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Complete Nucleotide Sequences of Functional Clones of the Virus Associated with the Acquired Immunodeficiency Syndrome, HTLV-III/LAV

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There is considerable evidence that the human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) is the etiological agent in the acquired immunodeficiency syndrome (AIDS) and AIDS-related syndromes [1]. The most convincing line of evidence is the recapitulation with in vitro infection of the major manifestation of the disease, depletion of T4 cells [2]. Definition of the viral determinants of this lymphocytopathic activity is critical to understanding the pathogenesis of AIDS. With this goal in mind we have established an in vitro model which will facilitate this analysis. In this system, plasmid clones with the full HTLV-III/LAV proviral sequence are transfected into normal human umbilical cord blood mononuclear cell cultures. This results in production of virus particles with a morphology typical of HTLV-III/LAV and in death of the cell culture (Fig. 1).

To provide the basic information for utilization of this assay system, we have determined the complete nucleotide sequence of the biologically active proviral clone HXB2 [3]. Eighty nucleotide substitutions are noted, compared to the previously reported HTLV-III/LAV sequence for clone BH10 [4]. Insertions of two and three nucleotides in HXB2 compared to BH10 were recognized in noncoding regions, as well as a deletion of one copy of a 36-nucleotide, tandemly repeated sequence in the overlap of *gag* and *pol*. Most notable is the lack of alterations in the size and location of each of the seven previously identified viral genes [3].

Polymorphism is also noted in the predicted amino acid sequences of the viral protein products of HXB2 compared to the other sequenced HTLV-III/LAV viruses. *Gag, pol,* and *sor* are relatively well conserved, with 0.6%–3.6%, 1.0%–4.0%, and 0.5%–10.9% amino acid substitutions respectively. *Tat, trs, env,* and *3'orf* are more polymorphic, with 0.0%–11.6%, 5.2%–16.3%, 1.7%–17.5%, and 2.9%–16.0% amino acid substitutions respectively. A number of amino acid insertions and deletions are also noted. The relationships of these sequence variations to alterations in neutralizing epitopes, receptor binding domains, and other biological characteristics of the virus remain to be determined. The use of molecularly cloned viruses generated from this in vitro system will provide reagents for approaching these problems.

The biological activity of several other HTLV-III/LAV clones was also tested in umbilical cord blood mononuclear cells and the T4+ cell line, ATH8 [3]. To test the functional capabilities of clone BH10, the missing portions of the provirus were complemented with long-terminal repeat sequences from HXB2. The resultant clone gave rise to a lymphocytopathic virus. Clone HXB3 has been partially sequenced and appears to be closely related to HXB2, differing at only 63 of 3890 positions in the 3' por-

