DEPARTMENT OF HEALTH & HUMAN SERVICES

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Memorandum

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From Dr. Marvin Reitz

F.L AIDS

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Subject Summary of Recent Work on DNA Sequence of HTLV-III Isolates

To Dr. Robert Gallo

Now that my group and I have virtually finished sequencing the SL and MN isolates of HTLV-III, I thought that it would be useful to provide you with a summary of our observations. As you know, in the recent paper in Science by Wong-Staal et al. on heterogeneity by restriction site maps of different HTLV-III isolates, MN and SL were unusually closely related, differing by only a single Bgl II site. Both were obtained from NY-NJ in 1984; one was a child with AIDS (the mother was a drug abuser) and the other a homosexual with ARC. They were selected for further study partly on the basis of their apparent close relatedness and were molecularly cloned and sequenced in order to ascertain what degree of similarity could be found between very similar isolates in different people. The clones were prepared from the DNA samples indicated as "SL" and "MN" in the Wong-Staal paper. Their SOR, tat, env, 3'orf, and LTR sequences have been entirely obtained. The gag and pol genes of SL have been completely sequenced while part of the same regions in MN have been sequenced. Our results indicate that the sequences of the clones obtained from these two DNAs are very closely related. There is perhaps 2% of differences between the env genes; about the same magnitude is found for the other genes as well. Moreover, as you know, there are usually hypervariable regions in the env region when sequences of two different HTLV-III isolates are compared. This is not the case with MN and SL. Differences seen in most regions of the currently available sequences of different HTLV-III isolates consist of point mutations leading to conservative amino acid substitutions. In contrast, the env genes usually also differ by nonconservative point mutations and additions (most of which are actually duplications). The greatest differences in the env genes of MN and SL, however, are occasional nonconservative point mutations and one or two deletions of a triplet.

I should stress that the high degree of similarity of these two isolates is likely very much the exception rather than the rule. The two viruses were in fact preselected from 18-20 isolates on the basis of strong similarity of restriction maps. The use of H9 as target cells for different isolates may constitute a strong form of selection. Indeed, since HTLV-III seems to grow in a variety of cell types, it seems that using one cell type as the target would preselect from the mixed virus populations that probably occur in one individual only those variants that have the most suitable envelope protein for binding to that cell type and the strongest promoter for that cell type. It may also be significant that both viruses were collected from the same approximate time and place (NY-NJ 1984), similar to the patients whose cells gave rise to LAV and HTLV-III-B (January 1983 in NY) although, of course, this does not always hold true.

There is one final point that I think is worth stressing to you, in view of some of the innuendoes concerning the degree of similarity between LAV and HTLV-III-B (BH10 clone), the HTLV-III prototype, LAV and HTLV-III-B (BH10) are actually somewhat less related than SL and MN. The most variable env region seems, from my impressions based on study of our sequence data, to be what has been designated as V1 in the paper by Starcich et al. (bases 6200-6300 from Ratner et al.).

LAV and HTLV-III-B (BH10) differ significantly in this region of the envelope. LAV has a 15 bp duplication lacking in HTLV-III-B (BH10), plus nonconservative changes in 4 out of 25 (16%) amino acids. Furthermore, the pattern of repeat sequences in the gag-pol overlap region (which also tends to vary between different isolates) is different in LAV and HTLV-III-B (BH10). LAV and HTLV-III-B (BH10) both lack a 12 bp repeat contained in SL, but HTLV-III-B (BH10) has a 36 bp repeat which SL and LAV both lack. In this pattern LAV actually most resembles ARV-2. I have studied the sequences of all of these viruses very closely, and the difference in these two regions leads me to conclude that on this basis alone it is extremely unlikely that BH10 and LAV could derive from the same source. While there is no way to prove or disprove something of this nature, I believe that as enough env sequence data becomes available, it will become more evident that LAV and BH10 are not the same virus.