cell for B19 a precursor lying between BFU-E and CFU-E in the erythropoietic pathway (*23*). This inhibition does not require coinfection with an exogenously added helper virus, but it remains to be determined whether this target cell supports a fully productive, lytic infection by B19. That B19 should show such an extreme target cell specificity is not surprising, since susceptibility to lytic parvovirus infection has been shown in several cases to be a function of host cell differentiation (*3, 4*). The identification of B19 as the causative agent of erythema infectiosum and its probable transmission by way of the upper respiratory tract suggest that this virus will also be found to replicate at other sites in the body. Now that cloned copies of the B19 genome are available many questions about the virus, its distribution, and its disease-producing potential become experimentally accessible.

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RESEARCH ARTICLE

Molecular Characterization of Human T-Cell Leukemia (Lymphotropic) Virus Type III in the Acquired Immune Deficiency Syndrome

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The human retrovirus that has been isolated repeatedly from patients with the acquired immune deficiency syndrome (AIDS) and from persons at risk for the disease (*1–5*) shares many of the biological and physicochemical properties common to the human T-cell leukemia (lymphotropic) viruses (HTLV). These properties include tropism for T-lymphocytes, induction of multinucleated giant cells, a Mg^{2+} preferring reverse transcriptase of high molecular weight, a relatively small major core protein (molecular weight 24,000; p24), distant antigenic and nucleic acid homology, and a likely African origin (*1–4, 6–10*). Because of these similarities, and because of the uniform nomenclature adopted for the HTLV family of retroviruses (*11*), the AIDS associated virus was called HTLV-III.

Detailed characterization of HTLV-III and serologic testing of large numbers of patients with AIDS or AIDS-related complex (ARC) became possible when it was found that the virus could be transmitted to a human T-cell line, H9, that is largely resistant to the cytopathic effects of the virus but is a good virus producer (*1*). This cell line has served as the principal source of viral reagents for several seroepidemiological studies of HTLV-III in AIDS (*3–5, 12–16*), and as the source of virus in the present study of the molecular biology of the AIDS agent. In this article, we describe the molecular cloning of two full-length integrated proviral DNA forms of HTLV-III and an analysis of the HTLV-III genome in cell lines and fresh tissues from patients with AIDS or ARC.

Molecular Cloning of the HTLV-III Provirus

Sequences of HTLV-III were first detected in DNA of infected H9 cells (H9/HTLV-III) by Southern blot analysis with the use of a ^{32}P-labeled complementary DNA (cDNA) probe prepared from HTLV-III virions. There was no evidence of such sequences in uninfected H9 cells. The identity of these sequences in H9/HTLV-III was subsequently confirmed by using as a probe the cloned genome of HTLV-III derived from unintegrated linear viral DNA (*9*). Preliminary analyses of Southern digests of H9/HTLV-III DNA revealed that the virus was present in this cell line both as unintegrated DNA and as proviral DNA integrated into the cellular genome at multiple different sites. Since the HTLV-III provirus was found to lack Xba I restriction sites, a genomic library was constructed by using Xba I–digested

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Abstract. *The human T-cell leukemia (lymphotropic) virus type III (HTLV-III) appears to be central to the causation of the acquired immune deficiency syndrome (AIDS). Two full-length integrated proviral DNA forms of HTLV-III have now been cloned and analyzed, and DNA sequences of the virus in cell lines and fresh tissues from patients with AIDS or AIDS-related complex (ARC) have been characterized. The results revealed that (i) HTLV-III is an exogenous human retrovirus, approximately 10 kilobases in length, that lacks nucleic acid sequences derived from normal human DNA; (ii) HTLV-III, unlike HTLV types I and II, shows substantial diversity in its genomic restriction enzyme cleavage pattern; (iii) HTLV-III persists in substantial amounts in cells as unintegrated linear DNA, an uncommon property that has been linked to the cytopathic effects of certain animal retroviruses; and (iv) HTLV-III viral DNA can be detected in low levels in fresh (primary) lymphoid tissue of a minority of patients with AIDS or ARC but appears not to be present in Kaposi's sarcoma tissue. These findings have important implications concerning the biological properties of HTLV-III and the pathophysiology of AIDS and Kaposi's sarcoma.*

H9/HTLV-III DNA, and this was screened with an HTLV-III cDNA probe (*8*) to obtain molecular clones of full-length integrated provirus with flanking cellular sequences. Fourteen such clones were obtained from an enriched library of 10^6 recombinant phage, and two of these were plaque-purified and characterized.

Figure 1 illustrates the restriction maps of these two clones, designated λHXB-2 and λHXB-3. The overall length of the HTLV-III provirus is approximately 10 kilobases (kb). The identical restriction cleavage site patterns (Bgl II–Sst I–Hind III) spaced 9 kb apart in the two clones suggested that these regions represent the viral long terminal repeat (LTR) elements. This was confirmed by hybridizing a cloned 0.9-kb cDNA fragment corresponding to the 3′ terminus of HTLV-III to these terminal regions (data not shown). To show that the restriction enzyme cleavage sites depicted in Fig. 1 for clones λHXB-2 and λHXB-3 were actually present in the viral DNA of HTLV-III–infected H9 cells, we digested DNA from the H9/HTLV-III cell line with various restriction enzymes and analyzed it by the Southern blot technique. As shown in Fig. 2 (lanes b), the restriction fragments for Sst I, Eco RI, Hind III, Pst I, Bam HI, and Bgl II predicted from the restriction maps of λHXB-2 and λHXB-3 (Fig. 1) are indeed present in the Southern blots of HTLV-III–infected cellular DNA.

