Lancet - 1st draft

DIFFERENT ISOLATES OF HTLV-III AND LYMPHADENOPATHY VIRUS (LAV) ARE GENETIC VARIANTS OF THE SAME VIRUS

F. Wong-Staal*, B. Hahn*, G. Shaw*, M. Popovic*,
R. C. Gallo*, S. Wain-Hobson†, F. Barré-Sinoussi†, J. C. Chermann†,
L. Montagnier†, and M. Allison†

*Laboratory of Tumor Cell Biology, National Cancer Institute,
Bethesda, Maryland 20205 USA

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Summary

The genome of a retrovirus isolated from a patient with lymphadenopathy called LAV was compared to the genomes of several isolates of another human retrovirus called HTLV-III. Both HTLV-III and LAV have been linked to the cause of the acquired immunodeficiency syndrome (AIDS), and previous comparison of biological and biochemical properties of these viruses showed them to be similar retroviruses. We have used a cloned genome of HTLV-III (BH10) as probe to anneal to DNA from a T lymphocyte cell line infected with LAV. Restriction enzyme fragments totaling 9.0 kb were detected even under conditions of high stringency, suggesting that LAV is highly homologous to HTLV-III (BH10). We have previously observed that different isolates of HTLV-III exhibit microheterogeneity. The enzymes Sst I and Bgl II yielded three distinct patterns each for analysis of eight HTLV-III genomes. With this limited analysis, LAV fits into one of these genotypes. Therefore, LAV and different isolates of HTLV-III are highly related variants of the same virus that is the causative agent of AIDS.

Introduction

All known exogenous human retroviruses are T lymphotropic and pathogenic. The first such viruses were isolated from patients with a mature T cell malignancy called adult T cell leukemia (ATL) and shown to be the etiological agent of this disease. These viruses were named human T cell leukemia (lymphotropic) virus, type I (HTLV-I).[1,2] A distantly related virus (HTLV-II) isolated subsequently displays similar cell tropism for the $OKT4^+$ T lymphocytes and biological activity *in vitro*, including cell transformation and some degrees of cytopathy.[1,2] When the acquired immunodeficiency syndrome (AIDS) was first recognized to be a transmissible disease, involving most likely the $OKT4^+$ helper-inducer T lymphocytes, it was speculated that a retrovirus related to HTLV-I and HTLV-II would be the agent in this disease.[3] Indeed, Essex and colleagues[4] detected the presence of antibodies in a large percentage of AIDS patients and populations at risk which reacted with the envelope glycoprotein of HTLV-I. However, retrovirus(es) identified in a patient with lymphadenopathy (LAV)[5] or two AIDS patients (IDAV1, IDAV2)[6] showed no extensive cross-reactivity with HTLV-I and HTLV-II. Until recently, continuous production of these viruses was difficult because of their highly cytopathic effect on the infected cells. Independently, Gallo and colleagues reported 48 isolates of a retrovirus from AIDS or pre-AIDS patients.[7] Transmission of this virus to an established neoplastic T cell line (HT)[8] facilitated large scale production for biochemical,[9] seroepidemiological[10] and molecular[11-13] analysis. This virus was called HTLV-III because of many common biological and biochemical properties with HTLV-I and HTLV-II: (i) tropism for the $OKT4^+$

lymphocyte, (ii) a relatively small major core protein (p24), (iii) a high molecular weight Mg^{++} preferring reverse transcriptase, (iv) induction of multinucleated giant cells and in vitro cytopathic effect, (v) one or more common epitopes of core and envelope proteins, (vi) similar organization of the virus genome, (vii) presence of a long open reading frame (LOR or pX) between the envelope gene and 3' LTR, and (viii) a likely African origin. Since there is now accumulating evidence indicating both HTLV-III and LAV to be etiologically associated with AIDS, it is important to determine whether they are the same agent. In this report, we present evidence based on DNA hybridization with cloned HTLV-III probes that LAV and different isolates of HTLV-III represent subtypes of the same virus.

