insertion of the copia element in the *white-apricot* allele; for a map of *white*, see R. Levis, P. M. Bingham, G. M. Rubin, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 564 (1982); for a map of the transposons, see (*5*). This probe hybridizes to a 5.9-kilobase (kb) fragment in this digest representing the 5′ half of the *white* DNA of the transposon. The w^{1118} allele does not hybridize with this probe. An identical DNA blot was hybridized with a probe containing both the Sal I(-2.7)–Sal I(-1.3) and Sal I(-1.3)–Sal I(−0.5) segments. This probe hybridizes to a 5.8-kb fragment in this digest representing the 3′ half of *white* in the transposon plus a 6.2-kb fragment from w^{1118}. For revertants of A^R4-3, genomic DNA digested with Xba I and Eco RI was hybridized with a probe containing the Xba I (+0.7)–Eco RI (+6.6) *white* segment. This segment hybridizes to the 5.9-kb fragment representing the 5′ half of the *white* DNA in the transposon. A Hind III digest of A^R4-3 revertants was hybridized with the Bam HI(+1.4)–Hind III(+3.3) *white* segment that hybridizes to the 6.2-kb band. This band represents the 3′ half of *white* in the A^R4-3 transposon and does not hybridize to w^{1118} DNA.

. The designation 24CD refers to a polytene chromosome region in the maps of C. B. Bridges (*24*).

. Both cytogenetic and blot analysis indicate that the three wild-type revertants of A^R4-3 having new sites of *white* on the second chromosome also retain the transposon at the original site (near the centromeric heterochromatin of chromosome arm 2L). This does not prove that transposition of P-transposons is replicative, however, since it might have been the copy of A^R4-3 on the other homolog or the sister chromatid of the injected embryo which transposed. For these three revertants, we have not constructed strains that carry *white* at these new sites alone. Therefore, we can only infer that the wild-type phenotype of each of these revertants was caused by the *white* gene at the new site.

. The chromosome containing this derivative also carried the parental A^R4-3 transposon. The presence of this chromosome resulted in a nearly wild-type eye color. The original and new sites were subsequently separated by recombination to reveal the novel mosaic phenotype conferred by the gene at the new site.

. Certain features of eye development demonstrate the existence of positional information in the eye. An equator of symmetry in photoreceptor arrangement bisects the eye into dorsal and ventral halves. A preferential boundary for cell clones is located parallel to the equator but does not always coincide with it [H. J. Becker, *Z. Indukt. Vererb. Lehre* **88**, 333 (1957); W. K. Baker, *Dev. Biol.* **62**, 447 (1978); D. F. Ready, T. E. Hanson, S. Benzer, *ibid.* **53**, 217 (1976); J. A. Campos-Ortega and M. Waitz, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **184**, 155 (1978)]. We do not know the precise position of the differential pigment border in the eyes of A^R4-24 flies (Fig. 4C) relative to these other two boundaries or whether there is a relation between them. It is also possible that there is a relation between the pigment pattern in the eyes of A^R4-3 flies (Fig. 4A) and the morphogenetic furrow, which moves from the posterior to the anterior across the eye-antennal imaginal disk of the third-instar larva.

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The 11 wild-type revertants with new insertions of *white* on autosomes have been tested for the interaction of a single copy of the P-*white* transposon with z^1. Males of the genotype $z^1w^{1118}cv$; P[*w*]/+ and females of the genotype $z^1w^{1118}cv/z^+w^{1118}$; P[*w*]/+ were constructed (*cv* is the genetic abbreviation for the *crossveinless* gene). All have a wild-type phenotype (do not interact with z^1 when present in a single copy) with the exception of the two transposons located in 92B, which had a red-brown eye color in the females. This eye color is indicative of a weak interaction.

Flies that are hemizygous or homozygous for z^1w^- and carry either one or two copies of the A^R4-24 transduced *white* gene have similar phenotypes.

