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LILLE, LE 25/6/91

Dr Joshua Lederberg

Dear Dr Lederberg,

Please find enclosed a copy of our revised manuscript. As I told you on the phone, it contains a significant amount of additional data, two new half figures (Fig 1 B and Fig 2 B) and a new Table (Table 1)

These include:

1) T cell proliferative response to PHA and PWM mitogens, to superantigens and to the tetanus recall antigen in 109 HIV-infected asymptomatic individuals and 49 HIV-seronegative healthy controls (instead of the previous 38 HIV-infected individuals and 20 healthy controls) (Fig 1). As controls, we have added 9 HIV-seronegative patients with infections or autoimmune diseases (Fig 1B), as asked by the two Science referees.

2) In addition to the T cell proliferation assays based on the use of ^{3}[H] thymidin of 1 Ci/mmol specific activity, we have also used ^{3}[H] thymidin of 25 Ci/mmol specific activity (Fig 1B). This leads to higher values of control T cells in response to mitogens (PHA and PWM), and to superantigens, while the values of HIV-infected individual T cells remain very low in response to PWM and to superantigens. Thus shows (as I indicated in my previous letter) that the low specific activity (1 Ci/mmol) of the thymidin we used accounted for the fact that "the mitogen responses are very small - something is suboptimal in the system" (first comment of the second PNAS referee).

3) Activation-induced T cell death assays have been performed in 59 HIV-infected individuals and 49 healthy controls (instead of the previous 20 HIV-infected individuals and 20 controls), as well as in the additional 9 HIV-seronegative patients with other diseases (Fig 2B). T cells from these 9 patients showed a reduced proliferative response (Fig 1B), but no activation-induced cell death was observed (Fig 2B).

4) Cell death was assayed in CD8+ T cell- or CD4+ T cell-depleted peripheral blood mononuclear cells (PBMC) from 12 HIV-infected individuals and from 4 healthy controls (instead of the previous one HIV-infected individual and one control). Cell death was also assayed in purified CD4+ and CD8+ T cells from 2 new HIV-infected individuals and from one new control in which the percentage of CD4+ T cells in unpurified PBMC were assessed. As asked by the Science referee, we have shown and discussed in the Result section (pages 10 and 11) the comparison between the percentage of CD4+ T cells in unpurified PBMC, and the percentage of cell death in the purified CD4+ T cell population and in the unpurified ~~mononucl~~ PBMC.

5) A table (Table 1) has been added, that shows that T cell proliferation and activation-induced death in T cells from 4 HIV-infected individuals (and 2 Controls) are identical in the absence or presence of in vitro addition of AZT. This suggests that apoptosis is not related to a cytopathic effect of HIV, such as that recently reported by Terai et al in the May issue of J. Clin. Invest, when T cell lines or mononuclear cells are incubated in vitro with certain strains of HTLV.

Finally, in order to reduce the lenghtth of this manuscript, I have deleted the final part of the discussion, that was mostly of a speculative nature.

I think that this revised manuscript provides an answer to the main questions that have been raised by the referees, and hope that you will find it suitable for publication in PNAS.

I thank you again for the time and effort you spend in this procen, and look forward to hearing from you.

Yours sincerely

J C Arnason