Materials and Methods

Transmission of LAV to an Established T Cell Line

A T cell line called Ti 7.4 was established from adult peripheral blood by Dr. A. Maisel. This cell line grows in the absence of exogenous T cell growth factor and expresses both OKT3 and OKT4 markers. Ti 7.4 cells were exposed to extracellular LAV and particles at high multiplicity of infection (10^3 virus particles/cell).[14] Two weeks after infection, virus expression was monitored by assaying particulate reverse transcriptase activity in the cultured fluids, and by immunofluorescence using specific monoclonal antibodies against viral core proteins.

HTLV-III Infected Cells and Tissues

The infected cell lines, H9/HTLV-III_B[8], H9/HTLV-III_{RF} and H9/HTLV-III_{MN} were established by infection of cloned H9 cells with supernatant virus from patient lymphocytes HTLV-III_B was from pooled material of several AIDS patients. RF was an AIDS patient from Haiti and MN was an American patient with AIDS related complex (ARC). Fresh tissues were obtained as mononuclear cells from a normal healthy carrier (RH) and biopsied lymphnodes of one ARC patient (HW) and two AIDS patients (FO and JR).

DNA Blot Hybridization

High molecular weight DNA was obtained from tissue and cell specimens. Restriction enzyme digest of the different DNA samples (30 µg each) were subjected to agarose gel electrophoresis and Southern blotting[15] to nitrocellulose filters. The filters were hybridized in 5 X SSC (1 X SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), 50% formamide (vol/vol), 5 X Denhardt's solution and 10% sextran sulfate at 37° overnight with ^{32}P-labeled BH10 clone.[12] The filters were then washed either in 5 X SSC or 1 X SSC at 63°C.

Results

DNA from the T cell line Ti 7.4,[14] infected with LAV and H9 infected with HTLV-III_B[c] was digested with the restriction enzyme Sst I, fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters as described by Southern.[15] Duplicate filters were hybridized to a

probe (BH10) consisting of 9.0 kb of HTLV-III sequences[12] and washed under two conditions of stringency: 5 X SSC/65° (low) or 1 X SSC/65° (high). H9/HTLV-III_B contains several distinct HTLV-III genomes[13] with two dominant Sst I genotypes. One is cleaved in the LTR and beginning of the gag gene, generating a 9.0 kb fragment equivalent to BH10 which comprises all but 180 nucleotides of the complete genome. Another genotype has an additional Sst I site internally, yielding two major fragments of 5.5 and 3.5 kb, respectively. Therefore, as seen in Figure 1A, H9/HTLV-III_B DNA yielded three Sst I bands. Under low stringency wash conditions, LAV DNA revealed two hybridizable bands of 5.5 and 3.5 kb of lower signal intensity as compared to the bands of H9/HTLV-III_B. However, the signal intensity of these bands was not significantly diminished under high stringency wash condition (Fig. 1B), suggesting the LAV genome is highly related to HTLV-III_B. The lower signal intensity can be accounted for by a lower copy number of viral DNA. This result is consistent with immunofluorescence studies which showed only 10-20% of Ti 7.4/LAV cells to be expressing virus.

We have shown previously that different isolates of HTLV-III may be distinguished based on restriction enzyme site polymorphism.[13] The questio arose as to whether LAV was more divergent from different HTLV-III isolates than they were from each other. We examined six HTLV-III proviruses and LAV with two enzymes: Sst I and Bgl II. Each of these enzymes yielded three genotypes for the HTLV-III proviruses. With Sst I, one obtained a 9.0 kb fragment, fragments of 5.5 and 3.5 kb, or fragments of 5.5, 1.8 and 1.7 kb (Fig. 2A). LAV generated Sst I fragments of 5.5 and 3.5 kb (lane 7, Fig. 2A). With Bgl II, one obtained fragments of 5.3, 1.6, 1.2, 0.6 and 0.5 kb

