by CEM/CBL1) with those of newer isolates of virus having apparently very different properties (*14*).

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Alterations in T4 (CD4) Protein and mRNA Synthesis in Cells Infected with HIV

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Cells infected with the human immunodeficiency virus (HIV) show decreased expression of the 58-kilodalton T4 (CD4) antigen on their surface. In this study, the effect of HIV infection on the synthesis of T4 messenger RNA (mRNA) and protein products was evaluated in T-cell lines. Metabolically labeled lysates from the $T4^+$ cell line Sup-T1 were immunoprecipitated with monoclonal antibodies to T4. Compared with uninfected cells, HIV-infected Sup-T1 cells showed decreased amounts of T4 that coprecipitated with both the 120-kilodalton viral envelope and the 150-kilodalton envelope precursor molecules. In four of five HIV-producing T-cell lines studied, the steady-state levels of T4 mRNA were also reduced. Thus, the decreased T4 antigen on HIV-infected cells is due to at least three factors: reduced steady-state levels of T4-specific mRNA, reduced amounts of immunoprecipitable T4 antigen, and the complexing of available T4 antigen with viral envelope gene products. The data suggested that the T4 protein produced after infection may be complexed with viral envelope gene products within infected cells. Retroviral envelope-receptor complexes may thus participate in a general mechanism by which receptors for retroviruses are downmodulated and alterations in cellular function develop after infection.

THE HUMAN IMMUNODEFICIENCY viruses (HIV) selectively infect T lymphocytes that express the 58- to 62-kilodalton (kD) glycoprotein T4 (CD4) (*1–5*) and produce characteristic cytopathic effects (*1, 2*). The acquired immune deficiency syndrome (AIDS), which may result from HIV infection, is characterized clinically by a progressive depletion of T4 cells (*6*). The tropism of HIV for T4 cells results, at least in part, from a direct interaction between the virion and the T4 molecule itself (*3, 7–9*). Monoclonal antibodies (Mab's) reactive with T4 can inhibit syncytia formation by HIV and neutralize infection of T-cell lines and peripheral blood T cells (*3*). Moreover, studies with concentrated preparations of HIV have demonstrated that Mab's to particular epitopes on the T4 molecule compete directly with the virus for binding to the cell (*7, 8*). McDougal *et al.* (*9*) have demonstrated that during the binding of HIV to the cell surface, a complex forms between the T4 molecule and the 110- to 120-kD glycoprotein that forms the externalized portion of the viral envelope (*9*). Because the T4 molecule appears to be important in determining the susceptibility of cells to infection, and because T4 expression may be modulated by virus after infection, we have studied the internal processing of T4 in normal and HIV-infected cells.

The alterations in expression of T4 that occur during viral binding to the cell surface are distinct from changes that occur after the production of viral antigens by infected T cells and cell lines (*3, 7, 8*). During the initial attachment of HIV to cells, the binding of monoclonal antibodies OKT4A, OKT4D, and Leu-3a is inhibited whereas the binding of OKT4 is not; thus, while some regions on T4 are required for viral binding and infection, the epitope defined by OKT4 is not (*7, 9–11*). However, after the production of viral antigens by infected cells the expression of all epitopes on T4, including those that are not involved in viral binding, are markedly diminished or absent, suggesting the loss of T4 from the cell surface (*2, 3, 7, 12*). This change in T4 expression could result from modulation of the T4 molecule at the cell surface or from the direct effects of viral products on the synthesis of T4 or its transport to the cell surface. We have evaluated both the T4 protein and T4 messenger RNA (mRNA) in infected T-cell lines. In some lines we found that the decreased expression of T4 on the cell surface is associated with reduced amounts of both the T4 protein and mRNA. We also found that the T4 molecule produced by these infected cells occurs as a complex with viral envelope and envelope precursor proteins.