To determine whether the HTLV-III genome contains sequences homologous to normal human DNA, the viral insert of λHXB-2 (5.5 kb and 3.5 kb Sst I–Sst I fragments) was isolated, nick translated, and used to probe HTLV-III–infected and uninfected cellular DNA. Under standard conditions of hybridization [washing conditions: 1 × SSC (standard saline citrate), 65°C; annealing temperature (T_m), −27°C], this probe hybridized to DNA from H9/HTLV-III cells as well

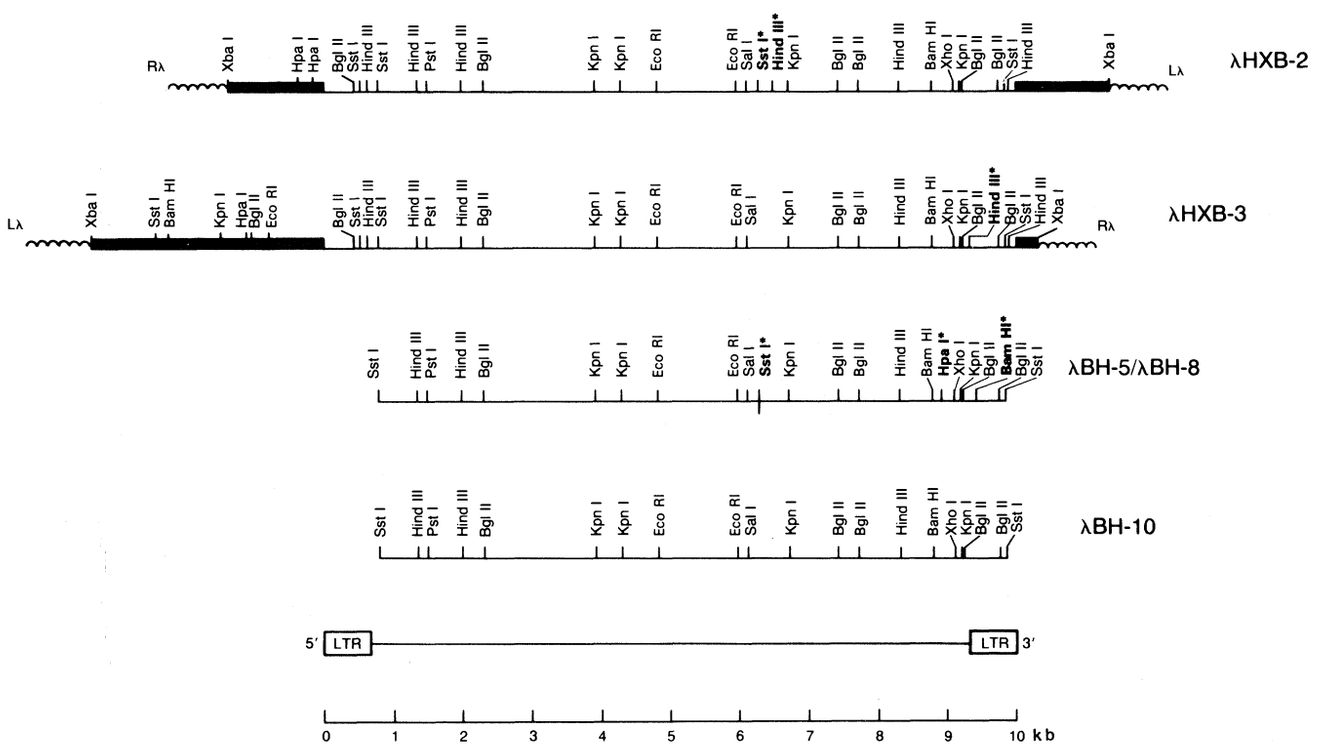

Fig. 1. Restriction endonuclease maps of four closely related clones of HTLV-III. λHXB-2 and λHXB-3 represent full-length integrated proviral forms of HTLV-III obtained from a λ phage library of H9/HTLV-III DNA (*36*). These clones contain the complete provirus (thin lines) including two LTR regions plus flanking cellular sequences (heavy lines). The LTR regions are known to contain the three restriction enzyme sites Bgl II, Sst I, and Hind III as shown, but their overall lengths are estimated. Clones λBH-10 and λBH-5/λBH-8 were derived from the linear unintegrated replicative intermediate form of HTLV-III in acutely infected H9 cells and have been reported elsewhere (*9*). Their restriction maps are shown here for comparison with λHXB-2 and λHXB-3 and with Southern blots of genomic DNA from other HTLV-III containing cells. It should be noted that λBH-5/λBH-8 consists of two separately cloned Sst I fragments (λBH-5 and λBH-8) which together constitute one HTLV-III genomic equivalent but which are not necessarily derived from the same viral molecule (*9*). Also, because λBH-10 and λBH-5 were cloned with the restriction enzyme Sst I (*9*), they lack 5′ LTR sequences as shown. Other differences in the restriction maps between these HTLV-III clones are indicated by bold letters and asterisks, with λBH-10 being used as a reference.