Intercalary heterochromatin may exist within otherwise euchromatic regions. However, 24CD has not been noted as a site of intercalary heterochromatin [E. V. Ananiev *et al.*, *Chromosoma* (Berl.) **70**, 1 (1978)]. Furthermore, there is no evidence for the induction by intercalary heterochromatin of mosaic position effects on rearranged euchromatic genes (*1*). We cannot now rule out the possibility that the P-*white* transposon in A^R4-3 may have carried flanking sequences with it in its transposition to 24CD. We have shown a difference in the distance between the transposon and the next adjacent Bam HI site in flanking DNA on one side of the sites of insertion in A^R4-3 and A^R4-24 (*12*). However, we have not proved that the DNA flanking the insertion in A^R4-24 originates from the 24CD region.

22. Transduced *white* genes may also be more sensitive to position effects in their interaction with z^1 than are rearranged *white* genes that are part of larger translocations (*5*).
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Hepatitis B Virus DNA Sequences in Lymphoid Cells from Patients with AIDS and AIDS-Related Complex

Abstract. *A lymphotropic virus HTLV-III/LAV was recently identified as the etiologic agent of the acquired immune deficiency syndrome (AIDS). In a study of concomitant hepatitis B infections in patients with AIDS or the AIDS-related complex, DNA sequences of hepatitis B virus (HBV) were found in fresh and cultured lymphocytes from patients with AIDS even in the absence of conventional HBV serological markers. Furthermore, the restriction DNA pattern was consistent with the integration of the viral DNA. These results should prompt additional studies to reevaluate a possible role of HBV as a cofactor in AIDS in addition to the HTLV-III/LAV causal agent.*

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A human T-lymphotropic retrovirus (HTLV-III, LAV) (*1–5*) has been isolated from patients with the acquired immune deficiency syndrome (AIDS) or AIDS-related complex (ARC) and from clinically asymptomatic individuals (*3, 6*). This virus has been causally related to AIDS and ARC, and it appears to be a necessary etiologic agent of these syndromes (*7*). However, infection by HTLV-III/LAV induces different responses depending on the individuals, suggesting that one or more other factors, such as hepatitis B virus (HBV), cytomegalovirus (CMV), or Epstein-Barr virus (EBV) (*8*), might also have a role in the pathogenic mechanism that leads to immune deficiency or might enhance the likelihood of disease manifestations of HTLV-III/LAV infection. Indeed, on the basis of epidemiological and biological considerations, several authors have proposed an etiologic role for HBV (*9*). Serological markers of HBV have frequently been detected in patients with AIDS and in people at high risk for AIDS (for example, homosexual men, hemophiliacs, and intravenous drug abusers), and HBV DNA sequences have been identified in bone marrow cells (*10*) and mononuclear blood cells of such subjects (*11, 12*). These data prompted us to investigate concomitant hepatitis B infections in patients with AIDS and ARC. We report here that HBV DNA sequences were present in lymphocytes derived from AIDS patients who were serologically HBV-positive or HBV-negative. In the lymphocyte populations assayed, we also detected a common pattern consistent with the integration of the HBV DNA and with free monomeric viral forms.

Lymphoid cell DNA from four patients, two with AIDS (one of them with Kaposi's sarcoma) and two with ARC, were assessed. That each of the patients was infected with HTLV-III/LAV was demonstrated by (i) the presence of antibodies to HTLV-III in the serum samples detected by indirect immunofluorescence as described (*13*), (ii) the identification of reverse transcriptase activity (RT) in the supernatant of primary mononuclear cell cultures and H9 cocultures, and (iii) the detection of HTLV-III antigens p15 and p24 in acetone-fixed cultured lymphocytes by indirect immunofluorescence in the presence of specific monoclonal antibodies (*1, 14*).

Serological markers for HBV were

Table 1. Results of serological and HBV DNA hybridization studies. HTLV-III/LAV was identified in mononuclear cells from AIDS or ARC patients by antibodies to HTLV-III in the serum samples revealed by an indirect immunofluorescence test, HTLV-III antigens detected by fixed-cell indirect immunofluorescence, and RT activity in the supernatant of primary mononuclear cells culture and H9 coculture (*1, 13, 14*). The HBV serological markers were identified by a commercial solid-phase radioimmunoassay (Abbott) and the HBV DNA sequences were detected as described in Fig. 1.

