is a unique protein, synthesized and secreted by retinal pigment epithelial cells, that could direct early development, even in early embryogenesis. It thus may be that PEDF is also present after the important developmental period and may help to maintain retinal cell viability in the adult retina.

Cloning of the PEDF gene.—We now have cloned the cDNA for the PEDF gene. The protein is a member of the serine protease inhibitor superfamily of genes. Some members of this family are known to promote cellular differentiation, so it is more probable that PEDF has a major, similar role in the retina.

Localization of the PEDF gene.—Using fluorescent in situ hybridization, polymerase chain reaction and Southern blotting, we have localized the PEDF gene to the short arm of human chromosome 17. By analysis of somatic cell hybrids containing only specific regions of 17p and 17q, we have further pinpointed PEDF to 17p13.1. It is important that PEDF colocalizes to the same chromosomal area as the Li-Fraumeni cancer gene; thus, PEDF may be part of an important cluster of genes involved in cell proliferation and cancer.

IGF-I.—IGF-I is involved in the development and function of many tissues. In the eye, IGF-I probably participates in attaining overall eye size and in the function of individual ocular tissues and cell types. Specific IGF-binding proteins (IGFBPs) control the bioavailability of the IGFs, thus are important regulators of IGF activity in health and disease. The vitreous and several ocular tissues, including the cornea, contain high levels of IGFBPs not derived from extraocular sources. The ciliary body is the probable site of synthesis for at least one of the vitreal BPs, specifically IGF-BP2. Other ocular tissues, such as the neural retina or pigment epithelium, do not contain IGF-BP2, thus the ciliary body probably secretes the BP into the vitreous where it could be a major factor in regulating developmental programs in the eye. It is interesting that the cornea exhibits exceptionally high amounts of binding protein activity. Their role in corneal metabolism is yet unknown but, because of their growth-regulating potential, BPs could be involved in such important processes as wound healing and corneal complications of diabetes.

Significance to Biomedical Research and the Program of the Institute

Determining the factors that affect normal ocular growth, differentiation, and function will aid us in understanding diseases of the eye, especially those of a hereditary or early developmental nature.

Proposed Course

The effects of growth factors on ocular development will be further examined. The factors that affect normal and abnormal growth of the sclera will be further investigated, with particular reference to myopia. The full PEDF gene (genomic) will be examined and analyzed to help to elucidate its presumptive role(s) in retinal development. The generality of the PEDF gene in other tissues will be examined, specifically in relation to its possible role in cancer growth.

NEI Research Program

Retinal Diseases—Retinitis Pigmentosa and Other Inherited Disorders

Publications

Steele FR, Chader GJ, Johnson LV, Tombran-Tink J: Pigment epithelium-derived factor (PEDF): Neurotrophic activity and identification as a unique member of the serine protease inhibitor (SERPIN) gene family. *Proc Natl Acad Sci USA*, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00148-19 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visual Control Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gerald J. Chader	Ph.D.	Chief	LRCMB, NEI
Others:	Paul Wong	Ph.D.	Visiting Fellow	LRCMB, NEI
	Tatiana Putilina	Ph.D.	Visiting Associate	LRCMB, NEI
	Ignacio Rodriguez	Ph.D.	Staff Fellow	LRCMB, NEI
	Jun Li	M.D.	Visiting Associate	LRCMB, NEI
	R. Theodore Fletcher	M.S.	Chemist	LRCMB, NEI

COOPERATING UNITS *(If any)*

School of Veterinary Medicine, University of Pennsylvania (G. Aguirre, D.V.M., Ph.D.); Department of Anatomy, Erasmus University, Rotterdam, The Netherlands (S. Sanyal, Ph.D.); Department of Zoology, University of Lund, Lund, Sweden (T. van Veen, Ph.D.); Instituto Nazionale per la Ricera sul Cancro, Genova, Italy (A. Albini, Ph.D., D. Noonan, Ph.D.)

LAB/BRANCH

Laboratory of Retinal Cell and Molecular Biology

SECTION

Section on Gene Regulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The function of neural tissues such as the retina depends upon the presence of normal complements of tissue-specific genes and upon the normal expression of the protein gene products. If these mechanisms fail, hereditary diseases of the retina, such as retinoblastoma or retinitis pigmentosa, will result. We have found that laminin, an important extracellular matrix protein, slows retinoblastoma cell growth and promotes differentiation. Specifically, it switches development from a photoreceptor pathway to a conventional neuronal-like pathway via a low affinity "differentiative" binding activity, which we have called laminin-binding molecule-100 (LBM-100). LBM-100 could have a major effect on early retinal cell development. In parallel, we have developed new techniques to clone and sequence retina-specific genes at a higher efficiency.

Project Description

Objectives

Normal development and expression of genes in the retinal photoreceptor neuron is crucial to visual function in the adult; thus, the factors that code for normal gene control and expression in human retina and in animal models of retinal degeneration are of primary interest. As a corollary, we have mounted a major effort to develop new molecular biological techniques such that unique retinal and retinal pigment epithelium (RPE) genes can be identified, cloned, and sequenced for ultimate use in screening human populations with inherited diseases of the visual system.

Methods

Standard molecular biological, biochemical, and neurochemical techniques are routinely employed. Histochemical techniques are used when necessary.

Major Findings

Laminin-binding molecule-100.—A ubiquitous extracellular matrix protein, laminin has profound effects on a variety of cell types. For example, both gene and protein expression in cultured human Y-79 retinoblastoma cells are switched from a photoreceptor to a conventional neuronal pathway by the addition of this basement membrane glycoprotein in culture. Unlike other cell systems in which laminin influences differentiation, Y-79 cells can neither attach to nor chemotactically respond to laminin. Affinity chromatography of radiolabeled Y-79 surface proteins revealed a single 100-kD laminin-binding protein. We have named the protein laminin-binding molecule 100 (LBM-100) and propose that it serves as an embryonic and/or tumor "differentiation receptor" through which laminin influences gene expression and development.

Advances in molecular biology.—Each tissue of the body expresses a unique complement of genes that are transcribed and translated at a high level. In the retina, for example, opsin and interphotoreceptor retinoid-binding protein (IRBP) are highly expressed, such that photoreception and the visual process can take place. Similarly, it is often a genetic defect in these tissue-specific genes that results in a hereditary degeneration such as retinitis pigmentosa. We are interested in developing new molecular biological techniques that will allow for more efficient identification of highly expressed genes of the RPE complex. In this, we have had two significant advances.

First, we developed a new method for rapid polymerase chain reaction-based construction of specifically enriched libraries from very small retinal samples. This development will be especially important when tissue samples are limited, eg, early development and rare pathology samples. Our second methodological advance involves subtractive cloning on an immobilizing Dynabead® base. One of the most common problems in subtraction cloning is that specific mRNAs or cDNAs of a particular tissue are subtracted by common sequences (eg, gene families) or repetitive elements (eg, Alu repeats), leaving little or no product for use as a probe. We synthesized single-strand antisense cDNA from retina on magnetic Dynabeads such that the mRNA could be easily destroyed and short, labeled sense fragments could be generated. We validated the technique by generating a number of clones, among which are opsin, IRBP, green visual pigment, and several RPE-specific genes not previously described.

Significance to Biomedical Research and the Program of the Institute

To understand and control or even reverse a hereditary disease process in a tissue, one must identify the normal complement of unique genes expressed in that tissue and on the functioning of the protein products. This is especially true in an early degenerative process, eg, retinitis pigmentosa, and in other hereditary diseases, such as retinoblastoma, in which abnormal changes are subtle and can be masked by normal developmental switches in gene expression. Thus, studying the molecular biological development and metabolic changes in retina-specific systems will lead to a better understanding of disease processes unique to the neural retina.

Proposed Course

Molecular biological and developmental control mechanisms in the retina and pigment epithelium will continue. In particular, we will investigate gene expression in normal retinas and in retinas affected with specific genetic diseases.

NEI Research Program

Retinal Diseases—Retinitis Pigmentosa and Other Inherited Disorders

Publications

Albini A, Noonan DM, Melchiori A, Fassina GF, Percario M, Gentleman S, Toffenetti J, Chader GJ: Laminin switches retinoblastoma cell differentiation from a photoreceptor- to a neuronal-like pathway via a low affinity "differentiative" binding activity. *Proc Natl Acad Sci USA* 89:2257-2261, 1992.

Aresu O, Nicolo G, Allavena G, Melchiori A, Schmidt J, Kopp JB, d'Amore E, Chader GJ, Albini A: Invasive activity, spreading on and chemotactic response to laminin are properties of high but not low metastatic mouse osteosarcoma cells. *Invasion Metastasis* 11:2-13, 1991.

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Hershfield B, Inouye L, Chader GJ, Ripps H, Aguirre G: Organization and transcription of canine $(CAC)_n$ sequences. *J Hered* 82:251-254, 1991.

Korf HW, Korf B, Schachenmayr W, Chader GJ, Wiggert B: Immunocytochemical demonstration of interphotoreceptor retinoid-binding protein in cerebellar medulloblastoma. *Acta Neuropathol* 83:482-487, 1992.

Kotake S, Sasamoto Y, Kawano Y, Sanui H, Wiggert B, Chader GJ, Gery I: The existence of two completely distinct antigenic sites with a decapeptide. *Cell Immunol* 140:123-129, 1992.

Kutty G, Hayden B, Osawa Y, Wiggert B, Chader GJ, Kutty RK: Heme oxygenase: Expression in human retina and modulation by stress agents in a human retinoblastoma cell model system. *Curr Eye Res* 11:153-160, 1992.

Kutty RK, Fletcher RT, Chader GJ, Krishna G: Expression of guanylate cyclase-A mRNA in the rat retina: Detection using polymerase chain reaction. *Biochem Biophys Res Commun* 182:851-857, 1992.

Pepperberg DR, Okajima TI, Ripps H, Chader GJ, Wiggert B: Functional properties of interphotoreceptor retinoid-binding protein (IRBP). *Photochem Photobiol* 54:1057-1060, 1991.

Robbins SG, Wiggert B, Kutty G, Chader GJ, Detrick B, Hooks JJ: Redistribution and reduction of interphotoreceptor retinoid-binding protein during ocular coronavirus infection. *Invest Ophthalmol Vis Sci* 33:60-67, 1992.

Rodriguez IR, Chader GJ: A novel method for the isolation of tissue specific genes. *Nucleic Acids Res* 20:3528, 1992.

Rodrigues MM, Rajagopalan S, Lee L, Nair CN, Advani SH, Donoso L, Chader GJ, Wiggert BN: Retinoblastoma: Differentiation and mRNA for interphotoreceptor retinoid-binding protein. *Curr Eye Res* 11:425-433, 1992.

Sasamoto Y, Kawano YI, Bouligny R, Wiggert B, Chader GJ, Gery I: Immunomodulation of experimental autoimmune uveoretinitis by intravenous injection of uveitogenic peptides. *Invest Ophthalmol Vis Sci* 33:2641-2649, 1992.

Smith SB, Lee L, Nickerson J, Si JS, Chader GJ, Wiggert B: Synthesis and secretion of interphotoreceptor retinoid-binding protein (IRBP) and developmental expression of IRBP mRNA in normal and rd mouse retinas. *Exp Eye Res* 54:957-963, 1992.

Wiggert B, Kutty G, Long KO, Inouye L, Gery I, Chader GJ, Aguirre GD: Interphotoreceptor retinoid-binding protein (IRBP) in progressive rod-cone degeneration (prcd)—Biochemical, immunocytochemical and immunologic studies. *Exp Eye Res* 53:389-398, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00260-03 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Outer Retina-Specific Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Michael Redmond	Ph.D.	Research Biologist	LRCMB, NEI
Others:	Christian P. Hamel	M.D.	Visiting Associate	LRCMB, NEI
	Ekaterina Tsilou	M.D.	Visiting Fellow	LRCMB, NEI

COOPERATING UNITS *(if any)*

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Section on Gene Regulation

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retinal pigment epithelial (RPE) cells and photoreceptor (PR) cells are functionally and developmentally closely integrated. We are characterizing a 65 kD RPE-specific protein that is membrane-associated, hydrophobic, and dependent upon detergent solubilization for its extraction. In cell fractionation experiments this protein is found in the microsomal pellet. We have obtained its amino acid analysis and the sequences of several tryptic and chymotryptic peptides. Its amino terminus is blocked. We have cloned cDNA for this protein, which encodes a 533-residue protein unlike any others in the data base.

The interphotoreceptor retinoid-binding protein (IRBP) is involved in the transport of retinoids, a functional relationship between the RPE and the PR. We have subcloned DNA fragments corresponding to integral repeats of bovine IRBP into a bacterial expression vector. The resultant expressed protein fragments were tested for their retinoid-binding functions. One of the fragments, which comprises the central two repeats, bound retinol. This finding indicates that all four repeats of IRBP are not required for retinoid-binding activity. The binding appears to be localized to the central region of the IRBP protein.

PHS 6040 (Rev. 5/92)

Project Description

Objectives

The retinal pigment epithelium (RPE) is essential for continued maintenance of the photoreceptor (PR) rod outer segments (ROS). These two components of the outer retina share a close developmental and functional relationship; however, knowledge of derangements of the RPE known to be involved in certain retinal dystrophies is, at the molecular level, lacking. We are trying to understand better the RPE/PR relationship by investigating the structure and function of outer retina-specific proteins: one, a 65-kD RPE-specific protein; another, interphotoreceptor retinoid-binding protein (IRBP) is involved in the transport of retinoids between the RPE and the ROS. In addition, we are isolating RPE-expressed cDNAs to generate other molecular probes.

Methods

Molecular cloning and biochemical and protein chemistry techniques are employed in this study. We also are using automated fluorescent DNA sequencing and gene mapping techniques.

Major Findings

A 65-kD outer retina-specific protein isolation and characterization.—The membrane-associated 65-kD protein is most effectively solubilized by the use of various detergents. We have found that the protein preferentially fractionates with the microsomal pellet when it is the major protein. Its affinity for phospholipids has facilitated partial purification of the protein. Approximately 40% of the protein has been sequenced by way of tryptic and chemotryptic peptides. The protein is not expressed by RPE cells in culture.

Cloning of the 65-kD protein.—We have cloned the 65-kD protein. Using peptide sequence-directed oligonucleotide probes we have isolated two overlapping clones covering 2.4 kb. The remaining 5′ sequence was obtained by polymerase chain reaction (PCR), coupled to reverse transcription of mRNA. The final mRNA is 3,115 bp long with a 1,602 bp open reading frame coding for 533 amino acids that match perfectly with the sequenced peptides. The amino acid sequence does not match any other in the database, definitively proving that it is a novel molecule.

Characterization of IRBP.—PCR-derived fragments of IRBP corresponding to repeats 1 + 2 and 2 + 3 have been characterized. It appears that only the 2 + 3 repeat fragment binds all-*trans*-retinol.

Significance to Biomedical Research and the Program of the Institute

There is a paucity of reliable RPE-specific proteins that have been characterized. This is especially onerous in view of the indispensable function of the RPE in such specialized functions as retinoid metabolism, outer segment phagocytosis, and various transport mechanisms. The 65-kD protein identified is a conserved, RPE-specific molecule found in mammal and bird RPE. Furthermore, it first appears in rat just prior to the appearance of the ROS suggesting a temporal or developmental control of expression. The loss of the protein in culture suggests that a continuous in vivo signal is required for its expression. Sequencing of the cDNA clones demonstrate that it is a novel protein. Elucidation of the function of the 65-kD protein may help our understanding of the RPE. Using this molecular probe, we will be able to dissect further the specific role of the RPE in the development and maintenance of processes important to vision.

Similarly, the relationship of structure to the function of IRBP, which is thought to transport retinoids between the RPE and the ROS, has not been fully elucidated. In particular, in the relationship of the fourfold repeat structure of IRBP to the ligand-binding properties is of major importance. A bacterially expressed fragment of IRBP consisting of the control two repeats will bind retinol, indicating that the whole protein is not required for binding, whereas a fragment consisting of 1 + 2 repeats does not appear to bind retinol. This finding will be important in understanding the trafficking of retinoids between the RPE and the ROS. The identification of further RPE-expressed cDNAs will markedly enhance our knowledge of the RPE in health and disease.

Proposed Course

1. Further characterization and purification of the 65-kD protein for structural, immunological, and biochemical analysis will continue. Elucidation of its function will involve a variety of approaches.

2. We will screen human and mouse genomic libraries for the respective genes, which will be sequenced and characterized by chromosomal localizations and possible association with disease. Transgenic studies will be undertaken.

3. The 65-kD protein will be overexpressed to provide material for structural, functional, and immunological studies.

4. Human RPE libraries will be screened using the expressed sequence tag approach to identify other RPE-specific genes.

5. We will continue using PCR techniques to produce defined sequences for expression of the repeats of IRBP. The expressed proteins will be purified and studied with respect to their ligand-binding properties. Certain expressed proteins will be supplied for analysis of uveitogenicity in the mouse model by Dr. Rachel Caspi (Laboratory of Immunology).

