DEPARTMENT OF HEALTH & HUMAN S

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From Chief, LMM, NIAID

Subject Additional Information regarding

To Lowell Harmison, Ph.D.
Science Advisor, OSAH

In November, 1984 my colleagues and I isolated a molecular clone of the LAV virus. We used this clone as a hybridization probe to assess similarities and differences among novel AIDS viral isolates we were collecting since reports were beginning to appear suggesting that individual isolates were quite heterogeneous.

On December 11, 1984 we initiated an experiment to assess whether a given AIDS virus isolate changed during passage in different human cells. For this analysis we used LAV and analyzed its restriction enzyme map following propagation in PHA stimulated lymphocytes or A3.01 cells, (the latter is a continuous T lymphocyte line we had developed for growing the AIDS retrovirus). Infected cellular DNA preparations were separately digested with different restriction enzymes, electrophoresed through 0.6% agarose gels, transferred to nitrocellulose membranes and then hybridized to a 6.5 kb Hind III fragment of the LAV clone which contained gag, pol, and env gene sequences. At the conclusion of the experiment the processed membranes were exposed to photographic film and the autoradiograms were developed for 1, 4 or 14 days. I have enclosed the 4 day exposure (12/20-12/24/84) of this experiment, as well as a map summarizing the results. We were surprised that instead of reacting with only the predicted 6.5 kb viral DNA segment, the labeled DNA probe (probe A in the drawing) also hybridized to two additional cleavage products (4.3 and 2.2 kb fragments). The interpretation of this result was not obvious from this particular experiment, but became clearer when other restriction digestions were carried out and different hybridization probes were used. Assembling all of our data, we concluded that at least two viral variants were present in the LAV virus stock we had received from Dr. Montagnier in late April 1984. These differed from one another by the presence of an additional Hind III site at map position 6.0 (indicated on the accompanying chart).

We were similarly surprised to learn that preparations of HTLV III also contained the same Hind III variant present in our stock of LAV that we had obtained from the Pasteur Institute. In Figure 3 of the Hahn, et al paper (Nature, vol 312, pages 166-169, 1984) the 4.3 and 2.2 Hind III cleavage products are clearly seen as well as the 6.5 kb reactive fragment. It should be noted that Hahn, et al employed a different hybridization probe than we used in our studies. I have indicated this on the accompanying diagram and designated it as "B". The extra fragments seen in Figure 3 of the Hahn paper represent other Hind III cleavage products of the AIDS virus (such as a 1.4 kb env segment) that are present in both virus variants but which would not

react with our probe. Shaw, Hahn, Arya, Groopman, Gallo and Wong-Staal published a paper in Science (vol 226, pages 1165-1171, 1984) describing a molecular clone (HXB-II, Figure 1) derived from HTLV III stocks which contains the aberrant Hind III site in question. This clearly indicates that the Hind III variant is present in HTLV III preparations.

If you have any further questions, please feel free to contact me.

Malcolm A. Martin, M.D.

Enc.