as other HTLV-III–infected cells, but not to DNA from uninfected H9 cells, uninfected HT cells (the parent cell line from which H9 was cloned), or normal human tissues (data not shown). This finding is in agreement with the results of other experiments in which the unintegrated (replicative intermediate) form of HTLV-III was used as probe (*9*) and demonstrates that HTLV-III, like HTLV-I and HTLV-II, is an exogenous retrovirus lacking nucleic acid sequences derived from human DNA.

As noted above, we previously cloned and analyzed the unintegrated linear DNA form of HTLV-III from H9 cells acutely infected with this virus (*9*). These clones, designated λBH-5, λBH-8, and λBH-10, were characterized in the same way as λHXB-2 and λHXB-3 and are shown for comparison in Fig. 1. The clones of integrated proviral DNA and unintegrated linear DNA are similar to each other yet are distinguishable by differences in several restriction cleavage sites. We know from recent analyses that λBH-10 and λBH-5/λBH-8 are incomplete viral clones that lack a short Sst I–Sst I segment of approximately 190 base pairs in the 5′ LTR-leader sequence region as a consequence of the use of Sst I in their cloning. The other differences between these clones must represent differences in the viral DNA restriction patterns since the predicted fragments unique to each clone can be identified in digests of H9/HTLV-III cellular DNA [see Fig. 2 and (*9*)].

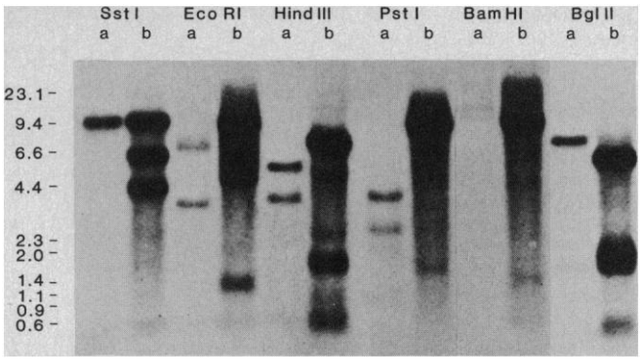

Fig. 2. Southern blot analysis of DNA from an HTLV-III–infected cell line established by coculturing uninfected H4 cells [another cloned cell line from HT cells; see (*1*)] with fresh peripheral blood mononuclear cells of a heterosexual Haitian man (R.F.) with AIDS (lanes a). For comparison, identical digestions of cellular DNA from the H9/HTLV-III line are shown (lanes b). The major internal restriction fragments predicted from clones λHXB-2, λHXB-3, λBH-10, λBH-5 and λBH-8 are apparent in blots of the H9/HTLV-III DNA (for example, see Sst I bands of 9, 5.5, and 3.5 kb, Eco RI band of 1.1 kb, and Hind III bands of 6.4, 4.5, 2, and 1 kb). In contrast, the restriction fragments generated by these same enzymes are quite different for HTLV-III$_{RF}$, indicating that the genome of this isolate is substantially different from the viral genomes in H9/HTLV-III cells. The experimental conditions have been described (*36–38*).

Genomic Diversity of HTLV-III

The finding of different forms of HTLV-III in a cell line originally infected with viral isolates from different patients suggested that the restriction pattern of the HTLV-III genome could vary from isolate to isolate. To test whether such diversity was a common occurrence, we examined the restriction patterns of HTLV-III in a number of different T-cell lines derived from individual patients with AIDS or ARC. In Fig. 2 we compare the restriction pattern of HTLV-III from a Haitian man (R.F.) with AIDS to that of the virus in the H9/HTLV-III cell line. As shown, the provirus from R.F. contains two Sst I sites, presumably in the LTR regions, which generate a 9-kb fragment indistinguishable in size from that in H9/HTLV-III, λHXB-3, or λBH-10 (Figs. 1 and 2). However, the HTLV-III in R.F. is substantially different from the predominant viral forms in H9/HTLV-III in most other restriction sites, including enzymes known to generate internal viral fragments in H9/HTLV-III DNA (for example, Eco RI, Hind III, and Bgl II). Because of these differences, and because most of the viral DNA in the HTLV-III$_{RF}$ line is polyclonally integrated (and therefore would not produce flanking bands), we conclude that the restriction map of HTLV-III$_{RF}$ must differ substantially from that of the viral forms in H9/HTLV-III cells.