NEI Research Program

Retinal Diseases—Photoreceptors and Retinal Pigment Epithelium

Publications

Redmond TM, Nickerson JM: Retinoid binding to a recombinant control two-repeat segment of IRBP. *Invest Ophthalmol Vis Sci* 33(4):2446, 1992.

Tsilou ET, Hamel CP, Harris E, Detrick B, Hooks JJ, Redmond TM: Partial purification, characterization and cloning of a RPE-specific protein. *Invest Ophthalmol Vis Sci* 33(4):1109, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00132-11 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Phototransduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Toshimichi Shinohara	Ph.D.	Head, Section on Molecular Biology	LRCMB, NEI
Others:	Thoru Abe	M.D., Ph.D.	Visiting Associate	LRCMB, NEI
	Takanobu Kikuchi	Ph.D.	Visiting Associate	LRCMB, NEI
	Shirley Yu	B.S.	Biologist	LRCMB, NEI

COOPERATING UNITS *(if any)*

Mount Sinai Hospital, Toronto, Canada (Martin Breitman, Ph.D.); Department of Anatomy, Nagoya University School of Medicine, Tsurumai, Showa-Ku, Nagoya, Japan (J. Usukura, M.D.)

LAB/BRANCH

Laboratory of Retinal Cell and Molecular Biology

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have characterized the human and mouse S-antigens and 33K protein genes and a human Shuzin gene, and we have determined the gene sequences of the human and mouse S-antigens. The S-antigen genes were approximately 50 kbp in length, contained 16 exons and 15 introns, and comprised of 97% intron and 3% exon. The 5′-flanking regions of the genes, approximately 1.5 kbp long, had no known regulatory elements for transcription, such as TATA, GC, or CCAAT boxes. Interestingly, the 5′ flanking regions of the human, bovine, and mouse genes expressed tissue-specific promoter activity in both in vitro and in vivo transcription assays as well as in transgenic mice.

Isolation of two 33K protein genes and determination of their DNA sequences revealed genes of approximately 10 kbp in length, containing four exons and three introns. The 33K protein gene seems to represent a family of genes: At least three genes have similar sequences.

The functional role of the retinal protein Shuzin is unknown. We isolated and sequenced several cDNAs each from humans and cows. The entire human Shuzin gene sequence also was determined. This gene, composed of two introns and three exons, has a highly repetitive sequence in the 5′ noncoding region.

We have constructed fusion genes containing a 5′-flanking S-antigen gene sequence upstream of the bacterial gene chloramphenicol acetyl transferase (CAT). A hybrid gene containing the 5′-flanking region of the mouse S-antigen gene and the CAT gene was microinjected into transgenic mice. The mice expressed CAT activity in the retina and pineal gland, suggesting that the 1,300 bp S-antigen promoter segment contains sufficient information to direct appropriate tissue-specific gene expression in transgenic mice. In addition, S-antigen was found in lens fiber and epithelial cells, the cerebellum, and the cerebral cortex. These results indicate that S-antigen is expressed in a wider spectrum of the cell types than previously recognized.

Project Description

Objectives

The objective of this project is to understand the basic mechanism of phototransduction in the retina and to understand the structure, function, and evolution of the proteins present in photoreceptor rod cells and pinealocytes.

Methods

Conventional methods for analysis of proteins and nucleic acids used include protein purification and RNA and DNA isolation, characterization, and sequence determination. Recombinant DNA techniques used include a baculovirus expression vector system, synthesis of point mutation clones, characterization of promoters, and transgenic animals. We also have synthesized and used purified oligopeptides and oligonucleotides.

Major Findings

1. We determined the gene sequences of S-antigen (S-Ag) from human and mouse. S-Ag is 50 kbp in length and has 15 introns and 16 exons. The smallest exon encodes for three amino acids.

2. We found that the intron-exon map sequence of the mouse S-Ag gene has been well conserved. Approximately 97% of this gene is intron; 3% is exon.

3. We subcloned the human and mouse S-Ag cDNAs into two expression vectors and expressed. The products of S-Ag cDNA were purified by column chromatography and prepared for crystallization.

4. We determined the 5′-flanking sequence of the human and mouse S-Ag genes and demonstrated promoter activity in in vivo and in vitro transcriptional assays.

5. Although the S-Ag promoter sequences are highly conserved between human and mouse, we found promoter activity at different locations of the 5′-flanking region in human and mouse genes. This result suggests that promoter activity is highly specific to tissues and species.

6. We fused the 1,300-bp mouse S-Ag promoter with the chloramphenicol acetyltransferase (CAT) gene and introduced it into transgenic mice. The transgenic animals expressed CAT activity only in the retina and pineal gland, indicating that the promoters have tissue-specific enhancer and promoter activity.

7. We fused the opsin promoter with a diphtheria toxin gene and introduced it into transgenic mice, which subsequently lost only their photoreceptor rod cell layers.

8. We isolated several cDNAs of Shuzin, a retinal photoreceptor protein, from human and cow retinal cDNA libraries (λ-gtll) and determined the entire DNA sequences. The deduced protein has sequence similarity with TFIID. We isolated its gene from a genomic library and determined the DNA sequence, showing it is composed of two introns and three exons.

9. We isolated two genes of 33-kD rod outer segment (ROS)-specific proteins from the retinal libraries of human and mouse and determined the entire DNA sequence of these genes. They have four exons and three introns.

Significance to Biomedical Research and the Program of the Institute

Eyes have remarkable properties in functioning efficiently over a wide range of illuminations. Rod cells having photosensitive rhodopsin, are more sensitive to dim light, and adapt in the dark to increase their sensitivity. However, rod cells cease their sensitive phototransduction in bright light. In contrast, cone cells do not operate in dim light but are operative in bright light.

Rhodopsin, transducin, phosphodiesterase, rhodopsin kinase, and S-Ag have been known to be associated with the phototransduction cascade. Rhodopsin kinase and S-Ag are considered to be the important proteins for light-dependent modulation of phototransduction. To understand this light-dependent modulatory mechanism in ROS, we have characterized S-Ag, Shuzin, and 33-kD protein as well as their genes. Interestingly, other signal transduction systems have cascades similar to that of phototransduction (one of the best characterized receptor-mediated signal transduction processes). In the phototransduction cascade, the shutoff mechanism appears to be modulated by the phosphorylation and dephosphorylation of rhodopsin. Studying this modulation mechanism is important for understanding phototransduction as well as for understanding signal transduction in general. In addition, we think that the night

blindness of vision may in part be associated with light adaptation.

Proposed Course

The following studies are in progress or have been proposed for Fiscal Year 1993: (1) identification of the S-Ag promoter using transgenic animals; (2) identification of *cis*-acting factors of the S-Ag and 33-kD protein promoter; (3) the crystallization of S-Ag and 33-kD protein; and (4) investigation of a functional role for S-Ag, 33-kD protein, and Shuzin: the homologous recombination between a mutant gene and a normal gene will be induced in ES cell culture. The recombinant ES cells will be introduced into a transgenic animal system to produce a mutant mouse.

NEI Research Program

Retinal Diseases—Photoreceptors and Retinal Pigment Epithelium

Publications

Breitman ML, Tsuda M, Kikuchi T, Zucconi A, Khoo W, Shinohara T: Expression of S-antigen in retina, pineal gland, lens and brain is directed to 5′ flanking sequences. *J Biol Chem* 266:15505-15510, 1991.

Dancinger M, Kozak CA, Abe T, Shinohara T, Farber DB: The gene for retinal rod 33-kDa protein is on mouse chromosome 1 near *lamb2*. *Genomics*, in press.

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Shinohara T, Kikuchi T, Tsuda M, Yamaki K: A family of retinal S-antigen (Arrestin) and their genes: Comparative analyses of human, mouse, rat, bovine, and *Drosophila*. *Comp Biochem Physiol*, in press.

Tsuda M, Kikuchi T, Yamaki K, Shinohara T: The mouse S-antigen gene; comparison with human and *Drosophila*. *Eur J Biochem* 200:95-101, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00250-05 LRCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Experimental Autoimmune Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Toshimichi Shinohara	Ph.D.	Head, Section on Molecular Biology	LRCMB, NEI
Others:	Kotaro Eto	M.D., Ph.D.	Visiting Associate	LRCMB, NEI
	Santhanakrishnan Sunil	M.D.	Visiting Fellow	LRCMB, NEI

COOPERATING UNITS *(If any)*

Department of Ophthalmology, Miami University, Miami, FL (D. Hamasaki, Ph.D.); Department of Anatomy, Nagoya University School of Medicine, Tsuramai, Showa-ku, Nagoya, Japan (Jiro Usukura, M.D.)

LAB/BRANCH

Laboratory of Retinal Cell and Molecular Biology

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously determined the amino acid sequences of human, mouse, rat, and bovine retinal S-antigen and rat pineal gland S-antigen. Immunogenic sites and four uveitopathogenic sites of S-antigen also were determined. Two of the immunogenic sequences were highly conserved among these species.

Many proteins in the National Biomedical Research Foundation data base have sequences similar to that of a uveitopathogenic site. We chemically synthesized many peptides, some of which induced experimental autoimmune uveitis (EAU) and experimental autoimmune pinealitis (EAP) in Lewis rats. The peptides include synthetic peptides from yeast (*Saccharomyces cerevisiae*) histone H3, *Escherichia coli* hypothetical protein, potato proteinase inhibitor, hepatitis virus protein, Moloney murine sarcoma virus protein, and Moloney murine leukemia virus protein. In addition, native yeast histone H3 was capable of inducing EAU.

The animals administered yeast histone by the oral route suppressed the induction of EAU and EAP by either the yeast histone H3 peptide or an S-antigen peptide. Thus, the peptides that have molecular mimicry cross-induced the tolerance. These findings provide a basis for autoimmune inflammatory diseases of the eye in humans.

To understand the role in autoimmunity of infectious microorganisms which have cross-reactive antigens, we injected Lewis rats with peptide M together with one of six different killed bacteria, either with or without incomplete Freund's adjuvant (IFA). The rats injected with IFA developed EAU, but most rats injected without IFA did not develop EAU. To assess the impact of infection by live microorganisms, we injected the rats several times with low doses of live *E. coli* expressing S-antigen and baker's yeast with a cross-reactive antigen. The rats injected with either live *E. coli* or live yeast developed EAU. We conclude that infection by microorganisms that have cross-reactive antigens can break immune tolerance to self-antigens and induce inflammatory autoimmune diseases.

Project Description

Objectives

The objectives of this project are to understand the basic etiology of autoimmune inflammation, including uveitis, and to find possible treatments for human uveitis.

Methods

Conventional methods for the analysis of proteins and nucleic acids used in this research include protein purification, RNA and DNA isolation, characterization and sequencing, molecular cloning, screening of clones, in situ hybridization, immunocytochemistry, and chromosome mapping. We also synthesized and used oligopeptides and oligonucleotides. Bovine, murine, primate, and human materials are used; animal experiments were conducted with Lewis rats and monkeys. T-cell response and adoptive transfer were done with lymph node or spleen cells of rat.

Major Findings

1. We found local sequence homology between peptide M and several other foreign proteins, including potato proteinase inhibitor IIa, *Escherichia coli* hypothetical protein, hepatitis B virus probable DNA polymerase, Moloney murine sarcoma virus gag-polyprotein, Moloney murine leukemia virus gag-pol polyprotein, baboon endogenous virus gag-pol polyprotein, and Baker's yeast histone H3.

2. The synthetic peptides of the above-mentioned proteins induced experimental autoimmune uveitis (EAU) in Lewis rats with pathology similar to that of EAU induced by peptide M or native S-antigen (S-Ag).

3. For the first time we proposed and showed the evidence that molecular mimicry plays a role in the process of pathogenesis of EAU and perhaps in autoimmune diseases in general.

4. Oral administration of histone H3 peptide suppressed EAU in the Lewis rats.

5. We also found the suppression of EAU by histone H3 in the EAU induced by S-Ag; thus, the tolerance also cross-reacted between the peptide which has molecular mimicry.

6. The T lymphocytes obtained from rats immunized with peptide M or yeast histone H3-transferred disease (EAU) in the naive rats (adoptive transfer) when stimulated with either peptide M or histone H3. In addition, oral tolerance was adoptively transferred from rats fed peptide M or histone H3 to the naive rats.

7. Infection by microorganisms that have cross-reactive antigens can break immune tolerance to a self antigen and induce inflammatory autoimmune diseases.

Significance to Biomedical Research and the Program of the Institute

Uveitis is a leading cause of visual handicap in the United States and throughout the world. Many physicians have suspected for decades that some types of uveitis are induced by bacterial and viral infections; however, there is no clear link between infection and disease.

Autoimmune processes are thought to play a significant role in the pathogenesis of disease. Molecular mimicry, a process by which an immune response, directed against a non-self protein, cross-reacts with a normal host protein, may play a role in autoimmunity. Here, we have proposed the idea of molecular mimicry and provided evidence that molecular mimicry plays a role in the pathogenesis of EAU. In addition, we have offered evidence that infection is a cause of autoimmune inflammation. These findings provide an important clue for understanding the etiology of autoimmune inflammatory diseases in human.

Proposed Course

The following studies are in progress or proposed for Fiscal Year 1993: (1) further evaluation of foreign proteins similar to S-Ag that induce EAU; (2) characterization of peptide M, with respect to the minimum number of amino acids required for induction of EAU; (3) study of the induction of EAU in transgenic mice that express foreign proteins in the photoreceptor cells; and (4) further characterization of molecular mimicry and its role in EAU and human uveitis.

NEI Research Program

Retinal Diseases—Inflammatory Diseases

Publications

Babila T, Schaad NC, Simonds WF, Shinohara T, Klein DC: Development of MEKA (phosducin), Gb, Gg and S-antigen in the rat pineal gland and retina. *Brain Res* 585:141-148, 1992.

Eto K, Suzuki S, Singh VK, Shinohara T: Immunization with recombinant *E. coli* expressing retinal S-antigen induced experimental autoimmune uveitis (EAU) in Lewis rats. *Cell Immunol*, in press.

Hamasaki DI, Sato H, Santhanakrishnan S, Shinohara T: Correlation between the physiological and morphological changes in the experimental autoimmune uveitis induced by peptide G of S-antigen. *Exp Eye Res*, in press.

Ni M, Yamaki K, Kikuchi T, Ferrick M, Shinohara T, Nussenblatt RB, Chan C-C: Developmental expression of S-antigen in fetal human and rat eye. *Curr Eye Res* 11:219-229, 1992.

Sai S, Usukura J, Shinohara T, Wakabayashi T, Awaya S: S-antigen localization in developing *rds* mouse retina. *J Ophthalmol* 36:331-341, 1992.

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Shinohara T, Kikuchi T, Tsuda M, Yamaki K: A family of retinal S-antigen (Arrestin) and their genes: Comparative analyses of human, mouse, rat, bovine, and *Drosophila*. *Comp Biochem Physiol*, in press.

Singh VK, Kalra HK, Yamaki K, Shinohara T: Suppression of experimental autoimmune uveitis in rats by the oral administration of the uveitopathogenic S-antigen fragment or a cross-reactive homologous peptide. *Cell Immunol* 139:81-90, 1992.

Singh VK, Usukura J, Shinohara T: Molecular mimicry: Uveitis induced in *Macaca fasicularis* by a microbial protein having sequence homology with retinal S-antigen. *Jpn J Ophthalmol* 36:108-117, 1992.

Sunil S, Ito K, Shinohara T: Immunogenic determinants common to cross-reactive self and nonself antigens in experimental autoimmune uveitis (EAU). *Cell Immunol*, in press.

Laboratory of Sensorimotor Research

Report of the Chief, Laboratory of Sensorimotor Research

Robert H. Wurtz, Ph.D.

The generation of movement and its sensory control are among the brain's most critical functions. In primates, including humans, the visual sense predominates and visually controlled movements are the most precise. The Laboratory of Sensorimotor Research concentrates its research effort on the brain mechanisms underlying these critical functions. We study neither the receptor input nor the muscular output but rather the intricate systems in between that lead to visual perception and the generation of movement. In this 14th annual report of the Laboratory of Sensorimotor Research one salient investigation in each of the five sections within the laboratory is used to illustrate the range of our studies on the visuomotor system.

Section on Neural Modeling

Dr. Lance M. Optican and his colleagues in the Section on Neural Modeling concentrated on the processing of visual input to the brain. Their studies indicate that different visual areas in the brain may communicate via temporally modulated messages. Their previous work had shown that neurons in different areas of the monkey brain encode and transmit information about stationary, two-dimensional pictures that vary in form, brightness, and duration. These investigators also had shown that information about remembered visual features is carried by a temporal code. Now they have extended those studies to show that neurons in visual areas of the cerebral cortex (areas V1, V2, V3, and V4) carry information about the form and color of a stimulus in a temporally modulated code. The results suggest that cortical neurons can convey information about many different features without confounding them. The mechanism for encoding these multiple messages uses temporal modulation to multiplex the different messages on the neuron's response in a separable way.