Patient number	HTLV III	Serological markers for HBV			Hepatitis B virus DNA sequences		
		HBsAg	Anti-HBc	Anti-HBs	Fresh PBL	Long-term cultures of T cells	Cocultured T cells with H9 cells
1 (AIDS)	+	−	−	−	NT*	+	+
2 (AIDS)	+	+	+	−	+	+	+
3 (ARC)	+	−	+	+	+	+	+
4 (ARC)	+	−	+	+	−	+	+
Control subjects (n = 5)	−	−	−	−	−	−	−

*NT, not tested.

identified by commercial solid-phase radioimmunoassay (Abbott). One patient was positive for hepatitis B surface antigen (HBsAg) and positive for antibodies to the core (HBc) protein. Two other patients were negative for HBsAg but positive for antibodies to HBs and HBc. The last patient and five normal individuals (control group) were negative for all HBV serological markers.

Fresh mononuclear cells from peripheral blood lymphocytes (PBL) or lymph nodes derived from patients with AIDS or ARC and from normal donors were obtained by separation through a Ficoll-Hypaque gradient. Primary cultures of 5×10^5 to 6×10^5 PBL were seeded in medium (RPMI 1640 with 20 percent fetal calf serum) containing phytohemagglutinin (PHA). After 2 to 3 days, T-cell growth-factor interleukin-2 (IL-2), antibody to human α-interferon (α-IFN) (neutralizing titer 6 IU at a dilution of 10^5), and hydrocortisone (5 μg) were added.

Long-term T-cell cultures were obtained as described (*15*). Briefly, 10^3 to 10^4 fresh PBL were cultured with a feeder cell layer consisting of irradiated (4000 rads) lymphoid cells in a medium containing PHA and, after 2 to 3 days, IL-2, antibody to α-IFN, and hydrocortisone. When maintained at a density of 0.3×10^6 to 1×10^6 cells per milliliter, the PBL proliferated in the presence of IL-2 for up to 2 months.

When RT activity was found (day 6 to 10), in the primary cultures from AIDS patients, portions of cells were cocultivated with uninfected H9 cells as described (*1, 14*). These cocultures, which proliferated in a medium deprived of IL-2 for long periods, were found to contain cells morphologically similar to H9 cells as well as multinuclear giant cells. The presence of retrovirus in the cocultures was demonstrated by finding RT activity in the culture fluids and HTLV-III p15 and p24 antigens in acetone-fixed cells by indirect immunofluorescence in the presence of specific monoclonal antibody (*14*).

Fresh mononuclear cells, long-term cultures of lymphocytes, and H9 cocultures were examined for the presence of HBV DNA sequences by Southern blotting (*16*). The purified viral HBV DNA probe was obtained from a recombinant plasmid after digestion with Eco RI, electrophoresis, and electroelution (*17*). The cellular DNA was studied before and after digestion (Fig. 1) with the following restriction enzymes: Eco RI and Xba I, which generally cut the HBV genome at one and two sites, respectively, and Hind III and Sac I, which usually do not cut the HBV DNA (*18*).

As shown in Table 1, HBV DNA sequences were detected in the long-term cultures of T lymphocytes from all four patients tested. We also tested cellular DNA from fresh mononuclear cells of three of these patients and identified viral DNA in cells from two of them. The samples of cellular DNA, both before and after digestion with Eco RI, showed a single band at the 3.2-kb position. After digestion with Hind III and Sac I, discrete bands corresponding to high molecular weight DNA (at the 12- and 23-kb positions, respectively) were associated with the 3.2-kb band.