We confirmed these differences by analyzing full-length clones of the HTLV-III$_{RF}$ provirus and showing that they differ from H9/HTLV-III in at least 19 out of 31 restriction sites. Despite these differences in restriction pattern, HTLV-III$_{RF}$ proviral DNA hybridized to λBH-10 probe under conditions of relatively high stringency (T_m − 27°C), indicating that the genomes of these viruses overall are similar. This conclusion is further supported by the finding that the major core protein of HTLV-III$_{RF}$ is similar in size (p24) and in antigenic reactivity to other HTLV-III isolates as determined by homologous radioimmunoprecipitation assays (*15*). Nevertheless, important differences may occur in the genomes of these viruses, for example, in the envelope glycoprotein which is a major determinant of antigenicity for most retroviruses.

In Fig. 3 we compare three more HTLV-III infected cell lines, one derived from a healthy homosexual man (R.H.) and two others from a child with ARC (M.N.). The isolates from M.N. were transmitted into the uninfected H9 cell line and also into the JM line (*17*), a human T-cell line that is unrelated to H9. Restriction digests with Hind III and Bgl II (as well as Eco RI, which is not shown), indicated that isolate HTLV-III$_{RH}$ is different from H9/HTLV-III. Similarly, HTLV-III$_{MN}$ is different from H9/HTLV-III in its restriction pattern for Sst I and Hind III as well as for Eco RI and Xho I. Moreover, these differences in restriction patterns are not the

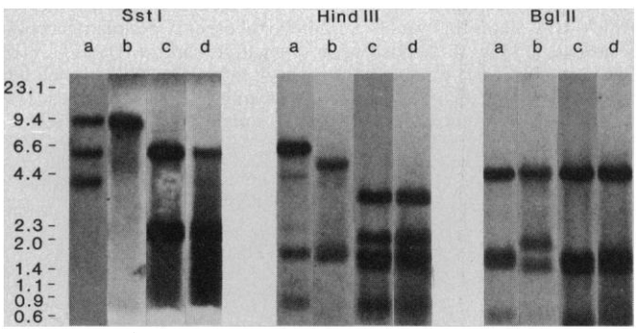

Fig. 3. Southern blot analysis of restriction endonuclease digested DNA from (lanes a) H9/HTLV-III cells and (lanes b, c, and d) from three other HTLV-III–infected cell lines. (Lanes b) DNA from a primary T-cell line established from peripheral blood lymphocytes of a healthy HTLV-III seropositive homosexual man (R.H.). (Lanes c and d) DNA from cell lines MN-1 and MN-2 established by transmission of the viral isolate HTLV-III$_{MN}$ into (lanes c) H9 cells or (lanes d) JM cells. As shown, the restriction patterns of the viral isolates from patients R.H. and M.N. are different from each other and from the H9/HTLV-III viral forms. HTLV-III$_{MN-1}$ and HTLV-III$_{MN-2}$ are identical to each other, indicating that the viral genome was not detectably altered as a consequence of its replication in a particular host cell type. The experimental conditions for this experiment, including the probe (λBH-10i), are the same as described for Fig. 2.

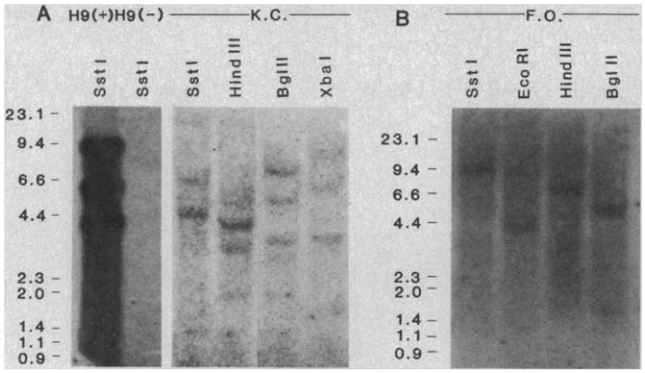

Fig. 4. Detection by Southern blot hybridization of HTLV-III sequences in DNA from freshly biopsied lymph nodes of (A) a patient with ARC (K.C.) and (B) another with AIDS (F.O.). (A) Five micrograms of DNA from cell line H9/HTLV-III (H9+), 25 μg of DNA from the uninfected cell line H9 (H9−), and 50 μg of DNA from the lymph node cells of patient K.C. were digested with the indicated restriction enzymes. HTLV-III sequences were detected in the DNA of H9/HTLV-III cells (H9+) and lymph node cells of K.C. but not in the DNA from uninfected H9 cells (H9−). (B) HTLV-III sequences were also detected in the DNA (25 μg per lane) from lymph node cells of patient F.O. [see (*35*) for methods]. The long exposure time (7 days) and the presence of some degraded DNA account for the heavy background smear in the first lane (H9+).

result of selective pressures associated with H9 cells, since similar patterns were observed for HTLV-III_{MN} grown in H9 or JM cells and digested with a total of seven different restriction enzymes, three of which are shown in Fig. 3.