Section on Neuro-Ophthalmologic Mechanisms

The effects of selective attention on visual processing have been investigated by Dr. Michael Goldberg and his colleagues in the Section on Neuro-Ophthalmologic Mechanisms. They studied neurons in the posterior cingulate cortex of the frontal lobe of the cerebral cortex during visuomotor tasks to determine their activity with respect to visual stimuli that were usually salient themselves or that were used as the targets for saccades. Neurons responding to salient visual stimuli such as checkerboards did not respond to small spots of light, even when those spots were used as targets for saccadic eye movements or as stimuli in a luminance-detection task. This result suggests that the brain treats voluntary attention differently from involuntary attention to salient and novel stimuli. The cingulate cortex is important in the generation of involuntary but not voluntary attention.

Section on Oculomotor Control

The influence of a motor control system on visual input has been the focus of Dr. Frederick Miles and his colleagues in the Section on Oculomotor Control. Primates have excellent binocular vision and use vergence movements to align the two eyes on a common object, thereby facilitating stereopsis, the visual perception of depth. Binocular disparity, which refers to the slight difference in the locations of the images on the two retinas resulting from the slight difference in the viewpoint of the two eyes, is known to provide an important drive for these vergence eye movements.

Using stimuli that extend over a much greater region of the visual field than had been employed in

most previous studies, Dr. Miles' group discovered that sudden changes in binocular disparity induce vigorous, machine-like vergence eye movements with a roughly exponential time course (time constant, 100-150 msec), at ultra-short latencies of approximately 60 msec. The obligate nature of these eye movements was evident from the fact that the animals were neither trained to make such responses nor reinforced for doing so. The latency is 100 msec or so less than values obtained using smaller visual targets reported in the literature. Such reflex-like vergence responses at ultra-short latencies in response to pure disparity stimuli have not been previously described; they are all the more surprising because the neural decoding of disparity is known to be a cortical function. These experiments suggest that large-field disparity stimuli provide a powerful yet simple new approach for probing the neural processing of disparity information and its role in the cortical control of vergence eye movements.

Section on Visuomotor Integration

In the Section on Visuomotor Integration, Dr. Robert H. Wurtz and his collaborators studied the generation of an eye movement, ie, the rapid or saccadic eye movements that move the eye from one object of interest in the visual field to another. Many neurons in the superior colliculus, a nucleus in the brain stem, give a burst of cell discharges before the onset of these saccades. Work over the last 20 years has progressively fit these neurons into models of a brain system for the control of this movement. This year Dr. Wurtz and coworkers found that another type of cell lying just below these burst cells is also related to the generation of saccades. These cells' behavior illustrates a method of motor processing only recently recognized. Instead of increasing their discharge before a given movement via the activity of a single group of cells (as burst cells do), these deeper cells support a moving front of activity that slides across the layer of their colliculus. This spread of activity may represent a type of neural processing not previously recognized in the brain.

Section on Visual Behavior

The control of movement centers not only on the eye but on the head as well. Dr. David Lee Robinson and his colleagues in the Section on Visual Behavior, studying the role of the brain stem reticular formation in the coordination of head and eye movements, have discovered that electrical stimulation of this region evokes brisk movements of the head to the ipsilateral side. These head movements are never associated with shifts in the direction of gaze although they are influenced by the starting position of the head, active fixation, and level of alertness. These observations suggest that this brain region is the central integrative site for head movements.

To enhance understanding of the global nature of this region, Dr. Robinson and coworkers have injected tracer substances into physiologically identified portions of this head movement area and discovered anatomical connections between the reticular formation and (1) those portions of the superior colliculus that drive head movements, (2) parts of the motor and premotor cortices that initiate head movements, and (3) regions of the midbrain that have limbic/emotional functions. In addition, the group has discovered efferent projections to the upper cervical spinal cord that end in the region of the neck motoneurons. These studies provide important information for understanding the neural mechanisms and sites for the control of head movements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00256-04 LSR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Information Processing by Visual System Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lance M. Optican	Ph.D.	Chief, NMS	LSR, NEI
Others:	John W. McClurkin	Ph.D.	Staff Fellow	LSR, NEI
	Arthur V. Hays	B.A.	Electronics Engineer	LSR, NEI
	Brad J. Zoltick	M.A.	Computer Programmer	LSR, NEI
	Jennifer A. Zarbock	B.A.	Electronics Engineer	LSR, NEI
	Merk Na Chee-Orts	Ph.D.	Visiting Associate	LSR, NEI
	Marc H. Cohen	M.S.E.	Visiting Associate	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Neural Modeling Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.6	4.0	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The brain requires many functions to span the gap from visual sensation to perception. Our studies indicate that different visual areas in the brain may communicate via temporally modulated messages. These findings suggest that understanding temporally encoded messages may give clues to the types of functional processes involved in visual information processing. New evidence suggests one such functional role for neurons in inferior temporal (IT) cortex during a short-term visual memory task.

In all visual areas studied, neurons encode information about pictures in a multidimensional temporal code. We are recording from individual neurons during a color-versus-form discrimination task. It appears that neurons in the IT cortex send four messages during a pattern recognition task: Two messages describe the color and form of the visual cue, and two describe the color and form of the visual target.

These results lead to a new hypothesis concerning the role of visual neurons in discrimination tasks. The multiplex-code hypothesis states that neurons carry separate color and form messages, multiplexed together. The temporal waveform of the response of visual neurons is formed by multiplying these two waveforms together.

Project Description

Objectives

Perception and recognition of complex visual pictures depend on the normal function of a hierarchically connected system of brain regions extending from the retina through the inferior temporal cortex. The properties of these regions are derived from the function of the single neurons within them. Thus, to understand how visual perception occurs, we must learn how neurons encode information in these successive stages of processing. If we could understand this neuronal code, it might become possible to distinguish between information related to the physical properties of a stimulus (eg, form, luminance, color) and that related to its behavioral significance (eg, leading to a reward).

Individual neurons in all the visual areas studied (retinal ganglion cell fibers, lateral geniculate nucleus neurons, pulvinar neurons, cortical neurons in visual areas V1, V2, V3 and V4, and inferior temporal cortical neurons) encode and transmit information about stationary, two-dimensional pictures that vary in form, color, brightness, and duration. The neurons use a multidimensional temporal code to represent and transmit their stimulus-dependent messages. We have shown that visual neurons convey complex messages about both a stimulus' physical parameters and its behavioral significance. Using information theory, we can begin to explore how physical and behavioral components of a neuron's response contribute to such higher visual cognitive functions as perception, attention, and memory.

Major Findings

We have developed a new approach to studying single neurons in which they are treated as communication channels that transmit information about visual pictures in their responses. This approach has allowed us to apply methods from signal processing, statistics, systems analysis, and information theory to understand single neurons.

According to a commonly held view of neuronal function, the strength of a neuron's response represents how closely the stimulus matches the receptive field's characteristics, eg, orientation or color. If response strength were the only parameter a neuron could use to encode information, different stimulus features would be confounded by individual neurons. Using informational analysis, we have shown that information about different stimulus parameters is not confounded but is carried across the different parts of the multidimensional neuronal code.

In recent experiments, responses of neurons in four visual cortical areas (V1, V2, V3, and V4) were recorded from a monkey trained to choose one of three parafoveal stimuli on the basis of whether their color or pattern matched that of a cue stimulus. These responses were modulated by the pattern and color of the stimulus on the receptive field and by the pattern or color of the preceding cue. Information about stimulus features developed continuously, but not uniformly, throughout the time course of neuronal responses. Most of the information was encoded in the initial 50-60 msec of the response. Some neurons also encoded a large amount of information in a second 50 msec interval, beginning 20-30 msec after the first.

These results show that neurons in V1-V4 carry information about the color and pattern of both current and remembered stimuli. Furthermore, the relative amount of information carried by a neuron depends upon the behavioral task. Finally, the development of information over time in different areas suggests that temporally modulated waves of activity may form a code for visual information. In fact, the response to each stimulus could be represented as the product of two waveforms, one for color and one for pattern. Feature-specific waveforms for each color and each pattern were isolated from the neuronal responses by a neural net. The product of these feature waveforms predicted the neuronal responses to stimuli with color and pattern combinations not used to train the neural net.

Feature waveforms were often similar for all neurons within a cortical area. To compare these waveforms across cortical areas, we pooled all the responses from neurons within each area. Waveforms encoding pattern were strikingly similar across all areas, irrespective of the behavioral task; those encoding color differed between cortical areas, depending on the behavioral task. The color waveforms V1 and V4 were different and showed no task dependence; those in V2 and V3 were different from those in V1 and V4 when pattern was the cue but identical to those in V1 when color was the cue. These results suggest that neurons convey information about compound visual features by multiplexing feature-specific messages. The invariance of pattern

waveforms suggests—at least for these stimuli—that the processing of pattern information is completed in V1.

Significance to Biomedical Research and the Program of the Institute

This project studies how visual information is encoded and transmitted by neurons. Knowledge of these fundamental processes is important for understanding deficits of visual processing, such as occur in amblyopia, and for developing visual prosthetic devices to compensate for field defects or blindness.

Proposed Course

Discovering that the responses of visual system neurons are multidimensional led to the discovery that information about multiple stimulus features may not be confounded by single neurons, a result with important, even revolutionary consequences. We now know that a substantial part of the temporal modulation arises after visual information has left the retina. Our latest results show that the neural code arises from the influence of feedback.

Since we found evidence of a neural code and saw a possible structure for it, we have been trying to delineate it. The properties of the code should give clues about the functions performed by the neurons. The structures of the spatial filters seen in lateral geniculate nuclei have already generated new ideas about the properties of encoded information there. Both issues are being pursued.

In addition, our findings suggest previously unconsidered principles as the basis for interactions among neurons. To investigate them, we need to collect and analyze data from several simultaneously recorded neurons. Thus, we have been developing the apparatus needed to make multiple, simultaneous single neuronal recordings. The apparatus should be completed sometime during the next year. We will relate the simultaneously recorded responses to each other using recent extensions to methods of signal identification that should allow us to develop models to describe relatively rapidly the roles of single neurons as components of larger networks. These studies should yield a better understanding of the information transmission mechanisms used for cognitive functions such as pattern perception and recognition.

Our findings suggest a completely new conceptional framework in which to investigate neuronal function. One presumed reason for the huge number of single neurons has been the need to unconfound stimulus features. However, we propose that the simultaneous messages about different features can be used as tags, so that messages arising in different processing regions of the visual system can be reunited in a unified percept, thus providing the mechanism to build a whole perception across many processing regions. Using new computational equipment, we are exploring this hypothesis both experimentally and theoretically.

NEI Research Program

Strabismus, Amblyopia, and Visual Processing—Visual Processing and Functional Organization (Structure and Function of Central Visual Pathways)

Publications

Eskandar EN, Hertz JA, Richmond BJ, Optican LM, Kjaer T: Decoding of neuronal signals in visual pattern recognition, in Moody JE, Hanson SJ, Lippmann RP (eds): *Neural Information Processing Systems 4*. San Mateo, Morgan Kaufmann, 1992, pp 356-363.

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Waitzman DM, Ma TP, Optican LM, Wurtz RH: Superior colliculus neurons mediate the dynamic characteristics of saccades. *J Neurophysiol* 66:1716-1737, 1991.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00049-14 LSR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cerebral Cortical Mechanisms for Eye Movements and Visual Attention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael E. Goldberg	M.D.	Chief, NMS	LSR, NEI
Others:	Edmond J. FitzGibbon	M.D.	Medical Officer	LSR, NEI
	Carol L. Colby	Ph.D.	Senior Staff Fellow	LSR, NEI
	Suzanne Y. Musil	Ph.D.	NRSA Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Neuro-Ophthalmologic Mechanisms Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.6	3.3	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two lines of inquiry were followed to determine how the cerebral cortex and its efferent regions control eye movements and visuospatial attention. In one, we studied the activity of neurons in the posterior cingulate during various visuomotor tasks to determine their activity with respect to visual stimuli that were either spontaneously salient or used as the targets for saccades. Neurons that responded to salient visual stimuli, such as checkerboards, did not respond to small spots of light, even when those spots were used as targets for saccadic eye movements or as stimuli in a luminance-detection task. These results suggest that the brain treats voluntary attention differently from involuntary attention to salient and novel stimuli. The cingulate cortex is important in the generation of voluntary but not involuntary attention.

The second line of inquiry was the study of visually responsive neurons in the superior colliculus. We used the visual stimulation engendered by eye movements to understand the effect of motor planning on the visual responsiveness of these neurons. For a distinct subpopulation of visuomovement neurons in the intermediate layers of the superior colliculus, the neurons would respond to visual stimuli that were about to be brought into their visual receptive fields by a saccadic eye movement. This predictive visual response resembled that previously described by this group for the lateral intraparietal area of the parietal cortex. It may well describe a general mechanism by which the brain achieves spatially accurate behavior.

Project Description

Objectives

This Section has concentrated on two aspects of the physiology and phenomenology of higher visual and oculomotor processing in monkey and man, especially as these functions relate to the parietal and frontal regions of the cerebral cortex, their afferent regions and their efferent targets. Previous work in this laboratory has shown that neurons in the parietal cortex have neurons which, paradoxically, maintain spatially accurate coding in a retinotopic framework by demonstrating presaccadic predictive responses: When a monkey makes a saccade that will bring a stimulus into the receptive field of a neuron, that neuron can start discharging in response to the stimulus before the saccade starts. In effect, this is a transient shift of visual receptive field, a projection target of the parietal cortex. We studied the superior colliculus to see whether it contains the presaccadic predictive responses discovered in the parietal cortex.

In a second series of experiments the cingulate cortex was shown to have neurons that discharged after eye movements and had visual responses. Work in the laboratory for the past year was concentrated on various aspects of neural function related to these earlier findings, ie, study of the physiology of the cingulate cortex in tasks designed to probe the nature of oculomotor and visuospatial processing in this region.

Methods

Monkeys implanted with magnetic search coils for the measurement of eye position, along with devices for temporary restraint and electrophysiological recording and stimulation, were trained to perform a number of visuomotor tasks, including fixation, saccades, and smooth pursuit. Microelectrodes were placed in the superior colliculus or posterior cingulate cortex, and single neurons were studied while the monkeys performed various visuomotor tasks.

Major Findings

Neurons in the posterior cingulate discharge after eye movements of a certain direction and amplitude. This discharge is modulated by target orbital position, saccade amplitude, saccade direction, and ambient illumination. Neurons that have no presaccadic response to saccade targets but are affected by ambient illumination can be shown to respond to the appearance of large, salient visual targets such as a black and white checkerboard. To determine whether voluntary attention is a component of the responsiveness of such neurons, we used two attentional tasks: (1) a peripheral attention task in which the monkey makes a luminance discrimination on a dim peripheral light and (2) a saccade task in which the monkey makes a saccade to the same target. Neurons that respond to the checkerboard fail to respond to these nonsalient but behaviorally significant targets. Targets that had the same luminance and total flux of the checkerboard but were dispersed and lacked the dramatic contrast of the checkerboard also failed to drive the neurons. These results suggest that the cingulate cortex is important in the analysis of the visual environment for novel, attention-grabbing stimuli but not for maintaining attention on behaviorally significant but visually nonsalient stimuli.

Neurons in the superficial and intermediate layers of the intermediate colliculus have visual responses. To see whether these visual responses had a presaccadic predictive study, we examined the neurons using a paradigm in which a saccade brought either a preexisting stimulus or the spatial location of a recently flashed stimulus into the neuron's receptive field. A special population of neurons in the intermediate layers demonstrated such predictive responses. Predominating in these neurons are partially clipped movement responses and large contralateral receptive fields. These neurons lie in the deeper parts of the intermediate layers of the colliculus, at least 0.5 mm below the stratum opticum. Neurons in the superficial layers of the colliculus do not show a predictive response.

Significance to Biomedical Research and the Program of the Institute

Understanding how the cerebral cortex and its afferent regions guide eye movements and modulate visual attention and learning is useful both as a model for the neural control of other, more complicated behaviors and as a key to understanding and developing treatments for disorders of the neural control of vision, eye movements, and attention.

Proposed Course

The examination of frontal eye fields will show whether they have predictive responses. We will examine the nature of the parietal cortical predictive responses to see if the responses occur in association with smooth pursuit eye movements as well as saccades, how they interact with orbital position planar gain fields, and whether the presaccadic predictive responses move continuously or discontinuously through the cortex.