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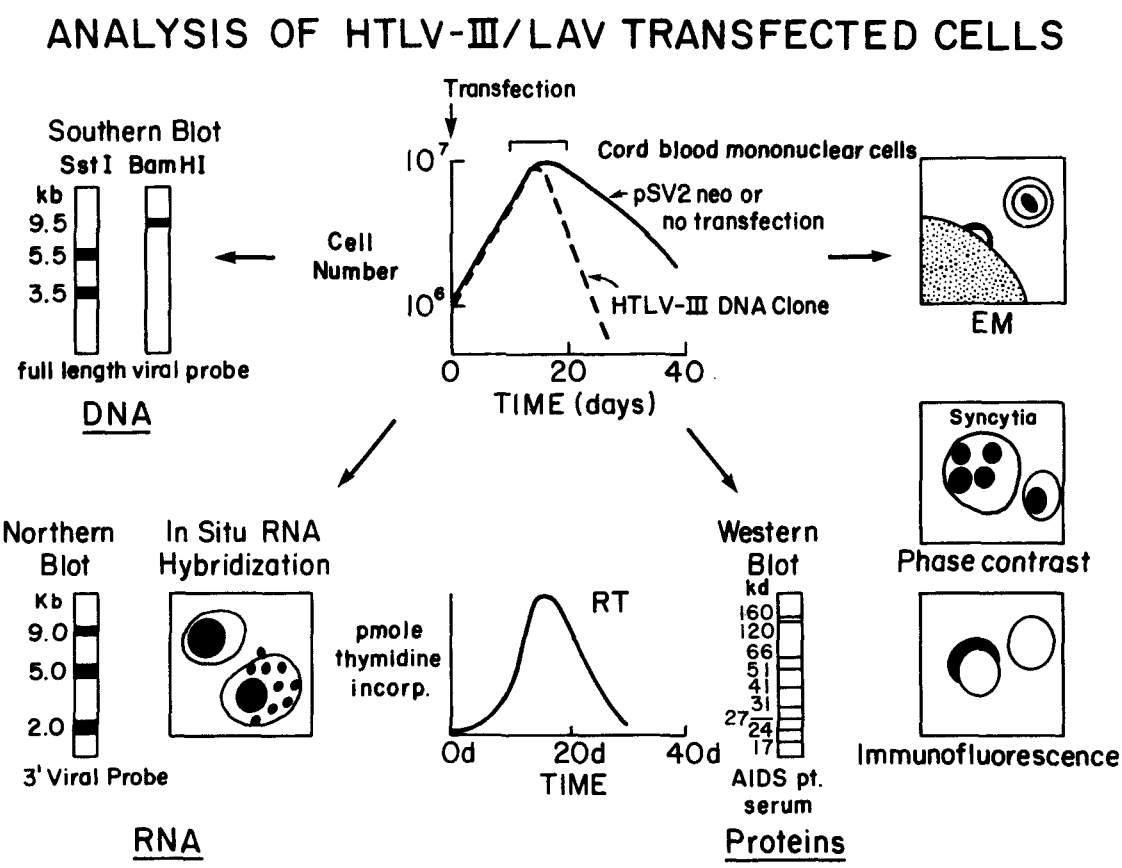

Fig. 1. An in vitro model system for AIDS. The schematic drawing depicts the major characteristics of the transfection system for analysis of the biological activity of HTLV-III/LAV DNA clones. The *top-center* drawing shows the growth curve of umbilical cord blood mononuclear cells after transfection with a plasmid lacking HTLV-III/LAV DNA sequences (pSV2neo) or a plasmid with the full HTLV-III/LAV provirus. Parameters measured 7–25 days after transfection include analysis of viral DNA by Southern blot, of viral RNA by Northern blot or in situ hybridization, of viral proteins by reverse transcriptase assays, Western blot, immunofluorescence for *gag* or *env* products, or phase-contrast microscopy for syncytia formation, and of the expression of particles with a morphology characteristic of HTLV-III/LAV

tion of the genome. A notable difference between HXB2 and HXB3, however, is the presence of a termination codon in *3'orf* of HXB2 and the lack of this sequence in HXB3. Viruses generated from HXB2 and HXB3 have similar replicative and cytopathic abilities. This finding, together with additional data on clones with deletions in *3'orf* (see Fisher et al., this volume), suggests that *3'orf* plays no essential role in the ability of HTLV-III/LAV either to replicate or to kill T4-lymphocytes.

Thus, these data provide the basic information essential for utilization of this system to construct clones of HTLV-III/LAV with alterations in the viral genome and for their assay in human lymphoid cells. Application of this system has yielded a variant with markedly attenuated cytopathic activities but normal replication (see Fisher et al., this volume). Further applications should provide information essential to understanding the pathogenesis of cell killing in AIDS and lead to approaches to the treatment and prevention of this disease.

References

1. Gallo RC, Wong-Staal F (1985) A human T-lymphotropic retrovirus (HTLV-III) as the cause of the acquired immunodeficiency syndrome. Ann Int Med 103:679–689
2. Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) Detection, isolation, and continu-

ous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497–500
3. Ratner L, Fisher A, Jagodzinski LJ, Mitsuya H, Liou R-S, Gallo RC, Wong-Staal F (1987) Complete nucleotide sequences of functional clones of the virus associated with the acquired immunodeficiency syndrome, HTLV-III/LAV. AIDS Res & Hum Retrov 3
4. Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich B, Josephs SF, Doran ER, Rafalski JA, Whitehorn EA, Baumeister K, Ivanoff L, Petteway SR, Pearson ML, Lautenberger JA, Papas TS, Ghrayeb J, Chang NT, Gallo RC, Wong-Staal F (1985) Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 313:277–284