To determine whether similar variability of the HTLV-III genome occurs in vivo, we examined the restriction enzyme cleavage patterns of HTLV-III DNA in freshly biopsied lymph nodes from one patient with ARC (K.C.) and another with AIDS (F.O.). In the HTLV-III viral DNA from patient K.C. (Fig. 4A), two major Sst I bands were present, as in H9/HTLV-III cells and in λHXB-2. However, the Hind III and Bgl II fragments did not correspond to fragments in any of the other isolates or clones of HTLV-III, and the cut made by Xba I within the provirus was unlike that in any other HTLV-III so far studied. In contrast to the viral DNA in isolate HTLV-III_{KC}, the viral DNA in HTLV-III_{FO} (Fig. 4B) corresponds in its major Sst I, Eco RI, Hind III, and Bgl II fragments to those in λBH-10 and λHXB-3. We assume that the small bands are present but too faint to be seen in this blot and that the Eco RI band is a doublet generated by cutting two internal Eco RI sites in predominantly unintegrated linear viral DNA (see Fig. 5). In one other HTLV-III–containing fresh tissue specimen the virus was similar to λHXB-2 in its Sst I and Bgl II patterns but different in its Hind III pattern (data not shown). In all, we examined 12 different HTLV-III isolates from either fresh or cultured cells. Five of them could be distinguished from each other by substantial differences in their restriction cleavage patterns. Seven others, including the four highly related forms cloned from the H9/HTLV-III cell line, were similar to (but not necessarily identical with) one of the first five. From these results it was not possible to differentiate between isolates from patients with AIDS and ARC on the basis of their restriction patterns.

The diversity in the genomic restriction maps of different HTLV-III isolates stands in contrast to the high degree of conservation in the genomes of HTLV-I and HTLV-II. For example, Wong-Staal *et al.* (*18*) reported that ten out of ten HTLV-I isolates from different regions of the world had conserved internal Pst I and Bam HI cleavage sites. Similarly, in a survey of 88 patients with adult T-cell leukemia (ATL) in Japan, Yoshida *et al.* (*19*) found that the Pst I sites in the HTLV-I proviruses were similar in 79 (90 percent) of them. Preliminary analysis of the exceptional nine cases indicated that these represented defective proviruses and not complete HTLV-I proviruses with divergent restriction patterns. These investigators also compared isolates of HTLV-I from Japan and the United States and found them to be quite similar (*19*). We have cloned and analyzed HTLV-I proviruses from two patients with ATL and have found them to be very similar to each other and to a Japanese clone of HTLV-I whose sequence has been reported (*20–22*). We have also compared the full-length clones of HTLV-II from two different individuals and have found them to be identical in 25 out of 25 restriction sites (*21*). Thus, we conclude that the genomes of different isolates of HTLV-III vary considerably more than do those of either HTLV-I or HTLV-II.

Table 1. Survey by Southern hybridization of fresh tissue specimens from patients with AIDS or ARC for HTLV-III DNA sequences. Tissue samples were obtained from 65 patients, and DNA was extracted as described (*35*). A sample of each DNA (25 to 50 μg) was digested with Sst I (alone) and with Bgl II (alone), both of which generate internal viral DNA fragments in all HTLV-III isolates that we have studied. All other experimental conditions and methods were as described for Fig. 4. A positive and negative control (H9/HTLV-III and normal lymphocyte DNA, respectively) was included on each filter. The DNA on the filters was also hybridized to nick-translated λ phage and pBR322 (lacking HTLV-III sequences) to rule out the possibility that the bands observed could be due to contamination of the fresh tissue DNA's with either of these vectors.

Clinical diagnosis	Number of patients		Number of tissue samples		Tissue tested*
	Tested	Positive	Tested	Positive	
ARC	26	3	7	0	PBC
			18	3	LNC
			1	0	BMC
AIDS	39	6	15	1	PBC
			16	4	LNC
			8	0	BMC
			11	2	SPL
			5	0	KS

*Abbreviations: PBC, peripheral blood mononuclear cells; BMC, bone marrow mononuclear cells; LNC, lymph node cells; SPL, spleen; KS, Kaposi's sarcoma.

Unintegrated Viral DNA and Cytopathic Effects

In the life cycle of a retrovirus, the single-stranded RNA genome must be copied by reverse transcriptase into an unintegrated linear DNA form prior to its integration into the host cell's chromosomal DNA (*23*). The linear unintegrated DNA duplex contains a copy of the viral genome in a nonpermuted order and can generally be detected only as a short-lived replication intermediate in the cytoplasm of infected cells shortly after viral infection. Persistence of the unintegrated form of viral DNA has been observed uncommonly in certain animal retroviral systems (*24, 25*) and has been correlated with the cytopathic effects of these retroviruses (*24*). In cells freshly infected with HTLV-III, in chronically infected cells, and even in biopsied

lymph node tissue from a patient with AIDS, we have found that a substantial amount of HTLV-III DNA persists in the unintegrated form, mostly as linear double-stranded DNA but also in much lesser amounts as closed and nicked circular DNA (Fig. 5). Although we have not quantified the actual numbers of unintegrated as opposed to integrated viral DNA forms per cell, we have found, in most cell lines examined, that the intensity of hybridization of the 10-kb band representing unintegrated linear DNA is much greater than that of the high molecular weight smear representing polyclonally integrated proviral DNA and is similar in intensity to internal viral bands generated by enzymes such as Sst I, Bgl II, and Hind III. We have found this relative abundance of unintegrated viral DNA in seven out of eight cell lines and in one out of three fresh tissue specimens, all of which contained detectable amounts of HTLV-III. The importance of this unintegrated DNA in the cytopathic effects of HTLV-III in vitro and in vivo requires further study.