(lanes 1, 4, 5 and 6 of Fig. 2B), of 5.3, 2.2, 1.2 and 0.5 kb (lane 2) and of 6.8, 1.6 and 0.6 kb (lane 3) for the various HTLV-III genotypes. LAV exhibited the more common pattern for Bgl II digestion (lane 7, Fig. 2B). Analogous results were obtained with other enzyme digestions which showed that although each virus isolate, including LAV, could be distinguished provided enough restriction enzyme cleavage sites were examined, it was also obvious that all these isolates are genetic variants of the same virus agent. LAV is not different from the other HTLV-III isolates in this respect. We conclude that LAV and HTLV-III are independent isolations of the same virus.

Discussion

The involvement of a human T lymphotropic retrovirus in the etiology of AIDS was first proposed when it was recognized that the disease was transmissible. There had been two leading retrovirus candidates for the AIDS agent, LAV and HTLV-III. Both are exogenous human T lymphotropic retroviruses that exhibit profound cytopathic effect. Comparative analyses of other biological, biochemical and immunological properties also indicate that they are closely related viruses (see accompanying paper by Chermann et al.). In the study reported here, we compared the genomes of LAV and several HTLV-III isolates, and showed that LAV was closely homologous to most, if not the entire genome of HTLV-III. Furthermore, different HTLV-III isolates exhibit some restriction enzyme site polymorphisms, and that LAV was not more divergent from these isolates than they were among each other. Therefore, as can be expected logically, there is only one etiological agent of AIDS, namely HTLV-III/LAV.

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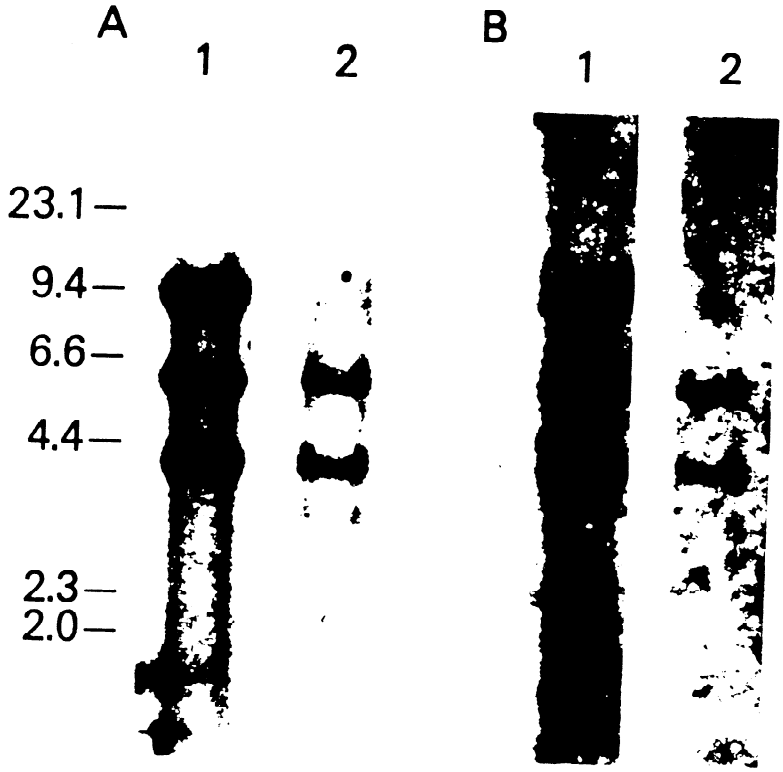
Figure Legends

Fig. 1. Homology of HTLV-III_B and LAV.

