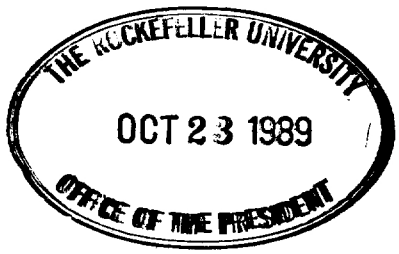
INSTITUT FÜR VIROLOGIE
Fachbereich Veterinärmedizin
(FB 18)
Justus-Liebig-Universität Gießen
Direktor: Prof. Dr. R. Rott

Prof. Dr. C. Scholtissek

D-6300 Gießen, den 11.10.89
Frankfurter Straße 107
Telefon 0641/702-4990/4991



Prof. Dr. Joshua Lederberg
President
The Rockefeller University
1230 York Avenue

New York, N.Y. 10021
USA

Dear Dr. Lederberg,

Thank you for your letter of September 27th, and for the interesting reprints. Concerning the history of the PR8 strain I am convinced that its nucleoprotein (NP) gene is still almost identical to the original isolate, which was obtained in 1934 from a person in Puerto Rico:

(1) The NP sequence of another strain (WSN) isolated by Wilson and Smith in 1933 is very similar to that of PR8 (see enclosed manuscript, which has been accepted by "Molecular Biology and Evolution"). When compared with the sequences of isolates from 1950 to 1983 the numbers of amino acid replacements fall on a straight line.

(2) Recombination (cross-over) between influenza RNA segments has not been observed yet, and no influenza virus has been isolated from a noninfected fertilized egg up to now. Thus, I do not think that recombination or reassortment with a passenger virus has occurred.

(3) The NP genes of the PR8 and fowl plague (FPV) strains have been sequenced in different laboratories using isolates of different passage histories: Only minor differences were observed, some of which are certainly due to errors introduced by reverse transcription and cloning and during sequencing (e.g. aa replacements in question in completely conserved regions).

(4) We have sequenced the NPs of four reassortants between virus N and FPV with different gene constellations, which were kept in our laboratory for 12 years (several plaque purifications and many egg passages): in none of the NP genes was a single base substitution when compared to the parent virus. Thus, inspite of the fact that the NP had to adapt to different gene constellations, its sequence was completely stable (see also enclosed reprint) for another example.

(5) There is evidence that, by changing the host, variants with mutations in the receptor pocket of the hemagglutinin can be isolated. However, in these variants maximally 2 to 3 aa replacements were discovered.

(6) Although the mutation rate of RNA viruses is extremely high and each egg fluid contains many different variants, the influenza viruses can be passaged quite stably, if one starts with an inoculum of 1000 to 10 000 pfu per egg, which is the usual practice. Then always the most efficient virus will be selected for the next passage. In summary, I am convinced that the sequence of the NP of the present PR8 strain is presumably not identical, but still very similar to that of the original human isolate. From the enclosed manuscript you can see why we think that these considerations are important. Together with NP sequences recently obtained in Rob Webster's laboratory (not yet published) more than 60 NP-sequences are available now.

Thank you for your interest in our studies.

Sincerely yours

(Christoph Scholtissek)