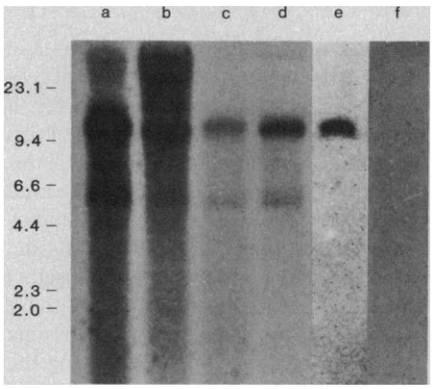

Fig. 5. Demonstration by Southern analysis of unintegrated HTLV-III DNA in cell lines and fresh tissue derived from patients with AIDS or ARC. High molecular weight DNA was extracted as described (*35, 37*). Samples (10 to 20 μg) were then subjected to electrophoresis in standard horizontal 0.8-cm-thick, 0.7 percent agarose gels without prior restriction enzyme digestion and blotted and hybridized to λBH-10i probe as described (*39*). The bands migrating at approximately 10 kb represent the unintegrated linear form of the virus and the smaller bands at approximately 5 kb, the unintegrated closed circular form. Identical bands were obtained when these DNA's were digested with Xba I (which does not cut the HTLV-III genomes in these samples) and when undigested low molelcular weight (Hirt) DNA was examined. (Lane a) Normal peripheral blood mononuclear cells 4 days after infection with concentrated H9/HTLV-III virions (*9*); (lane b) H9/HTLV-III cells 6 months after infection with HTLV-III (*1*); (lane c) JM cells 3 months after infection with HTLV-III (*17*); (lane d) primary RH lymphocytes after 3 months of culture; (lane e) lymphocytes from freshly biopsied lymph node of patient F.O.; and (lane f) normal uninfected human lymphocytes.

Detection of HTLV-III DNA in Fresh Tissue

Antibodies to HTLV-III can be detected in most patients with AIDS or ARC (*12*), and HTLV-III can be isolated from a large proportion of these patients (*1, 2, 13*). In the present study, we examined by Southern hybridization a variety of fresh tissue specimens from 65 patients with ARC or AIDS for the presence of HTLV-III DNA. Representative blots of lymph node DNA from a patient with ARC and another with AIDS are shown in Fig. 4. Sequences of HTLV-III were detectable in both tissue specimens but in small amounts. For example, in Fig. 4A, the signal intensity for 5 μg of H9/HTLV-III DNA is much greater than that for 50 μg of lymph node DNA. It should be noted, however, that H9/HTLV-III cells contain many HTLV-III DNA molecules per cell (*26*).

In all, HTLV-III sequences were detected in only 9 out of 65 patients evaluated (Table 1). Roughly the same proportion of ARC patients (3 out of 26) as AIDS patients (6 out of 39) was positive for HTLV-III, and viral sequences were detected most commonly in lymph node (7 of 34) and spleen (2 of 11). None of five Kaposi's sarcoma tissue specimens examined was positive for HTLV-III DNA sequences. These specimens had been obtained from the skin of three patients and from lymph node of two others and had been estimated by histopathologic examination to consist of between 30 and 90 percent Kaposi's sarcoma cells. Since we have found that our Southern hybridizations can detect less than one viral DNA copy per ten cells, we interpreted these data to indicate that the three Kaposi's sarcoma lesions did not contain HTLV-III sequences. To ensure that the Kaposi's sarcoma DNA was of good quality and was adequately digested, we rehybridized the DNA on the nitrocellulose filters to an HLA class I gene probe which readily detected the expected HLA gene fragments (data not shown). Of the nine patients positive for HTLV-III sequences in this fresh tissue survey, more than one tissue type was examined in three. In the first patient, HTLV-III DNA sequences were detected in a cervical lymph node on one occasion (see Fig. 4B) but not in an axillary lymph node or in peripheral blood lymphocytes from the same patient 2 months later. In another patient, HTLV-III DNA sequences were detected in both lymph node and spleen, and in the third patient, viral sequences were detected in spleen but not lymph node.

In most of the patients in which HTLV-III DNA sequences were detected in fresh tissue, the signal intensities were weak. From dilution experiments with known amounts of HTLV-III–containing DNA, we have estimated that, in the nine positive patients reported here, HTLV-III DNA was present at a level of less than one copy per 10 cells (data not shown). Theoretically, this low signal intensity could also be explained by the presence of a virus distantly homologous to HTLV-III in these cells. This is unlikely, however, because cell lines derived from these tissues are productively infected with HTLV-III (as determined by both nucleic acid and immunologic characterization) and because the restriction fragments in the Southern blots of DNA derived from fresh and cultured cells correspond to many of those present in the cloned genomes of HTLV-III. Furthermore, the genomes of HTLV-III and of the virus referred to as LAV [lymphadenopathy-associated virus (*27*)] are highly related, so any presumed differences between them could not account for the weak signals detected in AIDS and ARC tissues. We have also hybridized full-length HTLV-I and HTLV-II probes to these infected DNA's and they do not detect viral sequences, thus indicating that the weak signals detected by the HTLV-III probe are not due to hybridization to HTLV-I or HTLV-II sequences.