NEI Research Program

Strabismus, Amblyopia, and Visual Processing—Visual Processing and Functional Organization (Structure and Function of Central Visual Pathways)

Publications

Colby CL, Duhamel JR, Goldberg ME: Neurons in the lateral intraparietal area (LIP) of the monkey remap visual space in connection with saccadic eye movements: II. Remapping of a visual memory trace. *Soc Neurosci Abstr* 17:1282, 1991.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00153-10 LSR

PERIOD COVERED: October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Visual Motion and the Stabilization of Gaze

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederick A. Miles	D.Phil.	Senior Research Physiologist	LSR, NEI
Others:	Urs Schwarz	M.D.	Visiting Associate	LSR, NEI
	Claudio Busettini	Ph.D.	Visiting Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Sensorimotor Research

SECTION: Oculomotor Control Section

INSTITUTE AND LOCATION: NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.3	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primates have excellent binocular vision and use vergence eye movements to align the two eyes on a common object, thereby facilitating stereopsis, the visual perception of depth. Binocular disparity, which refers to the slight difference in the locations of the images on the two retinas resulting from the slight difference in the viewpoint of the two eyes, is known to provide an important drive for these vergence eye movements. Disparity-induced vergence eye movements have traditionally been studied using an optical arrangement that allows identical stimuli to be presented independently to the two eyes, thus allowing the application of pure disparity stimuli. In these experiments we studied disparity-induced vergence eye movements in monkeys by placing a red filter over one eye and a green one over the other. The animal faced a tangent screen onto which were projected two large, textured patterns that were identical except in color—one being red (therefore seen only by the eye with the red filter) and the other green (seen only by the eye with the green filter). Sudden changes in the horizontal alignment of the two images (disparity steps) induced vigorous, machine-like vergence eye movements with a roughly exponential time course (time constant, 100 to 150 msec), despite the fact that the animals were neither trained to make such responses nor reinforced for doing so. The latency of such vergence eye movements was typically about 60 msec, which is about 100 msec less than previous values reported in the literature for experiments using smaller visual targets. One possibility was that these ultra-short-latency vergence responses were actually akin to the short-latency ocular-following responses that we have previously reported in response to the motion of large, textured scenes. However, this possibility is unlikely because the short-latency vergence responses did not show postsaccadic enhancement, one of the characteristic features of short-latency ocular following. Whereas neural encoding of disparity is known to occur in the cortex, these vergence responses must be cortically mediated, despite their ultra-short latency.

Program Description

Objectives

The discovery reported here was entirely serendipitous and not the result of any prior hypothesis. The objective of the original study was concerned with the question of whether early ocular following is generated by a neuronal system that is sensitive to the binocular disparity of visual motion. Existing evidence that human optokinetic responses are better when the moving visual scene is binocularly fused than when disparate supported the idea that the visual stabilization mechanism can respond selectively to the motion of objects in the plane of fixation and can ignore the motion of objects that are nearer or further. However, the visual stimuli in those experiments were prolonged (ie, closed loop) and the apparent sensitivity to disparity might have been secondary to changes in the subject's attention level, a factor known to be very important for human optokinetic responses. The disparate images (double vision) in this situation simply could have failed to capture the subject's attention so that it was not necessary to invoke any sensitivity to disparity in the neurons decoding the visual motion. To eliminate this attentional problem, we used several disparities and introduced them randomly just prior to moving the visual scene so there would be insufficient time for the subject—a monkey in our experiments—to alter his/her attentional level in accordance with the disparity. We were surprised to find that the sudden changes in the disparity of the scene resulted in vergence eye movements at unexpectedly short latencies, severely contaminating the short-latency ocular following responses under study. At that point, we turned our attention to short-latency vergence responses.

Methods

The movements of both eyes were recorded in response to sudden changes in the disparity of the visual scene in three monkeys. The animals faced a tangent screen onto which were projected two independent images that were identical except in color, one being red and the other green. (When viewed through goggles with a green gelatine filter in front of one eye and a red one in front of the other, the observer sees a single fused image that can be positioned anywhere in front or behind the plane of the screen by merely offsetting the two images horizontally.) At the start of a trial, the two images were exactly superimposed and so were seen in the plane of the screen at a distance of 3 reciprocal meters. Each monkey was required to fixate a target spot projected onto the center of the scene, and, at a variable time after the spot was extinguished, the horizontal alignment of the two images was suddenly changed, introducing disparity steps of ±0.5, 1, 2, or 3 meter angles. We computed vergence eye movements by subtracting the position of the right eye from the position of the left eye.

Major Findings

Disparity steps evoked vigorous and machine-like vergence eye movements with a roughly exponential time course (time constant, 100-150 msec) at latencies of approximately 60 msec. These vergence responses were obtained despite the fact that the monkeys were neither trained to produce them nor reinforced for doing so: Reinforcement was based solely on appropriate prior fixation of the centered target spot. The short latency and consistency of the responses raised the possibility that they represent a disconjugate tracking response akin to short-latency ocular following. A characteristic of short-latency ocular following is that stimuli applied in the immediate wake of a saccadic eye movement are much more effective than identical stimuli applied some time later, ie, post-saccadic enhancement. However, the vergence responses induced by disparity steps were not subject to post-saccadic enhancement, pointing to an independent neural mechanism.

Significance to Biomedical Research and the Program of the Institute

The latency of the vergence eye movements reported here was approximately 100 msec less than the values reported in the literature. We attribute this difference to the fact that the disparity stimuli used in our experiments extended over a considerably larger area of the visual field than those employed in any previous experiment. Further, a clear difference between the present and all previous studies was that in our experiments training or reinforcement of the animal was unnecessary; vergence eye movements appeared to be obligate. Previously undescribed, such reflex-like vergence responses to pure disparity stimuli are all the more surprising because neural

decoding of disparity is known to be a cortical function. Our experiments suggest that large-field disparity stimuli provide a powerful yet simple new approach for probing the neural processing of disparity information and its role in the cortical control of vergence eye movements.

Proposed Course

Future experiments will examine visual and vestibular ocular stabilization in the presence of computer-generated optic flow patterns that simulate those experienced by the moving observer.

NEI Research Program

Strabismus, Amblyopia, and Visual Processing—Image Formation and Stabilization (Ocular Motility)

Publications

Busettini C, Miles FA, Schwarz U: Ocular responses to translation and their dependence on viewing distance: II. Motion of the scene. *J Neurophysiol* 66:865-878, 1991.

Kimmig HG, Miles FA, Schwarz U: Effects of stationary textured backgrounds on the initiation of pursuit eye movements in monkeys. *J Neurophysiol*, in press.

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Miles FA, Busettini C, Schwarz U: Ocular responses to linear motion, in Shimazu H, Shinoda Y (eds): *Vestibular and Brain Stem Control of Eye, Head and Body Movements*. Tokyo, Japanese Scientific Societies Press/Karger, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00045-14 LSR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visuomotor Properties of Neurons in the Thalamus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: David Lee Robinson Ph.D. Section Chief LSR, NEI

COOPERATING UNITS *(if any)*

Department of Anatomy, Howard University (Robert J. Cowie, Ph.D.)

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Visual Behavior Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	1.0	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Throughout our daily lives, there is continual movement of the head and eyes, and their coordination, which is essential for proper vision, requires complex interactions to deal with changes in the position of the head and shifts of gaze. We have discovered a region in the pontine and medullary reticular formation that, when electrically stimulated, leads to brisk head movements. The vast majority of these movements are in the ipsilateral direction. Horizontal rotatory movement predominates, but tilting and stabilizing movements also are elicited. Although eye movements frequently occur with head movements, stimulation of this region with the head restrained does not produce eye movements. Movements elicited from this area never change the direction of gaze, which is stablized by the vestibulo-ocular reflex. However, the elicited head movements are modulated by the initial position of the head, attentive fixation, and level of alertness.

To understand the role of this region of the brainstem in the control of head movements further, we injected tracer compounds to reveal afferent and efferent connections. Areas that send axons to our head movement area include our previously identified head movement portions of the superior colliculus, those parts of the motor and premotor cortices that initiate head movements, the periaqueductal gray (extensively connected to limbic/emotional areas), and the interstitial nucleus of Cajal (long suggested to have head movement function). Our data demonstrate a central role for this part of the brainstem reticular formation in the integration of head movements. We also studied the efferent projections from the reticular formation head movement area by means of localizing terminal fibers, learning that the reticular formation sends axons to the motoneuronal regions of the upper cervical spinal cord. These connections provide direct access to the motoneurons that move the head. Furthermore, axonal endings within the caudal medulla, vestibular nuclei, and parvocellular reticular fields integrate head movements with the vestibulo-ocular reflex and other movements of the eyes. Together these studies demonstrate for the first time the critical role that the medullary and pontine reticular formation plays in the control of head movement. They show how it integrates influences from voluntary, stabilizing, emotional, and orienting systems.

Project Description

Objectives

Primates are continually moving, which requires constant control of the position of the head and eyes. Precise coordination of the head and eyes is needed to maintain stable vision. Within the past two decades, there has been considerable progress in understanding the neural control of eye movements, but little attention has been directed toward head movements. The experiments described here have been directed toward understanding the ways in which the brain stem controls movements of the head and toward learning the afferent and efferent connections of the major centers for control of head movements.

Methods

To localize specific regions of the brain and reliably test certain regions of the central nervous system, we trained adult monkeys to enter a primate chair, sit quietly, and fixate on a spot of light. After the monkeys' adaptation to these situations, we performed sterile surgery to implant them with several devices for recording eye and head movements, electrical activity within the brain, and instruments for head restraint and recording. Once the monkeys had recovered from the surgery, we positioned fine wire electrodes into specific brain sites and electrically excited these loci. The evoked head movements, eye movements, and other behavioral responses were recorded and quantified.

At the sites from which we could elicit head movements, we injected a tracer compound (horseradish peroxidase [HRP]). Examination of histological sections of the prepared brains allowed us to localize those neurons that project to the injected head movement center and to identify the efferent target sites of this head movement area.

Major Findings

We have discovered within the pontine and medullary reticular formation regions that have significant relations to head movement. When we electrically stimulate these areas, we obtain brisk movements of the head in the ipsilateral direction. These head movements are found within a topographically organized map of the whole upper body. The predominant movement is a horizontal, rotary motion of the head, but flexion-extension, tilting, and stabilizing movements can also be evoked.

Since eye movements frequently accompany movements of the head, we wished to determine whether this region also had some involvement with eye movements. At the loci from which we could elicit head movements, we tested the stimulation procedures with the head restrained. In none of these situations was an eye movement ever produced. We conclude from these observations that this area is related to head motion, not eye movements.

Whereas the superior colliculus is a major afferent to this region and it is related to shifts of gaze, our goal was to determine how the reticular area related to gaze shifts. We tested reticular stimulation in a variety of conditions while the monkey was actively fixating or scanning in the light, as well as during its relaxed behavior in total darkness. In no situation did stimulation of the reticular formation evoke a gaze shift associated with the head movement. These observations led to our conclusion that this part of the reticular formation mediates movements of the head and that the vestibulo-ocular reflex compensates for the movements unless the reflex is suppressed.

We found several conditions that modulate the amplitude of the evoked head movement. The initial position of the head had a significant impact on evoked head movements. When the head was pointed in the contralateral direction, the elicited movement was larger than when the head pointed in the ipsilateral direction. Furthermore, when the monkey was actively fixating a spot of light, the amplitude and velocity of the movement was larger than when the animal sat quietly in total darkness. We also observed that the animal was able to suppress the effectiveness of the electrical stimulation voluntarily. From these observations we conclude that this reticular region is a central site for the integration of head movements and that many other neural systems interact with the head movement system at this site.

Because this portion of the brain stem appears to play such a critical role in the control and integration of head movements, we sought to determine the afferent and efferent connections of this part of the brain. To study such questions, we injected a tracer compound (HRP) into reticular formation sites we had physiologically characterized as head movement sites. Some of the HRP was transported orthogradely,

demonstrating the efferent sites of our head motion area; other aspects of the HRP were transported anterogradely, illustrating the afferent regions.

When we evaluated the sites that receive efferent projections from our reticular head movement area, there were four major targets. The most prominent pathway was to the upper levels of the cervical spinal cord. Axon terminals were localized in the ventral grey matter of the cord in the region of the motoneurons that would effect head movements. A second efferent pathway from the reticular region traveled medially in the brain stem, entered the ipsilateral medial longitudinal fasciculus, and ended in the caudal medullary supraspinal nucleus as well as the upper cervical ventral gray matter. A third projection pathway traveled laterally within the brain stem to end in the ipsilateral parvocellular reticular field, the vestibular nuclei, and the deep cerebellar white matter. Finally, some terminal labeling was found in the interstitial nucleus of Cajal and the caudal fields of Forel. Together, these efferent projections from the head movement area end in those sites that actually move the head or in sites that other data suggest have integrative functions in head movement.

To understand what types of information are integrated within the head movement area, we localized neurons that had accumulated HRP by means of retrograde transport. One of the major sites of labeled cells was the superior colliculus; labeled neurons in the intermediate and deep layers were numerous, located mainly in the caudal portions of the colliculus. Some of our previous studies of the colliculus demonstrated that coordinated movements of the head and eyes are elicited by stimulation of these regions of the colliculus. These then represent precise anatomical and physiological correlations of the tectoreticular spinal route for orienting movements; they suggest that the head movements evoked from the superior colliculus are mediated by the pontomedullary reticular formation.

A second important site of retrogradely labeled neurons was within the cortex; we localized neurons in the ipsilateral motor and premotor cortices. The neurons containing the HRP were large-diameter, pyramidal cells which were located in layer V. The sites of these labeled cells were contained appropriately within the neck representations of the motor maps. These connections provide pathways through which voluntary processes can influence movements of the head. Perhaps this is the pathway through which the monkeys were able to reduce the effects of reticular stimulation intentionally.

In addition to these major afferents to our head movement area, there is input from the ipsilateral interstitial nucleus of Cajal, the nucleus of the posterior commissure, and the nucleus subcoeruleus. Contralateral to the HRP injections, we observed labeled neurons in the vestibular nuclei, nucleus prepositus hypoglossi, and the paramedian reticular formation.

Also of great interest is the observation of a substantial number of labeled neurons in the dorsal, lateral, and ventrolateral subnuclei of the periaqueductal gray. This region of the brain stem is powerfully connected with various limbic sites. Finally, there were small-diameter neurons labeled within laminae VII and VIII of the ventral horn of the cervical levels of the spinal cord on the ipsilateral side. These observations demonstrate that our reticular head movement area is richly interconnected with several neural systems such that it can participate in head movements within the context of orienting, stabilization, or emotive behaviors.

Significance to Biomedical Research and the Program of the Institute

There is a continual interplay between movements of the head and eyes. When the coordination of these two functional systems deteriorates, there is a diminution of vision. Losses in this coordination can result from various brain lesions and certain degenerative diseases. Understanding each region of the brain that controls movements of the head and its specific contributions facilitates precise diagnosis of central nervous system disorders. In addition, fuller understanding of the mechanisms of control of the head and eyes could lead to new strategies in rehabilitating humans with this and similar motor disorders.

Proposed Course

Data from a variety of laboratories have led to the suggestion that there are several different mechanisms for initiating saccadic eye movements. Under certain conditions, which manipulate the state and direction of attention, saccadic eye movements can begin with extremely short latencies, termed "express saccades." Our previous studies have demonstrated

that certain regions of the parietal cortex and pulvinar are related to visuospatial attention. In future studies, we will attempt to determine the contributions of these portions of the brain to express saccades. We will train rhesus monkeys in a variety of eye movement tasks that reliably evoke express saccades. Subsequently, we will electrically excite or chemically inactivate the pulvinar and parietal cortex to learn how their attentional mechanisms contribute to the initiation of saccadic eye movements.

NEI Research Program

Strabismus, Amblyopia, and Visual Processing—Visual Processing and Functional Organization (Structure and Function of Central Visual Pathways)

Publications

McClurkin JW, Gawne TJ, Richmond BJ, Optican LM, Robinson DL: Lateral geniculate neurons in behaving primates: I. Responses to two-dimensional stimuli. *J Neurophysiol* 66:777-793, 1991.

Robinson DL: Functional contributions of the primate pulvinar. *Prog Brain Res*, in press.

Robinson DL, McClurkin JW, Kertzman C, Petersen SE: Visual responses of pulvinar and collicular neurons during eye movements of awake, trained monkeys. *J Neurophysiol* 66:485-496, 1991.

Robinson DL, Petersen SE: The pulvinar and visual salience. *Trends Neurosci* 15:127-132, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00109-12 LSR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visuomotor Processing in the Primate Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Wurtz	Ph.D.	Chief	LSR, NEI
Others:	Charles J. Duffy	M.D., Ph.D.	Staff Fellow	LSR, NEI
	Hiroshi Aizawa	Ph.D.	Visiting Fellow	LSR, NEI
	Gregg H. Recanzone	Ph.D.	Guest Researcher	LSR, NEI
	Douglas Munoz	Ph.D.	Guest Researcher	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Visuomotor Integration Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Among experiments concentrating on the visual motor system of monkeys and humans, the first set involved analysis of the mechanisms controlling the generation of rapid or saccadic eye movements in the superior colliculus of awake-behaving monkeys. Previous experiments had demonstrated that a subset of cells within the superior colliculus discharges with a burst of activity before the onset of saccadic eye movements. Cells just deeper to these burst cells also discharge in relationship to saccadic eye movements but without the crisply delineated high-frequency bursts. These cells, characterized by anticipatory discharges in addition to saccade-related bursts, have been called preparatory cells. In the preparatory cell layer, and once the saccade began, there was a continuous change in the spatial distribution of activity. Preparatory cells located progressively more rostral to the initially active zone were progressively activated until the activity reached the rostral pole of the superior colliculus. This moving edge of activity represents a potentially new mechanism for controlling the amplitude of the generated saccadic eye movement.