Taken together, these results are consistent with the integration of the viral genome with a dimeric or multimeric organization of HBV DNA. Since HBV DNA sequences were detected at the 3.2-kb DNA position both before digestion with restriction enzymes and after digestion with Hind III and Sac I, the results also suggest the presence of free monomeric viral DNA forms. However, the presence of discrete bands after digestion with Hind III and Sac I that were absent from the undigested DNA pattern is consistent with clonal proliferation of infected cells. These bands were observed at the same positions in the genomic DNA from different patients. When more cellular DNA becomes available it will be possible to clone the viral DNA sequences and determine the integration sites for the HBV DNA. It will also be possible to compare restriction patterns for such sequences with patterns from liver DNA (*19*). Free monomeric HBV DNA but no viral DNA replicative forms were identified in samples of the long-term cultures. It is interesting that HBV DNA, although absent from the reference H9 cell line, was detected in the cocultures of H9 cells with mononuclear

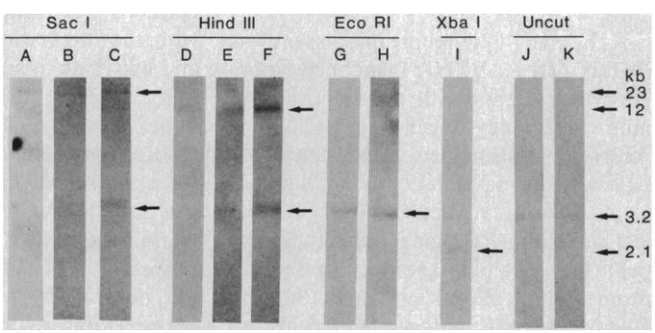

Fig. 1. Representative restriction pattern of HBV DNA hybridization in mononuclear cells from patient No. 1 (Fig. 1). Southern blot analysis of cellular DNA from fresh mononuclear cells (lanes A and D), long-term cultures of T lymphocytes (lanes B, E, G, and J), and T lymphocytes cocultivated with H9 cells (lanes C, F, H, I, and K). Samples (5 μg) of undigested or digested DNA's were separated through an 0.8 percent agarose gel and transferred to a nitrocellulose membrane. The filters were hybridized with a nick-translated HBV DNA (1.5×10^7 count/min) insert from pcP10 (3×10^8 count/min per microgram of DNA) in 15 ml of 50 percent formamide, 5× SSC (standard saline citrate), 1× Denhardt's solution, 10 percent of dextran sulfate, 20 mM Na_2HOP_4, pH 6.5, and sonicated salmon sperm DNA. After overnight incubation at 42°C, the filters were washed under stringent conditions in 2× SSC with 0.1 percent sodium dodecyl sulfate (SDS) for 5 minutes at room temperature and then in 0.1× SSC with SDS at 65°C for 3 hours. The specificity of these results was supported by the negativity of the H9 cells and the control samples. Bacterial DNA contamination was eliminated by the absence of hybridization with the ^{32}P-labeled PBR_{322} probe.

cells from the AIDS patients. If one assumes that the primary PBL had died after 3 weeks in coculture, which was when we detected the HBV DNA, this result implies that the H9 cells acquired the HBV from the patients' mononuclear blood cells.

Studies with five additional AIDS patients, two of whom were negative for conventional HBV markers, showed that HBV DNA sequences were present in cell populations derived from different lymphoid sources: bone marrow, semen, lymph node, as well as PBL. The concomitant infections of HBV-HTLV-III/LAV found in all of these patients, even in the absence of conventional serological HBV markers, are consistent with the possibility that HBV may be a cofactor in the development of AIDS, as previously suggested for CMV and EBV (*8*). Further studies are needed to assess this hypothesis, and it will be necessary to conduct similar studies with DNA probes for other viruses, such as EBV and CMV, and to determine the role of all of these viruses in the pathogenesis of AIDS.