Discussion

We have described here the molecular cloning and analysis of the full-length HTLV-III proviral genome from the cell line H9/HTLV-III. Since this cell line is the principal source of viral reagents for the seroepidemiological studies of HTLV-III in AIDS (*12*), and since these H9/HTLV-III–derived viral clones detect viral sequences in fresh and cultured tissues from patients with this disease, we conclude that λHXB-2 and λHXB-3 are representative of the virus that causes AIDS. This conclusion is further substantiated by the fact that these cloned HTLV-III probes detect viral sequences in cells infected with LAV under stringent hybridization conditions (*28*).

From an analysis of the HTLV-III proviral clones, it is apparent that the viral genome is approximately 10 kb in length (slightly larger than HTLV-I and

HTLV-II) and possesses redundant sequences, presumably the LTR elements, at each end. Comparison of these full-length proviral clones of HTLV-III with two clones of linear unintegrated viral DNA derived from the same HTLV-III–infected H9 cells (*9*) indicates that they are colinear and very similar in restriction pattern. These clones of HTLV-III lack any sequences homologous to DNA from uninfected human cells as determined by blot hybridization at moderately high stringency (T_m − 27°C).

We have used both biochemical (*9*) and electron microscopic (*26*) heteroduplex techniques to determine homology between HTLV-III and HTLV-I and HTLV-II and have found significant homology in the *gag-pol* region. This finding is in agreement with our earlier observations for which we used HTLV-III cDNA (*8*) and with the immunologic studies of Sarngadharan *et al.* (*7*). It is interesting that very minimal, if any, homology has been detected between the HTLV-III genome and the pX region of HTLV-I and HTLV-II, since it is the pX region which is most highly conserved between HTLV-I and HTLV-II and is thought to be involved in their transforming properties (*20, 29*). This difference in relative homologies may be related to the different biological properties of these viruses, HTLV-I and HTLV-II being primarily transforming and HTLV-III cytopathic.

The finding of substantial diversity in the restriction cleavage patterns of different HTLV-III isolates is probably related to the highly replicative nature of this virus (*1*) and the well-recognized infidelity of retroviral replication (*30, 31*). This characteristic distinguishes HTLV-III from HTLV-I, the latter being predominantly a transforming virus that exists for long periods in a proviral form that is generally not expressed (*32*). The true extent of the genomic differences in HTLV-III is not known, but for many of the isolates tested the majority of restriction enzyme cleavage sites were different from those found in the HTLV-III clones and in the H9/HTLV-III DNA. In fact, we recently cloned the complete HTLV-III provirus from cell line H9/HTLV-III$_{RF}$ (see Fig. 2) and found that this provirus differs from the proviruses in H9/HTLV-III cells in at least 19 out of 31 mapped restriction sites. Since even a single base pair substitution in a critical region of a retroviral genome can have drastic effects on its biologic function (*31*), further studies involving the expression of HTLV-III genes (such as attempts to express envelope sequences for vaccine production) will need to take into account the genomic diversity of different HTLV-III isolates. However, it may be possible to take advantage of this diversity by evaluating different isolates of HTLV-III for regions of conserved nucleotide sequences. Similar studies have been done with HTLV-I and HTLV-II and have led to new insights regarding the biologic properties of these viruses (*20, 29*). It may also be possible to exploit this diversity in following the transmission of specific HTLV-III viruses both in vivo and in vitro.

Unlike most other retroviruses, HTLV-III appears to persist in both integrated and unintegrated forms in chronically infected cells. We found proviral DNA integrated at multiple sites in eight chronically infected cell lines, indicating that the cell population was polyclonal with respect to the site of HTLV-III integration. These findings distinguish HTLV-III from HTLV-I and HTLV-II, both of which have predominant transforming effects and lead to the proliferation, in vitro and in vivo, of monoclonally expanded populations of cells (*6*). Since HTLV-III has not been shown to possess transforming properties but is cytopathic, it is not surprising that even chronically infected cells contain polyclonally integrated provirus. It is not known whether this polyclonality is due to the continuous reinfection of neighboring cells or to the stable growth of a population of immortalized cells having HTLV-III integrated at random sites. Seven of the eight cell lines, as well as one of the three fresh tissue samples, also contained unintegrated linear viral DNA. The relative proportion of unintegrated HTLV-III DNA and its location (that is, in the cytoplasm, nucleus, or both) have not been determined, and it is not known whether this form of the virus is responsible for its cytopathic effects. Such a mechanism has been proposed to explain the cytopathic effects of certain other retroviruses (*24*).