The second set of experiments concerned the use of optic flow stimuli, those stimuli that are generated as we move through the visual environment. We made several psychophysical observations on the human response to the simulated optic flow pattern that we had previously used to test the visual response of neurons in the monkey brain. We observed a striking illusion in the optic flow pattern. When we superimposed a field of moving dots on the pattern of radially moving dots, we found that human subjects mislocated the center of this radial pattern; they consistently indicated that the center was shifted in position. We think this illusion reveals a strategy used in separating the location of the center of the optic flow pattern from the confounding movement of other objects in the visual field as one moves through the visual environment.

Project Description

Objectives

The work performed this year centered on two functions of the visual-oculomotor system. The first is the saccadic system that moves the eye from one object of interest in the visual field to another. This is probably the best understood of all oculomotor systems, and our research has expanded on previous work in the monkey superior colliculus. The second experimental area concerns human behavior and the use of optic flow information, the visual motion that falls on the retina as a result of the observer moving through the environment. This higher order visual motion processing is likely to be used to provide visual information on the direction in which the observer heads through the environment.

Methods

In studies of the saccadic system, we have continued to use the rhesus monkey, *Macaca mulatta*. It is an ideal model of the visual-movement systems in humans, given that its eye movements and visual processing are so close to those in humans. In our experiments, the monkeys are awake and trained to perform visual motor tasks; their cooperation is an essential ingredient of the experiments. The entire experiment must be painless and stressless for the monkey because the visual tasks performed require a high level of cooperation that would deteriorate were any pain present. A monkey was trained to look forward at a small spot on a screen to obtain a liquid reward. A computer system measured the monkey's eye movements, registered the activity recorded from single cells in the brain, entirely controlled the experiment, and displayed experimental online progress.

In our work on humans, the subjects looked directly forward at a screen on which an optic flow stimulus was projected. Their task was to indicate with a light pointer where the center of this flow stimulus appeared to be located.

Major Findings

In the first set of experiments on the monkey, we studied the mechanisms controlling the generation of rapid or saccadic eye movements in the colliculus in awake behaving monkeys. Previous experiments had demonstrated that a subset of cells within the superior colliculus discharge with a burst of activity before the onset of saccadic eye movements. Analysis of these cells over the past 20 years has shown that they are critical for the generation of saccadic eye movements. Cells just deeper than these burst cells also discharge in relationship to saccadic eye movements but without the crisply delineated high-frequency bursts. These preparatory cells' characteristic anticipatory discharges occur in addition to the saccade-related bursts of the cells just above them in the superior colliculus.

We observed that the cells also have large movement fields that lead to activity of the cell preceding all saccades greater than a given amplitude. In preparatory cells, activity builds up at a specific point in the motor map that codes the amplitude and direction of the impending saccadic eye movement. Once the saccade begins, there is a continuous change in the spatial distribution of activity in these neurons across this motor map. Preparatory cells located progressively more rostral to the initially active zone are active until that activity reaches the rostral pole of the superior colliculus. Their activity locus moves across the collicular movement-related layer from the area representing large saccades to the area representing small saccades, in contrast to that of the burst cells, whose activity increases and then decreases with a saccade, all at only one location within the superior colliculus. This moving hill of activity represents a potentially new mechanism for controlling the amplitude of the generated saccadic eye movement.

The second area of investigation summarized here is related to the analysis of large-field visual motion, optic flow, that simulates the visual motion generated as an observer moves through the environment. We had previously found that neurons in an area of the cerebral cortex referred to as extrastriate area medial superior temporal (MST) discharge in response to large field stimulation and have other characteristics that would make them appropriate for the analysis of optic flow stimulation.

This year, while making several psychophysical observations on the human perception of the simulated optic flow pattern we had previously used to test the visual response of neurons in monkey brain, we noted a striking illusion in the optic flow pattern. When we superimposed a field of moving dots on the pattern of radially moving dots, we found that

human subjects mislocated the center of this radial pattern; they consistently indicated that the position of the center shifted in the direction in which the plane of dots moved. The illusory shift of this center, which persisted even when the observer was free to make eye movements, indicated that it was independent of any particular following eye movement. We think this illusion reveals a strategy used in separating the location of the focus of expansion from the confounding movement of other objects in the visual field as one moves through the visual environment.

Significance to Biomedical Research and the Program of the Institute

Saccades are necessary to move the eye from one point in the visual field to another. These movements must also be coordinated with head movements. Our experiments have revealed a new set of cells that may contribute to this control and coordination.

The contribution of optic flow stimulation to the guidance of our movement through the environment has been the subject of increasingly active psychophysical and neural modeling investigations in other laboratories. Our work on psychophysics this year suggests how the brain may separate the information contained in the optic flow stimulus that results from movement of the observer, as opposed to rotation of the observer's head and eyes.

Both the saccadic and the optic flow systems are functions essential to visual performance and the control of movement in normal humans and those with diseases or damage to the nervous system.

Proposed Course

Further experiments on saccadic eye movements will determine whether the preparatory cell function is related to head movement as well as eye movement. In further analysis of optic flow, we will return to the analysis of the response of neurons in MST in the monkey, in part to see whether the stimuli used in humans reveal more of the functional organization of cells in the brain.

NEI Research Program

Strabismus, Amblyopia, and Visual Processing—Visual Processing and Functional Organization (Structure and Function of Central Visual Pathways)

Publications

Yamasaki DS, Wurtz RH: Recovery of function after lesions in the superior temporal sulcus in the monkey. *J Neurophysiol* 66:651-673, 1991.

Wurtz RH, Duffy CJ: Neuronal correlates of optic flow stimulation, in Cohen B, Tomko DL, Guedry F (eds): Sensing and Controlling Motion: Vestibular and Sensorimotor Function. *Ann NY Acad Sci* 656:205-219, 1992.

Wurtz RH, Duffy CJ, Roy J-P: Motion processing for the control of movement in primate extrastriate area MST, in Ono T, Squire LR, Raichle ME, Perrett D, Fukuda M (eds): *Brain Mechanisms of Perception and Memory: From Neuron to Behavior*. New York, Oxford University Press, in press.

Roy J-P, Komatsu H, Wurtz RH: Disparity sensitivity of neurons in monkey extrastriate area MST. *J Neurosci* 12:2478-2492, 1992.

Ophthalmic Genetics and Clinical Services Branch

Report of the Chief, Ophthalmic Genetics and Clinical Services Branch

Muriel I. Kaiser-Kupfer, M.D.

The Ophthalmic Genetics and Clinical Services Branch within the National Eye Institute Intramural Research Program has been operational since February 1989. The Branch is organized into four sections: Ophthalmic Genetics, Acting Chief Muriel I. Kaiser-Kupfer, M.D.; Cataract and Corneal Diseases, Acting Chief Manuel B. Datiles, M.D.; Ophthalmic Pathology, Acting Chief W. Gerald Robison, M.D. Jr., Ph.D.; and Clinical Services, Acting Chief Rafael C. Caruso, M.D.

The purpose of the Branch is to conduct clinical and laboratory research on gene expression and molecular interactions important to the eye and to apply clinically relevant research findings to the prevention, diagnosis, and treatment of diseases affecting the eye and visual system. Such disorders include corneal and retinal diseases, cataract, and visual pathway abnormalities.

The Branch is responsible for the essential psychophysical and electrophysiologic diagnostic tests of visual function required by clinical intramural research programs of all the Institutes. In addition, it processes ocular clinical biopsy and autopsy materials. The Branch differs from other NEI laboratories engaged in molecular investigations because its emphasis is the translation of appropriate research findings directly to the clinical setting. This Branch is also a point of focus for the trans-NIH emphasis on research in genetics, more effectively aligning its organizational structure within the Institute's intramural research program.

Since beginning its operation, the Branch has shown considerable growth and productivity.

Section on Cataract and Corneal Diseases

The Section on Cataract and Corneal Diseases continued to pursue research on the anterior segment, especially the short-term and long-term effects of contact lens wear on the cornea. Analysis of the data may be helpful in understanding the dynamics of contact lens-cornea interaction, the risk to corneal tissues, and how systemic or local ocular disorders may increase the risk of wearing contact lenses. Corneal endothelial morphology is being studied by specular microscopy to compare the endothelial status in patients wearing different types of lenses. The development of automated computer analysis is under way to facilitate the analysis of data, which when performed by hand is laborious and time consuming.

This Section has been particularly productive in studies using different systems to develop objective and subjective methods of monitoring and documenting opacities in the human lens. Reproducibility studies on objective systems include the use of the Scheimpflug cameras (Zeiss and Oxford) and the retroillumination camera (Neitz and Oxford). Subjective systems or methods such as the LOCS II grading system and the effects of cataracts on visual perception, contrast sensitivity, and glare may be useful in identifying additional parameters. These systems are used to study the natural history of various cataracts, eg, pre-senile, senile, or age-related, steroid-induced, radiation, diabetic, retinitis pigmentosa, gyrate atrophy (GA), and neurofibromatosis 2. Genetic linkage studies are under way to pursue the gene(s) of congenital cataracts. Monitoring and documenting human cataract development is a crucial step toward the ultimate testing of several medications that might be helpful in preventing or reversing human cataracts.

Research on cataractogenesis has been hampered by the extreme scarcity of tissue and an abrupt shift in surgical technique from intracapsular (intact lens) to extracapsular (fragmented lens). Through the collaborative efforts of cataract surgeons and basic researchers, we have made efforts to develop and modify techniques to study materials that become available at surgery and can be well documented clinically. We are now carefully documenting cataracts in preoperative patients using clinical and photographic LOCS II grading, Zeiss Scheimpflug and Oxford retroillumination videophotography, and

image analysis. Cataracts are extracted extracapsularly with implantation of an intraocular lens. The specimens obtained are examined histologically via light and electron microscopy and biochemically by two-dimensional gel electrophoresis (PHAST and LSB systems). Cataractous specimens are compared with normal tissues obtained from eye bank eyes. Abnormal proteins are identified by immunoblotting techniques as well as by protein sequencing.

With aging there is an acidic shift of proteins, and an increased number of polypeptide species are found in the molecular weight range of the crystallins. These aging changes need to be differentiated from changes occurring in cataract formation.

Investigators in this Section have been in the forefront of recognizing the role of the neural crest in normal and abnormal development of the anterior segment. Studies continue on anterior chamber abnormalities and iridocorneal endothelial syndrome patients.

Section on Ophthalmic Genetics

Studies by the Ophthalmic Genetics Section have emphasized retinal degeneration and ophthalmic involvement in systemic genetic diseases. This Section has been a leader in studying GA of the choroid and retina. The accumulation of natural history data and the work on the definition of genetic abnormalities has been unique. Confirmation of evidence for biochemical, clinical, and molecular heterogeneity continues. There appear to be many different single-point mutations in the ornithine aminotransferase gene in GA patients. Dietary intervention studies that use an arginine deficient diet have been very promising, especially in young patients, in whom a delay in the onset of pathologic changes has been demonstrated.

Foveal cone sensitivity (assessed by measurements of increment thresholds) and orientation (estimated with measurements of the Stiles-Crawford effect) were found to be abnormal in a group of patients with GA. These results suggest that foveal cones are altered in orientation and sensitivity before the encroachment on the foveal area by the atrophic lesions of GA.

Albinism in animals has been associated with an anatomic anomaly of the visual pathways characterized by excessive crossing of the retinogeniculate fibers with two different modes of geniculocortical projection. In humans, indirect evidence of the same anomaly is demonstrated by asymmetry in visually evoked potentials (VEPs) elicited by pattern reversal stimulation. Recent studies using appearing-disappearing patterns claim VEP asymmetry is diagnostic; they propose a uniform type of asymmetry. We used the same recording conditions to determine the diagnostic value of VEP in albinism and to attempt correlation of the VEP results with clinical features.

This study shows that two different patterns of VEP asymmetry in albinism. They may be explained by differences in the reorganization of the geniculocortical pathway. Although VEP asymmetry is very frequent in this condition, it may not be constant. However, its value is decreased in some cases in which the low amplitude of the responses makes interpretation difficult. Furthermore, there is no correlation of the type of asymmetry with any other feature of albinism.

Collaboration with the Interinstitute Genetics Program has continued, with active participation by the Genetics Clinic. During the past year, we saw approximately 200 individuals representing approximately 60 different disease categories. Because of the high frequency of ocular involvement in these cases, almost all of these patients were evaluated by the Ophthalmic Genetics staff.

Neurofibromatosis 2 (NF2), otherwise known as bilateral acoustic neuroma, is inherited as an autosomal dominant disorder. Multiple members of several large pedigrees as well as a large number of unrelated families have been studied in collaboration with Dr. Roswell Eldridge (National Institute of Neurologic Diseases) and Dr. Dilys Parry (National Cancer Institute). An important original observation was the striking frequency of posterior capsular cataract in patients with NF2 (80-85%). In addition, 30% of patients have shown an associated cortical cataracts. These findings are helpful in establishing a diagnosis of NF2 in at-risk patients. The etiology of the cataract is unclear; however, it is interesting that the gene locus for bilateral acoustic neuromas is on chromosome 22 as is the gene for β-crystalline.

In a collaborative study with Dr. Larry Charnas of the National Institute of Child Health and Human Development, data from patients with Lowe's syndrome and the patients' mothers have confirmed the

usefulness of detecting lens changes in making the diagnosis of the carrier state.

Finally, the results from the continuing double-masked control clinical trial of topical cysteamine in patients with nephropathic cystinosis are very exciting. After confirming the usefulness of 0.5% cysteamine eye drops in the young patients, we expanded our study to include older patients, and the results were similarly striking. Particularly important is the fact that these patients have shown dramatic relief from their ocular symptoms, with a decrease in crystals in the treated eye and a significant improvement in quality of life.

Section on Clinical Services

The Clinical Services Section has been very active in characterizing psychophysical and electrodiagnostic findings in patients with diseases that affect the eye and the visual system. Continued documentation by noninvasive techniques has shown that more and more refined and accurate classification of diseases is possible. Psychophysical and electrodiagnostic information is particularly helpful in understanding the pathogenesis of disease, as well as being available for use as a marker in various treatment modalities. The results of specially designed studies have demonstrated a strong positive correlation between electroretinogram (ERG) amplitude and electrooculogram (EOG) results in both patients with retinal diseases and normal subjects. These findings confirm that EOG results are not independent of ERG amplitude and emphasize the need for a diagnostic test of retinal pigment epithelium (RPE) integrity that does not depend on light-induced RPE responses.

Other studies described a reduction in the amplitude of the ERG responses correlated with an increase in ocular length. These findings suggest that the effect of ocular length on ERG amplitude should be taken into consideration with ERG results from patients with high refractive error.

Section on Ophthalmic Pathology

The Section on Ophthalmic Pathology has provided technical support services to investigators involved in clinical and basic research as well as to those performing routine pathology. Careful monitoring of the volume of material handled shows a steady increase in processing by the laboratory, with excellent results. Considerable savings to the Institute have resulted from the elimination of costly contract services.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00187-09 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effects of Corneal Contact Lenses on the Cornea

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Manuel B. Datiles	M.D.	Medical Officer	OGCSB, NEI
Others:	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Louella Lopez	M.D.	Visiting Associate	OGCSB, NEI
	Sailaja Chintalagiri	M.S.	Visiting Associate	OGCSB, NEI

COOPERATING UNITS *(if any)*

Image Processing and Analysis Laboratory, Division of Computer Research and Technology, NIH (Mark Vivino, B.S.)

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Cataract and Corneal Diseases

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.225	0.200	0.025

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This investigation of short-term as well as long-term effects of contact lens wear on the cornea includes specular microscopy studies of changes in corneal curvature, corneal epithelial morphology, and corneal endothelial cell morphology. Analysis of the data obtained will help us understand the dynamics involved in the interaction between a contact lens and the cornea, the risk to corneal tissues, and how a systemic or local disorder may increase these risks. In addition, we are studying the differences in corneal endothelial status in wearers of soft compared with hard contact lenses.

Animal models showing corneal endothelial abnormalities similar to those in long-term contact lens wearers are also being explored in diabetic and galactosemic animal models. Treatment with aldose reductase inhibitors helps prevent these corneal abnormalities.

Project Description

Clinical Protocol Number

84 EI-133

Objectives

The objective of this project is to investigate the effects of contact lens wear on corneal tissues, including the study of factors that increase or decrease the potential risk of injury to corneal tissues by contact lens wear.

Methods

Each patient's complete history, ophthalmologic examination, photography, keratotometry, pachymetry, and specular microscopy of the corneal endothelium are used.

Major Findings

We have found that diabetes may increase the risk of complications from contact lenses in the first 6 months of wear. In addition, we have found changes in the corneal endothelium after long-term wear of contact lenses. These changes include polymegathism and pleomorphism. Furthermore, 2 years after some of our patients discontinued wearing contact lenses, we found a trend toward recovery but no statistically significant change.

In addition, we found that diabetic and galactosemic animals have these endothelial abnormalities and that treatment with aldose reductase inhibitors prevented these abnormalities.