Southern hybridization of cellular DNA from H9/HTLV-III_B (lanes 1) and Ti 7.4/LAV cells (lanes 2) to labeled insert DNA from λBH10, a recombinant phage containing a complete HTLV-III genome less 180 basepairs of LTR sequences,[12] was carried out as described in Materials and Methods, and washed under two conditions of stringency (A) 5 X SSC, 65°C. (B) 1 X SSC, 63°C.

Fig. 2. Comparison of LAV and subtypes of HTLV-III.

DNA from different HTLV-III positive cell lines or patients was analyzed as described in Materials and Methods. Panel A, SSt I, Panel B, Bgl II. Lanes: 1, H9/HTLV-III_B; 2, RH (healthy carrier); 3, RF (AIDS); 4, HW (ARC); 5, MN (ARC); 6, FO (AIDS); 7, Ti 7.4/LAV; and 8, normal peripheral blood lymphocytes as negative control.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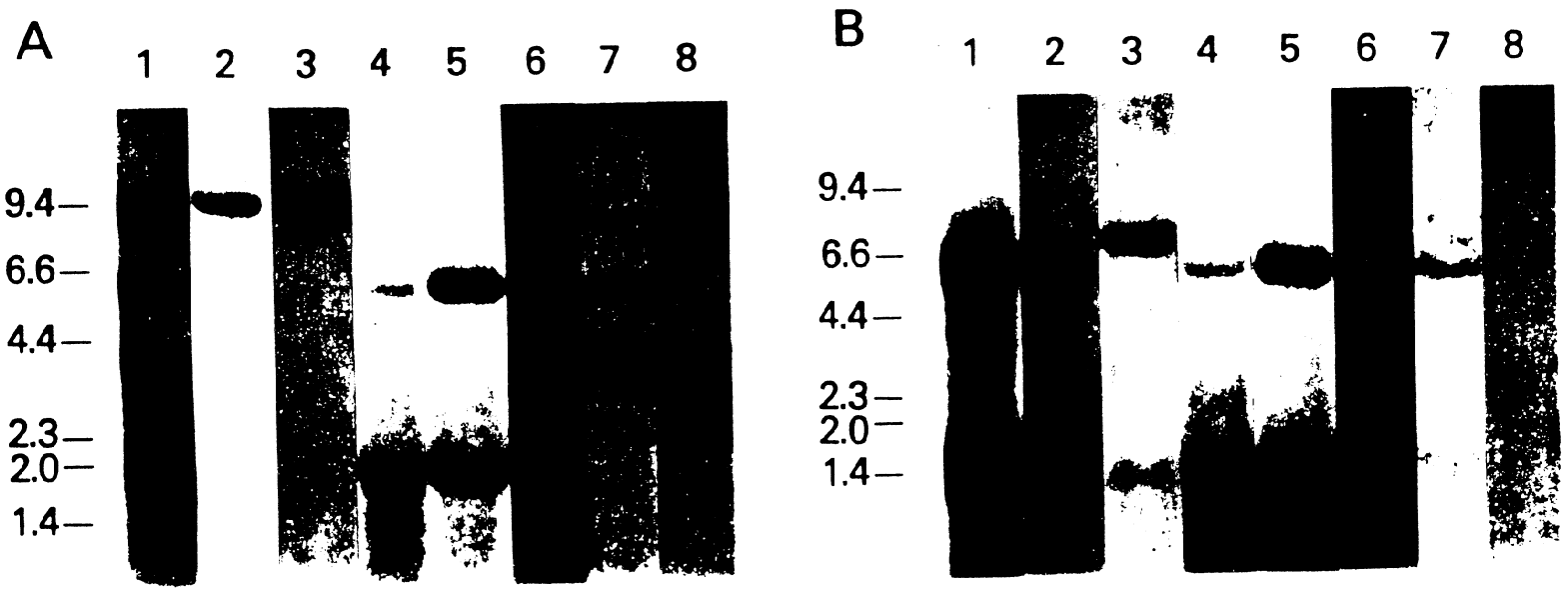
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