We have previously been able to isolate HTLV-III from peripheral blood or lymph node tissue from most patients with AIDS or ARC (*12*), in concordance with the 85 to 100 percent seropositivity for HTLV-III in these groups. However, as shown herein, HTLV-III DNA is usually not detected by standard Southern hybridization of these same tissues and, when it is, the bands are often faint. This must mean that only a minor population of cells is infected with HTLV-III at any one time. Thus, the lymph node enlargement commonly found in ARC or AIDS patients cannot be due directly to the proliferation of HTLV-III–infected cells (as occurs with HTLV-I in adult T-cell leukemia). Whether the lymphocytic proliferation in lymph nodes occurs in response to infection with HTLV-III or another agent, or both, is not known. Similarly, the absence of detectable HTLV-III sequences in Kaposi's sarcoma tissue of AIDS patients suggests that this tumor is not directly induced by infection of each tumor cell with HTLV-III. Furthermore, the observation that HTLV-III sequences are found rarely, if at all, in peripheral blood mononuclear cells, bone marrow, and spleen provides the first direct evidence that these tissues are not heavily or widely infected with HTLV-III in either AIDS or ARC. However, on the basis of our ability to culture virus successfully from these various tissues (*13*), it is apparent that each does harbor the virus in a small population of cells. It is likely that the use of more sensitive techniques will increase the frequency of detection of HTLV-III sequences in fresh tissues from patients.

The availability of molecular clones of HTLV-III will now permit the direct comparison of this virus to other retroviruses detected in patients with AIDS or ARC. These viruses, named variously as LAV, IDAV$_1$, IDAV$_2$, and ARV (*27, 33*), are morphologically indistinguishable from HTLV-III and, for those isolates tested, immunologically indistinguishable as well (*34*). On the basis of these results and of our detecting viral sequences in LAV-infected cells using HTLV-III probes (*28*), we believe that HTLV-III, LAV, IDAV$_1$, IDAV$_2$, and the most recently described AIDS-associated viral isolate, ARV, are all essentially the same virus. However, given the diversity in the genomic restriction pattern of HTLV-III reported herein, we would expect similar differences to be present in these other viral isolates.

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sexual men possess serum antibodies to HTLV-III, whereas less than 1 percent of normal heterosexuals have these antibodies (*3–5, 13–15*). Furthermore, antibodies to HTLV-III have been detected in most hemophiliacs and children with AIDS and in ten out of ten pairs of transfusion-associated donor-recipient AIDS cases (*4, 13, 15, 16*).
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35. Freshly biopsied lymph nodes were minced with a scalpel in 2 ml of isotonic phosphate buffered saline (*p*H 7.4). This cell suspension was made up to 20 ml in tris (20 m*M*, *p*H 7.4), EDTA (5 m*M*), sodium dodecyl sulfate (5 mg/ml), and proteinase K (100 μg/ml) and incubated at 50°C for 3 hours. The DNA was then extracted three times with phenol and chloroform (1:1 by volume) saturated with 50 m*M* tris (*p*H 9.0) and once with chloroform alone, adjusted to 0.3*M* Na^+ with sodium acetate (*p*H 6.0), and precipitated with two volumes of absolute ethanol. High molecular weight DNA was dissolved in TE buffer (20 m*M* tris, *p*H 7.4; and 1 m*M* EDTA). The indicated amount of DNA was digested with 150 units of each restriction enzyme for 8 hours at 37°C and then subjected to electrophoresis in 0.7 percent agarose. The probe (λBH-10i) and other experimental conditions were the same as described for Fig. 2 except that 10×10^6 dpm of probe per milliliter of hybridization solution was used and the blots were exposed to x-ray film for 7 days.
36. A λ phage library was constructed according to standard methods (*37*) using the cloning vector J1λ (*38*) and Xba I digested H9/HTLV-III DNA which had been enriched by sucrose gradient centrifugation for 10- to 15-kb fragments. Phage plaques (10^6) were screened with cDNA prepared from doubly banded HTLV-III virions (*8*), and 14 positive signals were obtained. Two of these were plaque purified and their restriction maps determined according to standard methods (*37*).
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39. High molecular weight DNA was prepared from RF and H9/HTLV-III cells by standard methods (*37*). We used 15 μg of DNA for each restriction enzyme digestion, performed according to manufacturers' recommendations; the DNA was subjected to electrophoresis through 0.8-cm-thick 0.7 percent agarose slab gels. Gels were blotted in 10× SSC onto 0.1-μm nitrocellulose filters (Schleicher and Schuell) (*37*). Hybridizations were performed at 37°C for 18 hours in 2.4× SSC, 40 percent formamide, 10 percent dextran sulfate, 1 mg/ml each of bovine serum albumin, polyvinylpyrollidone, and Ficoll, and 20 μg/ml transfer RNA. Filters were washed for 2 hours at 65°C in 1× SSC. The probe used was the Sst I/Sst I insert from λBH-10, 3×10^6 dpm/ml (specific activity approximately 2×10^8 dpm/μg). Blots were exposed to Kodak XAR-5 film for 2 days.
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