Significance to Biomedical Research and the Program of the Institute

Contact lenses are commonly used for correction of errors of refraction as well as for therapy. However, our knowledge of the interaction of contact lenses with the cornea and tears is limited. In addition, risks associated with wearing contact lenses are also poorly understood. Understanding the interaction between contact lenses and corneal tissues will allow us to determine why some patients cannot wear contact lenses and provide methods to avoid some of the complications associated with contact lens wear.

Proposed Course

The following studies are in progress or proposed for next year: (1) continued examination of patients who have worn hard contact lenses and have now shifted to gas-permeable or soft contact lenses; (2) recruitment of patients who plan to discontinue contact lens wear or to shift from one type of contact lens to another; and (3) development of automated computer analysis of all types to facilitate the data analysis (under way).

NEI Research Program

Corneal Diseases—Corneal Edema, Endothelial Dysfunction, Dystrophies and Inherited Disease

Publications

Sibug M, Datiles M, McCain LM, Kashima K, Kracher G: Specular microscopic studies on the corneal endothelium after cessation of contact lens wear. *Cornea* 10:395-401, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00188-09 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Documentation and Monitoring of Opacities in the Human Lens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Manuel B. Datiles	M.D.	Medical Officer	OGCSB, NEI
Others:	Rafael C. Caruso	M.D.	Visiting Scientist	OGCSB, NEI
	Benjamin Magno	M.D.	Visiting Associate	OGCSB, NEI
	Susan M. Lasa	M.D.	Visiting Associate	OGCSB, NEI
	Sailaja Chintalagiri	M.S.	Visiting Scientist	OGCSB, NEI
	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Louella Lopez	M.D.	Visiting Associate	OGCSB, NEI

COOPERATING UNITS *(If any)*

Image Processing and Analysis Laboratory, Division of Computer Research and Technology (DCRT), NIH (Benes Trus, Ph.D.; Mark Vivino, M.S.; Hal Frederickson, B.S.); Biomedical, Engineering and Instrumentation Branch, DCRT, NIH (Michael Unser, Ph.D.); Epidemiology Branch, NEI, NIH (Robert Sperduto, M.D.; Marvin Podgor, Ph.D.; Valeria Friedlin, Ph.D.; Nancy Remaley, B.S.; Roy Milton, Ph.D.)

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Cataract and Corneal Diseases

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.305	2.280	0.025

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project uses different systems to develop objective and subjective methods to monitor and document opacities in the human lens. We are actively recruiting patients with and without cataracts for reproducibility studies on the objective systems—the Scheimpflug (Zeiss and Oxford) and retroillumination (Neitz and Oxford) cameras. Our study of subjective systems or methods such as the LOCS II grading system and the effects of cataracts on visual perception, contrast sensitivity, and glare may be useful in identifying additional parameters for monitoring cataract presence, progression, or regression. We are now using these systems to study the natural history of various cataracts, such as presenile, senile, or age-related, steroid-induced, radiation, diabetic, retinitis pigmentosa, gyrate atrophy, and neurofibromatosis 2 cataracts. This study will prepare the way for eventual clinical trials of anticataract drugs.

Genetic linkage studies now under way are pursuing the gene(s) of congenital cataract.

Project Description

Additional Personnel

Maria A. Drews-Bankiewicz	M.D.	Visiting Fellow, OGCSB, NEI
Yvonne Duglas-Tabor	B.S.	Biologist, OGCSB, NEI
Marvin Podgor	M.D.	Epidemiologist, NEI
Rita Hiller	M.S.	Epidemiologist, NEI
Robert Sperduto	M.D.	Chief, Epidemiology Section, NEI
Doretha Leftwood	B.S.	Computer Specialist, OGCSB, NEI
Anup Mahurkar	B.S.E.	Computer Engineer, OGCSB, NEI
Laura Wozencraft		Genetic Counselor, OGCSB, NEI
Mark H. Scott	M.D.	Senior Staff Fellow, OGCSB, NEI
J. Fielding Hejtmancik	Ph.D.	LMOD, NEI

Clinical Protocol Number

84-EI-132

Objectives

The objective of this project is to formulate means of documenting cataract formation and progression. This is an important step prior to undertaking clinical trials of drugs purported to prevent cataract and cataract progression. Family studies are involved in looking for the gene for congenital cataract via linkage studies.

Methods

Complete ophthalmologic examination, including contrast sensitivity, glare testing, and potential acuity testing, are performed for each person in the study. Techniques used to measure and evaluate cataracts include Scheimpflug photography, retroillumination photography, specular microscopy, and laser light-scanning spectroscopy.

Major Findings

We have found that clinical grading of cataracts using the LOCS II system can quantitatively detect the progression of age-related cataracts within 1 year. In addition, we found that in various types of cataracts, glare and contrast sensitivity testing shows abnormal results only in the severe or more advanced grades. The only exception was in posterior subcapsular cataracts, which showed an abnormality in contrast and glare sensitivity in the early stages. In a study of pure nuclear cataracts, we found a significant correlation between lens nuclear density (measured by either LOCS II grading or Scheimpflug photography) and contrast sensitivity loss of intermediate and high spatial frequencies.

In our continued development of objective, semiautomated methods of detecting and following cataracts, we now are able to quickly perform densitometry of Scheimpflug nuclear cataract images and compare them to previous images to detect significant changes, which are expressed in optical density units. For posterior subcapsular and cortical cataract, we also have developed a semiautomated method of quantitating the cataracts in square millimeters using retroillumination photographs.

Significance to Biomedical Research and the Program of the Institute

Monitoring and documenting human cataract progression is a crucial step toward the ultimate testing of several medications believed capable of preventing or reversing human cataracts. This step is also important in categorizing types of cataracts in various parts of the world and correlating them with the physical and genetic factors within specific geographic regions.

Subjective methods of determining visual function are also important to determine the degree of handicap cataract patients have in coping with daily activities. Since in our studies none of the subjective methods could demonstrate subjective experiences in early cataracts, research is needed to develop more sensitive techniques.

Proposed Course

We will continue the study and development of subjective and objective methods of documenting and monitoring human cataracts. We will pursue the improvement and automation of present systems of lens photography (eg, such as Scheimpflug, retroillumination, and laser-light spectroscopy), as well as exploration of possible applications of new technological advances. Appropriate population groups for study will be identified.

NEI Research Program

Cataracts—Epidemiology of Cataract

Publications

Datiles M: Clinical evaluation of cataracts, in Tasman W, Jaeger E (eds): *Duane's Clinical Ophthalmology* Philadelphia, Lippincott Co., in press.

Datiles M, Kinoshita J: Pathogenesis of cataracts, in Tasman W, Jaeger E (eds): *Duane's Clinical Ophthalmology*. Philadelphia, Lippincott Co. 1:72B:1-14, 1991.

Drews-Bankiewicz MA, Caruso RC, Datiles MB, Kaiser-Kupfer MI: Contrast sensitivity in patients with nuclear cataracts. *Arch Ophthalmol* 110:953-959, 1992.

Lasa S, Datiles M, Podgor M, Magno B, Lee J: Contrast and glare sensitivity: Association with the type and severity of the cataract. *Ophthalmology* 99:1045-1049, 1992.

Magno B, Datiles M, Sperduto R, Podgor M, Lasa S, Lee J: Cataract progression rates using the lens opacity classification system. *Invest Ophthalmol Vis Sci*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00212-07 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Human Lens Material for Determining Possible Causes of Cataracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Manuel B. Datiles	M.D.	Medical Officer	OGCSB, NEI
Others:	Susan M. Lasa	M.D.	Visiting Associate	OGCSB, NEI
	Benjamin Magno	M.D.	Visiting Associate	OGCSB, NEI
	Yvonne Tabor	B.S.	Biological Technician	OGCSB, NEI
	Pushpa Sran	M.D.	Medical Officer	OGCSB, NEI
	Louella Lopez	M.D.	Visiting Associate	OGCSB, NEI
	Miguel Burnier, Jr.	M.D.	Visiting Scientist	LI, NEI

COOPERATING UNITS *(if any)*

Laboratory of Mechanisms of Ocular Disease, NEI, NIH (Donita Garland, Ph.D.; J. Samuel Zigler, Jr., Ph.D.; Paul Russell, Ph.D.); Image Processing and Analysis Laboratory, Division of Computer Research and Technology, NIH (Benes Trus, Ph.D.; Mark Vivino, M.S.)

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Cataract and Corneal Diseases

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.55	1.55	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is an extreme scarcity of human cataract material because of an abrupt shift of cataract surgical technique from intracapsular (intact lens) to extracapsular (fragmented lens), with the advent of the use of intraocular lens. We are exploring ways by which fragmented lens materials can be maximally used in cataract basic research through close collaboration with cataract surgeons and basic researchers and through modification of techniques by both groups.

We are now carefully documenting the cataracts in patients preoperatively using clinical and photographic LOCS II grading and Zeiss Scheimpflug and Oxford retroillumination video photography and image analysis. Cataracts are extracted extracapsularly with implantation of an intraocular lens. Specimens obtained are examined histologically using light and electron microscopy and biochemically using two-dimensional gel electrophoresis (PHAST and LSB systems). Cataractous specimens are compared to normal tissues obtained from eye bank eyes. Abnormal proteins are identified using immunoblotting techniques as well as protein sequencing.

Project Description

Additional Personnel

Samuel Zigler	Ph.D.	Head, Section on Cataracts, LMOD, NEI

Clinical Protocol Number

84-EI-194

Objectives

In light of the present and future scarcity of intact human cataracts for basic research, this study was designed to develop methods in which fragmented lens materials can be maximally used for biochemical and genetic research.

Methods

We examined patients who have different forms of cataracts and documented their cataracts as described in the project Documentation and Monitoring of Opacities in the Human Lens (Z01 EY 00188). After extracapsular cataract extraction, fragmented lens materials—including the anterior lens capsule, lens nucleus, and aspirated lens cortical material—undergo protein and biochemical analyses. Some of the specimens are frozen for future genetic studies. We also have undertaken studies to determine the visual results of current techniques of cataract surgery and how to modify and improve them further.

Major Findings

We have found that a successful collaboration needs a close professional relationship between the clinician-surgeon and the basic researcher. Although the two professionals have different immediate priorities (one being patient care; the other, adequacy of tissue sample for laboratory studies), the ultimate goal of alleviating human suffering is the same.

The two-dimensional gel electrophoresis technique may be extremely useful in determining lens protein changes using very small amounts of time (300 mcg). Aging results in acidic shifting of proteins, an increased number of polypeptide species in the molecular weight range of the crystallins, and covalent cross-linking of crystallins—changes that need to be differentiated from changes occurring in cataract formation. We also have found that lens material aspirated through the irrigator-aspirator or phakoemulsifier lose some crystallins; optimum samples are those we obtain separately, thus avoiding oxidation.

Significance to Biomedical Research and the Program of the Institute

A severe setback is being dealt many cataract projects because of the lack of human cataract material available for basic research studies. Whereas the current technique, which involves fragmenting the cataract during extraction, is extremely successful and effective, there is no foreseeable change back to utilization of the intracapsular method (removal of the lens in toto). Hence, it is imperative that we modify our basic research methodology to adapt to the use of fragmented lens materials in order to continue the various basic lens research projects that deal with human materials.

Proposed Course

We will continue to pursue development of the use of fragmented lens material in basic research experiments. Using two-dimensional gel electrophoresis, we will study ways in which surgeons can modify their surgical techniques without compromising patient care while providing scientists with critical lens tissue for basic research. In addition, we will investigate ways in which scientists can work with surgeons in handling lens materials to maximize the quality of specimens for basic research.

NEI Research Program

Cataract—Treatment of Cataract and Correction of Aphakia

Publications

Datiles M, Schumer J, Zigler S, Russell P, Anderson L: Two dimensional gel electrophoretic analysis of human lens proteins. *Curr Eye Res* 11:669-677, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00084-14 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carl Kupfer	M.D.	Director	NEI
Others:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Manuel B. Datiles	M.D.	Medical Officer	OGCSB, NEI
	Maria Susan M. Lasa	M.D.	Visiting Associate	OGCSB, NEI
	Benjamin V. Magno	M.D.	Visiting Associate	OGCSB, NEI
	Louella Lopez	M.D.	Visiting Associate	OGCSB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Cataract and Corneal Diseases

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.45	0.35	0.10

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent embryological research has indicated the role of the neural crest in contributing to all connective tissues anterior to the lens epithelium. Therefore, the group of developmental anomalies of the anterior chamber with glaucoma or ocular hypertension is being reviewed.

Project Description

Clinical Protocol Number

77-EI-119

Objectives

The object of this study is to determine whether congenital or developmental anomalies of the anterior chamber are related to faulty migration or terminal differentiation of neural crest tissue.

Methods

Patients of all ages with congenital or developmental anomalies of the anterior chamber are being examined to determine the involvement of cornea, trabecular meshwork, iris stroma, lens, and ciliary body. When intractable glaucoma cannot be controlled with medication, surgery is performed, and the specimens are examined histologically. When the lenses become cataractous, cataract extractions are performed and the lens epithelium is grown in tissue culture. When the cornea is opaque and corneal transplantation indicated, that procedure is performed and the corneal specimen is examined histologically.

Major Findings

It appears that in this group of anomalies of anterior chamber development, there are pathological changes in one or several tissues derived from neural crest. These changes include corneal stroma, corneal endothelium, anterior iris stroma, Descemet's membrane, and trabecular meshwork endothelium.

We recently performed trabeculectomies on patients with the irido-corneal-endothelial syndrome. Histopathologically, we found a membrane covering the trabecular meshwork. That membrane may have caused peripheral anterior synechias and glaucoma.

Significance to Biomedical Research and the Program of the Institute

A better understanding of the pathogenesis of this glaucoma may help by improving diagnosis and treatment. The presence of this membrane may explain the glaucoma's progressive nature and suggest possible surgical or laser treatments as a way to control or prevent the progression of the disease.

Proposed Course

Patients with other anomalies of the anterior chamber, including congenital cataracts, will be examined for abnormalities in tissue derived from neural crests. We will continue to study cases of congenital corneal disorders to uncover the cause and to determine treatment choices for these cases.

NEI Research Program

Glaucoma—Other Glaucoma (Developmental, Congenital, and Infantile Glaucoma)

Publications

Kupfer C, Chan C-C, Burnier M Jr, Kaiser-Kupfer MI: Histopathology of the ICE syndrome. *Trans Am Ophthalmol Soc*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00123-12 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Psychophysics of the Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rafael Caruso	M.D.	Visiting Scientist	OGCSB, NEI
Others:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
	Doris J. Collie	A.A.	Ophthalmic Technician	OGCSB, NEI
	Maria Bankiewicz	M.D.	Visiting Associate	OGCSB, NEI
	Patricia A. Mercer	M.P.A.	Ophthalmic Technician	OGCSB, NEI
	Leanne M. Reuter	B.S.	Ophthalmic Technician	OGCSB, NEI

COOPERATING UNITS *(If any)*

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Eye Services Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.31	0.68	0.63

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The visual function of patients with ocular diseases or lesions in the visual pathways and of normal subjects is measured using psychophysical techniques. These data are correlated with those obtained with electrophysiological tests of visual function. The results obtained contribute to the diagnosis of ocular and neural disorders that affect vision and are needed to characterize their nature and evolution. They are also valuable in the assessment of how different forms of treatment affect the outcome of these diseases.

Project Description

Clinical Protocol Number

81-EI-108

Objectives

The aims of this project are to apply and develop psychophysical techniques for the study of vision in the clinical setting, to characterize the human visual system's normal function, and to analyze the patterns of its alteration in ocular diseases and lesions of the visual pathways.

Methods

Several psychophysical techniques are employed: (1) Perimetry: Visual fields are explored with kinetic quantitative perimetry and static quantitative perimetry. (2) Color vision: Central color vision is estimated using HRR pseudoisochromatic plates, Ishihara pseudoisochromatic plates, Farnsworth's Tritan plate, Farnsworth-Munsell D-15 panel, Lanthony's desaturated D-15 panel, Farnsworth-Munsell 100 hue test, and the Nagel anomaloscope. (3) Adaptometry: Dark-adapted rod and cone thresholds are measured with a modified Goldmann-Weekers adaptometer. (4) Spatial vision: The spatial contrast sensitivity function is determined using sinusoidal luminance gratings. A two-alternative temporal forced-choice technique is used for a criterion-free judgement of threshold visibility. (5) Luminance and chromatic increment thresholds are measured with a two-channel Maxwellian view instrument. This instrument also is used to assess retinal receptor orientation by measuring the Stiles-Crawford effect (SCE of the first kind).

Major Findings

We described abnormalities of foveal cone thresholds and directional sensitivity in eight patients (five females and three males, ages 12-48 years). The diagnostic criteria were biochemical (hyperornithinemia) and ophthalmoscopic (presence of classical chorioretinal atrophic lesions). We conducted a complete ophthalmological examination and evaluation of visual function (ie, visual acuity, contrast sensitivity, visual fields, color vision, dark adaptation, Ganzfeld and focal electroretinogram, and electrooculogram). The SCE of the first kind was used as an indicator of foveal cone orientation, and increment threshold (IT) measurements were used to asses foveal cone sensitivity. Both measurements were obtained using a two-channel Maxwellian-view instrument, varying background intensity for IT determination, and background position for SCE measurements.