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Infection of HTLV-III/LAV in HTLV-I–Carrying Cells MT-2 and MT-4 and Application in a Plaque Assay

Abstract. *The human T-cell lines MT-2 and MT-4 carry the human T-cell leukemia virus type I (HTLV-I). When MT-2 and MT-4 were infected with HTLV-III, the probable etiologic agent of the acquired immune deficiency syndrome (AIDS), rapid cytopathogenic effects and cytotoxicity were observed that made it possible to titrate the biologically active virus in a plaque-forming assay. The cytopathogenic effects were preceded by the rapid induction and increase of HTLV-III antigens as revealed by immunofluorescence and immunoprecipitation. Activities of HTLV-III were neutralized by the human antibodies against the virus when immunofluorescence and plaque assays were used. Essentially the same results were obtained with the lymphadenopathy-associated virus (LAV_1).*

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In 1984, retroviruses termed HTLV-III and AIDS-related virus (ARV) were isolated in the United States from the peripheral blood lymphocytes of patients with AIDS or pre-AIDS (*1*). A similar virus, LAV_1, had been isolated in France from patients with lymphadenopathy syndrome (*2*). Determination of the complete nucleotide sequences of the genome of these viruses revealed that they were variants of the same virus (*3*). That this virus is the causative agent of AIDS is indicated by studies showing that patients with AIDS or AIDS-related complex (ARC) frequently possess serum antibodies against this virus (*4*) and that the virus is found with high frequency in AIDS and ARC patients (*5*), causes specific cytopathogenic changes in $OKT4^+$ cells (*6*), is transmissible through blood transfusions (*7*), and causes a similar disease in chimpanzees (*8*).

Transmission of HTLV-III to an established T-cell line, H9, was first achieved by Popovic *et al.* (*1*). The same group of investigators subsequently showed that several $OKT4^+$ cell lines were susceptible to HTLV-III infection (*9*). The discovery of cell lines that continuously grow and produce the virus after infection greatly facilitated further studies of this virus. It was also reported that LAV could be adapted to grow in Epstein-Barr virus–transformed B lymphoblastoid cell lines or in the CCRF-CEM T-cell line (*10*). However, viral replication in all of these cell lines, and in primary human lymphocytes, requires a considerable time lag after infection. (Growth of the virus in primary human lymphocytes usually results in cell lysis.) In H9 cells infected with concentrated HTLV-III, viral activity is detectable 6

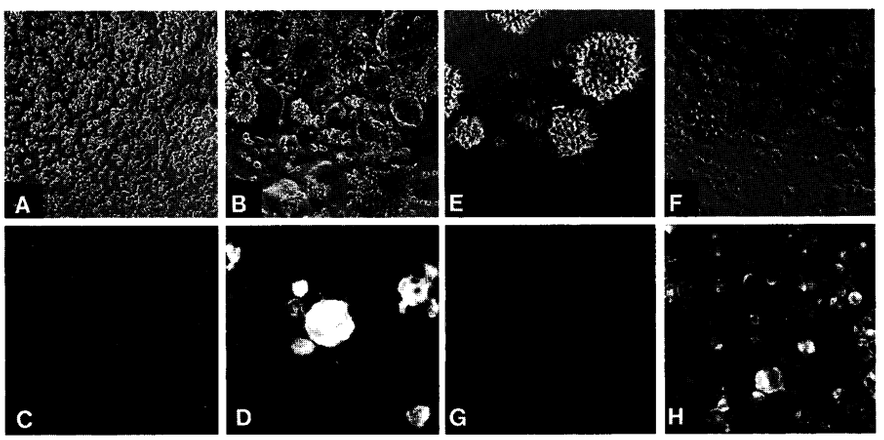

Fig. 1. HTLV-III–infected MT-2 (B and D) and MT-4 (F and H) cells and uninfected MT-2 (A and C) and MT-4 (E and G) cells. Virus was obtained from H9/HTLV-III culture medium after incubation for 4 days at 37°C. After removal of cells by centrifugation at 900*g* for 10 minutes, the supernatant was passed through a 0.22-μm Millipore membrane and stored at −80°C. Cells were infected with tenfold diluted virus preparation and photographed 3 days later by phase-contrast microscopy (A and B, ×60; E and F, ×120) or by immunofluorescent microscopy (C, D, G, and H, ×240) after staining.