All patients showed abnormal IT results. In three of them, we observed only a sensitivity loss, while the remaining five showed both sensitivity loss and alteration in effective adaptation status. The shape of the SCE function (estimated by Stiles' rho parameter) was normal for the same three patients whose IT measurements showed only sensitivity loss. Four patients' data showed flat SCE functions (low rho values), results that agree with the previous report on a gyrate atrophy (GA) patient (Tasuma T, et al: *Clin Vis Sci* 1:93-102, 1986). The remaining patient, who had the most severe chorioretinal atrophy, failed to show directional sensitivity. These results suggest that foveal cones are altered in their orientation and sensitivity before the encroachment on the foveal area by the atrophic lesions of GA.

Significance to Biomedical Research and the Program of the Institute

Psychophysical techniques are noninvasive methods useful in the diagnosis and management of ocular diseases and visual pathway lesions. In addition to the application of validated tests, the development of new techniques contributes to the elucidation of the pathophysiological mechanisms of visual disorders.

Proposed Course

Clinical psychophysical studies of visual function in diseases of the eye and visual pathways will be continued. We will introduce modifications that are expected to enhance the diagnostic value of the techniques described.

NEI Research Program

Retinal and Choroidal Diseases—Noninvasive Techniques in the Study of Retinal Disorders Strabismus Amblyopia, and Visual Processing—Visual Processing and Amblyopia

Publications

Drews-Bankiewicz MA, Caruso RC, Datiles MB, Kaiser-Kupfer MI: Contrast sensitivity in patients with nuclear cataracts. *Arch Ophthalmol* 110:953-959, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00144-11 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Electrophysiology of the Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rafael Caruso	M.D.	Visiting Scientist	OGCSB, NEI
Others:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
	Maria Bankiewicz	M.D.	Visiting Associate	OGCSB, NEI
	Patricia A. Mercer	M.P.A.	Ophthalmic Technician	OGCSB, NEI
	Doris J. Collie	A.A.	Ophthalmic Technician	OGCSB, NEI
	Leanne M. Reuter	B.S.	Ophthalmic Technician	OGCSB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Eye Services Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.85	0.68	1.17

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The visual function of both patients with ocular diseases or lesions in the visual pathways and normal subjects is measured objectively with electrophysiological techniques. The data are correlated with those obtained with psychophysical tests of visual function. The results obtained contribute to the diagnosis of ocular and neural disorders that affect vision and are needed to characterize their nature and evolution. They are also valuable in the assessment of the effect of different forms of treatment on the outcome of these diseases.

Project Description

Clinical Protocol Numbers

91-EI-26
82-EI-55

Objectives

The aims of this project are to apply and develop electrophysiological techniques for the study of visual function in the clinical setting, to characterize the normal electrical activity of the human visual system, and to analyze the patterns of its alteration in ocular diseases and lesions of the visual pathways.

Methods

The electrophysiological techniques employed involve recording potentials generated by the retinal pigment epithelium (RPE) (electrooculogram [EOG]), the neural retina (electroretinogram [ERG]), and the central visual pathways (visually evoked potentials [VEPs]). These potentials are elicited by unstructured stimuli (Ganzfeld full-field or focal stimulation) and spatially structured stimuli (sinusoidal gratings or checkerboard patterns).

Major Findings

The ERG is used as the standard electrophysiological test of RPE function. Unfortunately, EOG results also are affected by the functional status of photoreceptors, which can be assessed by the ERG. We found a strong positive correlation between ERG amplitude and EOG results in both patients with retinal diseases and normal subjects. Five groups (83 subjects) were included in the study: patients with retinitis pigmentosa (RP, 21), gyrate atrophy (GA, 21), juvenile macular dystrophy (JMD, 12), Best's vitelliform dystrophy (BVD, 2), and normal subjects (27). We analyzed the results obtained in one eye chosen at random for each subject.

We recorded ERGs using the international standard protocol and analyzed EOGs using the light peak/dark trough (Arden) ratio. Correlation analysis showed a statistically significant relationship between the Arden ratio and the amplitude of the rod-mediated b-wave (r:0.822), the maximal retinal response (r:0.841), the cone mediated b-wave (r:0.827) when patients with RP, GA, JMD, and normal subjects were included in the analysis. For these subjects, the ratio between normalized ERG amplitude and normalized Arden ratio was approximately constant. As expected, BVD patients (with normal ERGs and subnormal EOGs) fell outside the "main sequence" of the EOG versus ERG relationship, and their ERG/EOG ratio was unusually high.

ERG results were more useful than EOG results to assign a given patient to a diagnostic group (nearest neighbor analysis). When ERG and EOG results were used simultaneously to classify a patient, the improvement in diagnostic specificity over the ERG alone was not meaningful. These findings confirm that EOG results are not independent of ERG amplitude and emphasize the need for a diagnostic test of RPE integrity that does not depend on light-induced RPE responses.

We described a reduction in the amplitude of ERG responses correlated with an increase in ocular length. Previous studies have yielded contradictory results regarding this relationship. One of these reports used a nonstandard ERG recording technique, and another included only seven ametropic subjects. To minimize the effect of known sources of ERG variance (age and sex), we included in the study only women in the 20-49 age range; 28 normal women were evaluated. A complete ophthalmologic examination was performed to rule out ocular disease. Axial length and vitreous segment length were measured using A-mode ultrasonography. An expanded version of the international standard ERG protocol was used for Ganzfeld ERG recordings in one eye, and an intensity-response function was obtained in the fellow eye. Regression analysis was performed to describe and quantify the relationship between ERG findings and ocular measurements.

Our principal finding was a statistically significant inverse correlation between ocular length and the amplitude of rod-mediated b-waves, dark-adapted cone-mediated b-waves, maximal retinal responses, light-adapted cone-mediated b-waves, and 30 Hz flicker responses. In contrast, no significant association was found between b-wave implicit time and ocular length. This change in amplitude without variation in implicit time suggests that our results are due to the difference in change in retinal sensitivity. Our findings suggest that when ERG results from patients with high refractive error are analyzed, the effect of ocular length on ERG amplitude should be taken into consideration and the normative data adjusted.

Albinism in animals has been associated with an anatomic anomaly of the visual pathways characterized by excessive crossing of the retinogeniculate fibers with two different modes of geniculocortical projection. In humans, indirect evidence of the same anomaly is demonstrated by asymmetry in VEPs elicited by pattern reversal stimulation. Recent studies using appearing-disappearing patterns claim VEP asymmetry is diagnostic; they propose a uniform type of asymmetry. We used the same recording conditions To determine the diagnostic value of VEP in albinism and to attempt correlation of VEP results with clinical features.

Eighteen subjects (6-42 years of age) with different types of albinism (oculocutaneous and ocular) underwent complete ocular examination and VEP recording. VEPs were generally of low amplitude, interfering with the interpretation in two cases. One patients with albinism without nystagmus had symmetric responses; 15 showed asymmetry of two distinct types. In eight, the major positivity of the response was distributed in the channels contralateral to the stimulated eye and, in seven, it was ipsilateral to the stimulated eye. There was no correlation of either of these two types of asymmetry with race, sex, type of albinism, or any particular clinical finding.

This study shows that there are two different patterns of VEP asymmetry in albinism. They may be explained by differences in the reorganization of the geniculocortical pathway. Although VEP asymmetry is very frequent in this condition, it may not be constant. However, its value is decreased in some cases in which the low amplitude of the responses makes interpretation difficult. Furthermore, there is no correlation of the type of asymmetry with any other feature of albinism.

Significance to Biomedical Research and the Program of the Institute

Electrophysiological techniques are noninvasive methods useful in the diagnosis and management of ocular diseases and visual pathway lesions. In addition to validated tests, the new techniques developed contribute to the elucidation of the pathophysiological mechanisms of visual disorders.

Proposed Course

We will continue clinical electrophysiological studies of visual function in diseases of the eye and visual pathways, introducing modifications expected to enhance the diagnostic value of the techniques described.

NEI Research Program

Retinal and Choroidal Diseases—Noninvasive Techniques in the Study of Retinal Disorders
Strabismus, Amblyopia, and Visual Processing—Visual Processing and Amblyopia

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00257-04 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visual Function Diagnosis Service

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rafael Caruso	M.D.	Visiting Scientist	OGCSB, NEI
Others:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
	Tracy T. Nolan	M.A.	Health Technician	OGCSB, NEI
	Dessie Koutsandreas	B.S.	Ophthalmic Technician	OGCSB, NEI
	Donna M. Chandler		Health Technician	OGCSB, NEI
	Lorraine Kendra	B.A.	Health Technician	OGCSB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Eye Services Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	0.1	3.4

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This general service project provides diagnostic support for all research protocols conducted by the Clinical Sections of the National Eye Institute and other Institutes that require an assessment of visual function. Psychophysical and electrophysiological techniques are used to detect and quantify visual loss due to disorders of the ocular media, uvea, retina, optic nerve, and central visual pathways.

Project Description

Additional Personnel

Linda Goodman	Ophthalmic Technician OGCSB, NEI
Antoinnette Laren	Ophthalmic Technician OGCSB, NEI
R. Patrick McDaniel	Ophthalmic Technician OGCSB, NEI
Anne Randall	Ophthalmic Technician OGCSB, NEI
Sueli Mueller	Special Volunteer OGCSB, NEI

Objectives

The aim of this project is to provide accurate measurements of visual function for the differential diagnosis of visual loss. The first step in this process is detection of a visual deficit (ie, determining whether visual loss is present). The second step is quantification of a detected deficit. The third is analysis of the characteristics of the visual deficit to determine the site of the lesion responsible for this symptom (topographic diagnosis). The final step is correlation with other clinical findings to ascribe the visual deficit to a given pathological process.

Methods

The psychophysical techniques employed include commercially available and laboratory-developed techniques for the measurement of visual acuity, visual fields, color vision, dark adaptation, spatial contrast sensitivity, and glare disability.

The electrophysiological techniques used include recording potentials generated by the retinal pigment epithelium (electrooculogram), the neural retina (electroretinogram), and the central visual pathways (visually evoked potentials).

Major Findings

During the period October 1, 1991, through September 30, 1992, we performed the following tests:

Kinetic perimetry	216
Static perimetry	276
Screening perimetry	109
Manifest refraction	450
Color vision tests	146
Adaptometry	46
Contrast sensitivity tests	226
Glare disability tests	294
Ganzfeld electroretinography	110
Focal electroretinography	33
Electrooculography	93
Visually evoked potentials	30

This represents an increase of 57% over the tests performed during the same period in Fiscal Year 1991.

Significance to Biomedical Research and the Program of the Institute

This project provides all tests of visual function for patients who visit the NEI Eye Clinic. In the majority of ophthalmologic diseases, visual loss is the most meaningful finding. In most clinical research protocols involving diseases of the eye and visual pathways, visual deficit is used as an indicator of the progress of a disease or the effect of a treatment. Therefore, sensitive and accurate measurements of visual function are essential for these clinical research projects.

Proposed Course

The provision of clinical electrophysiologic and psychophysical tests of visual function for patients with diseases of the eye and visual pathways will be continued. We will introduce modifications that are expected to enhance the diagnostic value of the techniques described.

NEI Research Program

Retinal and Choroidal Diseases—Noninvasive Techniques in the Study of Retinal Disorders; Strabismus, Amblyopia and Visual Processing—Visual Processing and Amblyopia

Publications

The nature of this project is such that the results obtained are included in publications listed under other projects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00011-18 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pigment Dispersion With and Without Glaucoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
Others:	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Ophthalmic Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the risks of patients with pigment dispersion syndrome for glaucoma. Comparisons of patients with and without glaucoma are made on the basis of diagnostic tests, genetic screening, and aqueous humor dynamics. The data acquired may enable determination of pigment dispersion syndrome patients' risk of developing glaucoma, as well as adding to the understanding of the pathology of the disease.

Project Description

Additional Personnel

Marvin Podgor	M.S.	Statistician Biometry and Epidemiology Program, NEI

Clinical Protocol Number

76-EI-189

Objectives

This project was designed (1) to compare patients with and without glaucoma who have pigment dispersion by documenting and following the clinical features and courses of disease and by evaluating performance on a variety of diagnostic tests; (2) to determine the presence of abnormal aqueous humor dynamics in glaucoma and nonglaucoma patients with pigmentary dispersion; and (3) to compare the association of pigment dispersion, with and without glaucoma, with possible genetic markers.

Methods

A complete evaluation included the following: complete family history with detailed pedigree; best-corrected visual acuity with manifest refraction; slit-lamp biomicroscopy; visual field examination (Goldmann I_2e and I_4e); applanation Goldmann tension; photography of iris color, iris transillumination, and Krukenberg spindle; A-scan, anterior chamber depth, and anterior chamber volume measurements; goniophotography; static perimetry; baseline tonography and water-drinking tonography 1 hour later, when indicated; fasting blood sugar, when indicated; dilated ophthalmoscopic examination (using 2.5% phenylephrine and 1% cyclogel); and stereophotographs of the optic nervehead.

Major Findings

One hundred and sixty-four patients were classified into three groups: (1) pigment dispersion syndrome (PDS) without abnormal ocular pressure, (2) PDS with ocular hypertension, and (3) PDS with glaucoma (PDS+GL). Analysis of baseline characteristics with respect to anatomical and physiological parameters has yielded the following conclusions:

1. It appears that the majority of patients recruited have PDS with a benign course; they do not develop ocular hypertension or glaucoma.

2. Consequently, family members of PDS patients should be alerted and appropriately screened. PDS may be familial and can show a dominant inheritance pattern.

3. Analyses of graded iris transillumination, the amount of pigment deposited on the trabecular meshwork, and the anterior chamber depth have demonstrated no significant differences among the three categories of PDS. Thus, pigment deposited in the angle may be only a secondary factor adversely ·ffecting an already compromised outflow facility that is primarily a result of open-angle glaucoma.

4. It also appears that those patients who develop ocular hypertension and demonstrate early field changes can be managed medically by control of intraocular pressure and reversal of early field loss. Patients who develop glaucoma do not appear to be more difficult to treat than patients with open-angle glaucoma.

5. The phenomenon of unilateral or asymmetric pigment dispersion syndrome with little difference between the measurements of the two eyes is being investigated in followup studies.

6. In our series, retinal detachment does not appear to occur with any greater frequency than with high myopia. A history of asymptomatic and nonprogressive peripheral retinal holes was noted in two patients.

7. The data from this study have been computerized, and an indepth analysis is under way.

Significance to Biomedical Research and the Program of the Institute

These results may facilitate determination of the risk of the development of glaucoma for patients with pigment dispersion. Specifically, it may be possible to identify which features have predictive value in forecasting which PDS patients will develop visual field defects. In addition, the data will aid investigation of the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma.

Proposed Course

This project will be continued for 3 more years to obtain additional data on the patients enrolled in the study and to recruit more patients to add to the knowledge about pigment dispersion syndrome.

NEI Research Program

Glaucoma—Other Glaucomas (Developmental, Congenital, and Infantile Glaucomas)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00060-14 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visual Function and Ocular Pigmentation in Albinism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
Others:	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Rafael Caruso	M.D.	Visiting Scientist	OGCSB, NEI
	Evrydiki Bouza	M.D.	Visiting Scientist	OGCSB, NEI
	Malina Bankiewicz	M.D.	Visiting Associate	OGCSB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Ophthalmic Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40	0.35	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with hypomelanotic disorders, such as ocular albinism, oculocutaneous albinism, Chediak-Higashi disease, Hermansky-Pudlak syndrome, and iris transillumination defects, are being recruited to determine visual function with these conditions and to evaluate the changes in visual function over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.

Project Description

Clinical Protocol Number

76-EI-207

Objectives

The objectives of this study are (1) to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; (2) to correlate the amount of nystagmus with visual acuity and iris pigmentation; (3) to determine whether ocular pigmentation, visual acuity, and nystagmus change with age; (4) to identify the heterozygous state of family members; and (5) to determine whether abnormalities of crossing of the optic nerve fibers can be correlated with the lack of pigmentation and whether previous reports in abnormalities of crossing can be confirmed.

Methods

For each patient, we compile a complete family history with detailed pedigree and perform the following procedures: best-corrected visual acuity near and at distance with refraction; slit-lamp examination; psychophysical testing, including D-15 and Munsell 100 hue as well as rod and cone thresholds; dilated ophthalmoscopic examination; photography to document hair color, eye color, iris transillumination, and the status of the disc and macula; visually evoked response testing; and contrast sensitivity measurements, in selected patients. Information on family members is collected by examination of best-corrected visual acuity, slit-lamp examination of iris, photography of iris transillumination, and fundus examination when vision is not corrected to 20/20.

Major Findings

1. Examination of patients and family members indicated that transillumination of the iris may be seen in the absence of recognized albinism. The pattern, which appears to be punctate, may be present in a diffuse manner or limited to the 6 o'clock sector. The finding is not associated with nystagmus.

2. Three patients presented with marked iris transillumination, reduced pigmentation of the fundus, and no nystagmus, but they had decreased visual acuity, which has improved in conjunction with an increase of the pigmentation of the fundae.

3. Visually evoked responses were normal in some patients, but in a subset of albinos, there was evidence of abnormalities, such as crossing of optic nerve fibers of the chiasm. These findings are being pursued with more refined and updated equipment.

Significance to Biomedical Research and the Program of the Institute

These data may allow identification of the carrier state of albinism, which would be important in genetic counseling. Determination of whether the development of the fovea is abnormal in albinism, whether this abnormal foveal development is the cause of the decreased visual acuity in albinism, or alternatively, whether decreased visual acuity is secondary to hypopigmentation and the resultant light scatter and glare, may be possible. Collection of these data also will facilitate ascertainment of whether visual acuity improves with age and whether this correlates with changes in pigmentation.

In addition, studies are being conducted to verify the reported findings of abnormalities of the crossing fibers, as measured by visually evoked responses, contrast sensitivity, degree of nystagmus, and amount of pigmentation.

Proposed Course

This project will be continued for 5 more years to obtain additional data.

NEI Research Program

Retinal and Choroidal Diseases—Developmental and Hereditary Disorders

Publications

Bouzas EA, Caruso RC, Bankiewicz MA, Kaiser-Kupfer MI: Two different VEP patterns demonstrated in albinism unrelated to clinical type. *Invest Ophthalmol Vis Sci* 33(4):964, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00083-15 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gyrate Atrophy of the Choroid and Retina and Other Retinal Degenerations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
Others:	Evrydiki Bouza	M.D.	Visiting Scientist	OGCSB, NEI
	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Rafael Caruso	M.D.	Visiting Scientist	OGCSB, NEI
	Pushpa K. Sran	M.D.	Medical Officer	OGCSB, NEI
	Doris Collie	A.A.	Ophthalmic Technician	OGCSB, NEI

COOPERATING UNITS *(if any)*

The Howard Hughes Medical Institute Laboratory and the Department of Pediatrics, The Johns Hopkins University, School of Medicine, Baltimore, MD (David L. Valle, M.D.)

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Ophthalmic Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with gyrate atrophy of the choroid and retina are examined systematically to confirm the diagnosis. Skin fibroblasts from affected patients and family members are grown in tissue culture and assayed for ornithine aminotransferase activity. The results are evaluated for correlation with the presence of homozygosity or heterozygosity for the disease trait. Each patient is given a trial of pyridoxine to see whether serum concentration of ornithine can be reduced; if so, the patient is classified as a "responder" and treatment with pyridoxine is continued. Nonresponder and responder patients are then placed on a low-arginine, low-protein diet with supplemental amino acids and observed for arrest or improvement of the disease. If patients are not considered eligible for the diet, or if they appear unable to comply with the dietary regimen, we follow them to record the natural progression of the condition. Patients with other forms of retinal degeneration such as retinitis pigmentosa, fundus flavimaculatus, juvenile retinoschisis, and Usher's syndrome are also examined. The courses of their diseases are compared with those of gyrate atrophy patients.

Project Description

Additional Personnel

Laura Wozencraft		Genetic Counselor OGCSB, NEI
J. Fielding Hejtmancik	Ph.D.	Medical Officer LMOD, NEI
Susan Gentleman	Ph.D.	Biologist LRCMB, NEI

Clinical Protocol Number

78 EI-01

Objectives

This project is being conducted (1) to determine the biochemical processes responsible for the elevated plasma ornithine and the chorioretinal lesions that occur in gyrate atrophy (GA) of the choroid and retina; (2) to determine which patients respond to pyridoxine treatment with a decrease in plasma ornithine concentration; (3) to determine whether treating "responders" with pyridoxine and nonresponders with an arginine-deficient diet will arrest the progress of chorioretinal atrophy; (4) to study the natural history of this condition when intervention is not undertaken and to determine the degree of heterogeneity; (5) to define the molecular mutations and compare the molecular defect with the clinical features of the disease; and (6) to characterize and follow the progression of lens opacities, obtaining lens specimens at the time of cataract extraction for protein analysis.

Methods

Patients suspected of having GA of the choroid and retina are examined according to a standard set of procedures to confirm the diagnosis. Plasma ornithine concentration is measured periodically. Punch biopsies of the skin are grown in tissue culture; ornithine aminotransferase activity is measured, and patient molecular defect is characterized. Complete evaluation of ocular function in these patients includes best-corrected visual acuity, Goldmann visual fields, color vision, cone thresholds, dark adaptation, electroretinogram (ERG), foveal electroretinogram (FERG), electrooculogram (EOG), contrast sensitivity, and Stiles-Crawford effect.

Major Findings

GA, a rare autosomal recessive disorder, is associated with hyperornithinemia, overflow ornithinuria, and a deficiency of activity of the mitochondrial enzyme ornithine-δ-aminotransferase (OAT). Although rare, the condition has been described worldwide in all races. Thirty-six patients have been recruited and evaluated in this study. The patients' ethnic origins vary, including Scottish, English, Welsh, Portuguese, Finnish, Lebanese, American Black, Asian Indian, German, and Israeli.

In this study, the 19 females and 17 males range in age from 2.5 to 65 years, with 9 children less than 12 years old at the time of recruitment. Observations of these patients have enabled documentation of both clinical evidence and laboratory heterogeneity.

Analysis of the mutation that causes GA of the choroid and retina has been undertaken by Drs. David Valle, Grant Mitchell, and colleagues of The Johns Hopkins University. They have analyzed probands from 72 GA pedigrees. No gross structural alterations of the OAT gene have been detected; 85% of the probands express nearly normal amounts of normal-sized OAT mRNA. The remainder express little or no OAT mRNA (n = 5) or an mRNA with an altered size (n = 2). Western blot studies showed the OAT antigen was absent in 67% of the mRNA+ mutants and all of the mRNA– mutants. A total of 14 mutations have been delineated at the molecular level: 10 missense mutations (M1I, R180T, L402P, C93F, Y55H, R154L, A270P, R271KL, G375V, and P417L/L437F), a single nucleotide deletion at cDNA position +159 (H53fs), an interesting in-frame three-nucleotide deletion of A1a-184 (A185F0), and a nonsense mutation at a CpG dinucleotide (R396ter).

The functional consequences of several mutations have been examined by substituting the mutations into otherwise wild-type OAT cDNA in the expression vector P91023b and transfecting the recombinant constructs into CHO-K1 cells that lack endogenous OAT mRNA or protein. Three (R180T, L402P, A184D0) have been shown to encode a CRM+, enzymatically inactive protein, while M1I—as expected for an initiation codon alteration—has a CRM– phenotype. Studies are under way to correlate mutational heterogeneity with clinical and biochemical heterogeneity.

The earliest clinical and electrophysiologic features were documented in the two youngest patients (ages 2.5 and 3 years). The minimal evidence of clinical retinal changes when significant reduction of rod and cone function is seen by electroretinographic studies is noteworthy.

Clinical and biochemical evidence of genetic heterogeneity is present in these patients. Fewer than 10% of patients have been reported to have a 30-50% decrease in plasma ornithine following treatment with vitamin B_6. Only one of our patients showed an in vivo response to this treatment.

Whereas arginine is the precursor of ornithine in the metabolic pathway of ornithine metabolism, we have undertaken a dietary intervention study limiting arginine. Of 23 patients placed on a low-protein (low-arginine) diet, all sustained significant reduction of ornithine during hospitalization; however, the diet was discontinued in 4 Finnish patients following their discharge because of poor compliance and in 7 other patients because of a variety of factors. Of 13 patients remaining on the diet, 4 have excellent control; 4, fair control; 4, erratic control. One young child was followed for too short a period of time to assess control. Ophthalmologic evaluations are performed on all patients every 6-12 months, travel permitting.

In the two patients with the best biochemical control for the longest time (11 and 12 years old, respectively), there was evidence of improved visual function. One patient, after being on the diet for 14 months, showed improved dark adaption, averaged ERG, and color vision. This improvement was sustained for 30 months, then the ERG amplitude showed a small but definite reduction. The second patient with lowered plasma ornithine levels, who had been on the diet for 11 years, showed progressive improvement in visual field and color vision and has since remained stable. A third patient, despite fair control, was stable for 36 months but has deteriorated for the past 18 months. It should be noted that she was the oldest patient and had the most advanced disease at the outset. Other patients followed for various periods of time appear stable. Of particular interest are the children who were ages 2.5 to 9 years at the outset of the low-arginine diet. The results indicate that as a result of dietary intervention, the course of the disease in the younger of each sibship has been improved, compared with that of the older sibling.

All but one patient over age 11 have had progressive cataracts in the posterior capsule. They present a uniform histologic picture and can be identified by their characteristic pattern in image analysis. Studies with Usher's syndrome are continuing for the purpose of identifying the gene for Usher's syndrome through molecular genetic applications.

Significance to Biomedical Research and the Program of the Institute

GA of the choroid and retina is the first of the genetically determined isolated severe retinal degenerations for which a specific biochemical marker and concomitant enzyme defect have been demonstrated. This study to test the efficacy of treatment for this blinding eye disease will serve as a model for the investigation of other genetically determined retinal degenerations. Study of the two young patients is the best opportunity for the evaluation of diet control.

Proposed Course

This project will be continued for 3 more years to assess further the knowledge concerning reduced ornithine in halting chorioretinal degeneration.

NEI Research Program

Retinal and Choroidal Disease—Development and Hereditary Disorders

Publications

Kaiser-Kupfer MI, Caruso RC, Valle D: Gyrate atrophy of the choroid and retina: Long-term reduction of ornithine slows retinal degeneration. *Arch Ophthalmol* 109:1539-1548, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00163-10 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NIH Interinstitute Genetics Program: The Genetics Clinic

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
Others:	Evrydiki Bouza	M.D.	Visiting Scientist	OGCSB, NEI
	Mark Scott	M.D.	Senior Staff Fellow	OGCSB, NEI
	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Anren Li	M.D.	Visiting Associate	OGCSB, NEI
	Laura Wozencraft		Genetic Counselor	OGCSB, NEI
	Steve Bernstein	M.D.	Senior Staff Fellow	LRCMB, NEI
	Robert Kim	M.D.	Senior Staff Fellow	LMDB, NEI

COOPERATING UNITS *(if any)*

Interinstitute Medical Genetics Program, NIH

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Ophthalmic Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.30	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Interinstitute Genetics Program and the Genetics Clinic supported by the Clinical Center offer a multidisciplinary approach to patients with genetic disease (Z01 CP 05139-06 CEB). Involved in the program are researchers from all Institutes. Patients evaluated in the clinic represent a broad spectrum of genetic diseases. During the past year, approximately 200 persons seen represented about 60 distinct disease categories. Due to the high frequency of ocular involvement in many of the cases, almost all the patients were evaluated by Clinical Branch staff or were discussed in consultation. The clinic serves as a source of interesting case material concerning patients with inherited or developmental abnormalities of the visual system.

Project Description

Clinical Protocol Number

Interinstitute Medical Genetics Program

Objectives

The objectives of this Program are (1) to evaluate patients with ocular abnormalities associated with genetic disease in the context of a multidisciplinary approach to the patient; (2) to provide genetic counseling to patients at risk for inherited ocular disease; (3) to recommend and advise appropriate evaluation for the ocular problem; (4) to provide training in the diagnosis, counseling, and treatment of individuals with or at risk for genetic disease, as well as in the research approach to genetic disease.

Methods

Referred patients are examined, and the appropriate diagnostic ophthalmologic workup is recommended.

Major Findings

1. Iris nodules were seen commonly in the classic cases of neurofibromatosis (NF1) and less frequent in patients with less-well-defined disease. They were seen rarely in patients with bilateral acoustic neuroma (BAN or NF2). Patients with NF2 showed increased frequency of posterior capsular cataracts, which serve as an excellent marker, being present in 29 of 30 patients with NF2. A new finding is the association of congenital cortical cataracts in NF2 patients.

2. Serious ocular complications were observed in 13 long-term postrenal transplantation nephropathic cystinosis patients. These complications included decreased visual acuity and visual function, as measured by psychophysical and electrodiagnostic tests, band keratopathy, and posterior synechia. Corneal transplantation may be necessary in cases with debilitating symptoms from recurrent erosion after all other treatment modalities have failed. In two such patients, the corneal grafts have remained clear for as long as 5 years.

Significance to Biomedical Research and the Program of the Institute

Genetic and developmental anomalies of the eye are a major cause of blindness and visual disability, and they are responsible for about 35% of the cases of blindness in developed nations. Involvement with the Interinstitute Genetics Program affords a systematic approach to studying these and other conditions associated with genetic diseases.

Proposed Course

The project is in a growth phase and will be expanding in future years.

NEI Research Program

Retinal and Choroidal Disease—Development and Hereditary Disorders

Publications

Bouzas EA, Kransewich D, Koutroumanidis M, Papadimitriou A, Marini JC, Kaiser-Kupfer MI: Ophthalmological examination in the diagnosis of Proteus syndrome. *Ophthalmology*, in press.

Bouzas EA, Parry DM, Eldridge R, Kaiser-Kupfer MI: Familial occurrence of combined pigment epithelial and retinal hamartomas associated with neurofibromatosis 2. *Retina* 12(2):103-107, 1992.

Parry DM, Kaiser-Kupfer MI, Sherman JL, Pikus A, Eldridge R: Neurofibromatosis 2 (bilateral acoustic or central neurofibromatosis), a treatable cause of deafness, in Ruben, RJ, de Water TR, Steel KP (eds): *Genetics of Hearing Impairment. Ann NY Acad Sci* 630:305-307, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00211-07 OGCSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Double-Masked Controlled Randomized Clinical Trial of Topical Cysteamine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Chief	OGCSB, NEI
Others:	Lessie McCain	R.N.	Nurse Specialist	OGCSB, NEI
	Manuel Datiles	M.D.	Medical Officer	OGCSB, NEI
	Evrydiki Bouza	M.D.	Visiting Scientist	OGCSB, NEI
	Mark Scott	M.D.	Senior Staff Fellow	OGCSB, NEI

COOPERATING UNITS *(if any)*

Human Genetics Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD (William Gahl, M.D., Ph.D.)

LAB/BRANCH

Ophthalmic Genetics and Clinical Services Branch

SECTION

Section on Ophthalmic Genetics

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nephropathic cystinosis is an autosomal, recessively inherited storage disease in which nonprotein cystine accumulates within cellular lysosomes due to a defect in lysosomal cystine transport. Ocular manifestations include photophobia; crystal deposits in the cornea, conjunctiva, and iris; and depigmentation of the retina. Systemic complications include the Fanconi syndrome and renal failure.

Ten years ago cysteamine, a free thiol that depletes cystine from cells, was introduced in the therapy of cystinotic patients. Although patients had improved growth and stabilized renal function, there was no noticeable effect on the accumulation of corneal crystals. Recent studies showed that corneal cells in tissue culture are readily depleted of cystine by the introduction of cysteamine, making feasible the use of topical ophthalmic cysteamine to circumvent the humoral route. After appropriate animal studies to test for complications revealed none, we began a double-masked clinical trial to test the efficacy of topical cysteamine (0.1%) in humans. Fourteen patients of ages less than 3 years were enrolled and randomized to 0.1% cysteamine. Five patients showed a significant decrease in crystals in the cysteamine-treated eyes.

To test the effects of increasing the concentration of cysteamine eye drops in humans, a toxicity study was performed in rabbits. It showed no adverse reactions. The results permitted an increase in concentration to 0.5% for human use, and all patients receiving 0.1% cysteamine were switched to 0.5%. An additional five young patients showed a significant decrease in treated eyes. Thus, of 28 young patients, 14 successfully had the code broken; of the 14 remaining, 2 died, 1 discontinued medication, 2 are still in the trial with poor compliance, and 9 have been receiving treatment too short a time to tell. Because of the success in the younger patients, this study was expanded to include older patients, 3 to 31 years of age. The findings have been most exciting: Twenty-one patients have shown a significant decrease in crystals in treated eyes as well as improvements in comfort, i.e., relief of pain and photophobia. This study has resulted in significantly improved quality of life for the successfully treated patients.

Project Description

Additional Personnel

Ernest M. Kuehl	Chief, Photography Section OGCSB, NEI

Clinical Protocol Number

86-EI-62

Objectives

The purpose of this project is to test the efficacy of topical cysteamine in patients with nephropathic cystinosis.

Methods

Slit-lamp examination and photography of the cornea are performed by a masked observer to determine whether there is a difference in the quantity of crystals seen in the cornea.

Major Findings

Topical cysteamine eyedrops (0.5%) are well tolerated. The crystal accumulation is reversible in very young patients, who do not have crystals packing the cornea, as well as in older patients.

Significance to Biomedical Research and the Program of the Institute

The continued accumulation of crystals in the cornea appears to lead to increasing discomfort in cystinosis patients, who develop severe photophobia with recurrent corneal erosions. Topical cysteamine treatment, which has been found to halt the process, has led to an improvement in the quality of life of these patients.

Proposed Course

This study is planned for 1- to 3-years' continuation.

NEI Research Program

Corneal Diseases—Ocular Surface Problems (Drug Delivery and Toxicity)

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The authors of the reports in this volume contributed to the index by submitting their own lists of descriptor terms.

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