In the first experiment, virus obtained from supernatants of productively infected H9 cells was concentrated 100-fold by ultracentrifugation and added to MOLT-4 cultures. At various times after the addition of virus the reactivity of Mab's OKT4 and OKT4A with the MOLT-4 cell surface was determined by flow cytometry. Control MOLT-4 cells were cultured with supernatant prepared from uninfected H9 cells. As early as 1 hour after the addition of viral concentrate the binding of OKT4A was reduced compared to control cells as indicated by the mean level of fluorescence intensity (Fig. 1) or by the percentage of cells considered positive. Inhibition of OKT4A binding was maximal 24 hours after the addition of virus, when the fluorescence intensity was 45% of that of control cells.

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Table 1. Reactivity of monoclonal antibodies to T4 with T-cell lines before and after infection with different isolates of HIV. T-cell lines were cultured with cell-free supernatants from HTLV-III$_B$ or ARV-2–producing T cells, and viable cells were analyzed 1 to 4 months later for the presence of surface T4 by immunofluorescence flow cytometry with OKT4 and OKT4A, as described (*27*). The nonreactive antibody P3X63 was used as a negative control. Results for pairs of infected and uninfected cell lines are expressed for each antibody as the percentage of cells positive, and the mean channel of fluorescence intensity, on a linear scale, for the entire population of cells. All T-cell lines inoculated with HIV, with the exception of CEM/III$_B$ (see text), were shown to be infected by the detection of reverse transcriptase activity in culture supernatants and the presence of viral antigens by immunofluorescence microscopy in >75% of cells. Each experiment was repeated three times and the results of representative experiments are shown.

Cell line	Number of cells positive (%)			Mean fluorescence channel		
	P3	OKT4	OKT4A	P3	OKT4	OKT4A
Sup-T1	1	99	100	6	160	159
Sup-T1/III$_B$*	2	7	1	8	14	7
MOLT	1	82	76	8	93	84
MOLT/III$_B$*	1	1	0	10	11	7
Jurkat	0	35	36	7	40	41
Jurkat/III$_B$*	1	3	3	7	10	12
HUT-78	1	92	90	13	69	63
HUT-78/ARV-2*	1	11	3	13	23	16
H9	0	86	83	9	63	56
H9/III$_B$*	1	1	1	8	9	8
CEM	1	100	100	8	127	119
CEM/III$_B$*	1	5	2	8	10	9

*Indicates a cell line infected with either HTLV-III$_B$ or ARV-2.

Relatively little inhibition in the binding of OKT4 occurred during this time. From day 1 through day 7 the reactivity of cells with OKT4A increased as the original inoculum of concentrated virus was diluted by the addition of medium during cell culture. However, from days 7 to 14, when viral antigens were appearing on the MOLT-4 cells, the reactivity of the cells with both OKT4 and OKT4A was markedly reduced. No differences were observed during this time period in the reactivity of MOLT-4 cells with OKT8. These findings confirm previous observations that the binding of HIV to T4 is competitive with OKT4A but not OKT4, and that a loss in detectable T4 from the cell surface occurs with the production of virus in the infected cells (*3, 7–9, 12*).

To study the synthesis and processing of the T4 molecule, we used several human T-cell lines that were infected with different isolates of HIV (HTLV-III$_B$ or ARV-2). The reactivity of OKT4 and OKT4A with these lines was determined before infection and between 1 and 4 months after infection (Table 1). All cells were >95% viable at the time of staining (as determined by trypan blue dye exclusion), and in five of six infected cell lines we detected viral antigens on 75 to 98% of cells. One T-cell line, CEM, that was analyzed at 1 and 4 months after infection showed no evidence of viral production (as determined by fluorescence microscopic detection of viral antigens or by the presence of reverse transcriptase activity in culture fluids), even though >80% of the cells showed viral antigens at 1 month after infection. The transient production of HIV after infection was described previously for another subclone of the CEM line (*13*). In all lines infected with HIV there was a marked reduction in reactivity with both OKT4A and OKT4 to low or undetectable levels (Table 1). These results, consistent with those of others (*3, 7–9, 12*), demonstrate that HIV-infected T-cell lines produce very little or no T4 antigen that can be detected by OKT4.

We studied the production of T4 protein in HIV-infected cells by immunoprecipitating the protein from infected and uninfected Sup-T1 cells (*14*). This cell line produced large amounts of T4 relative to other T-cell lines as determined by the increased fluorescence intensity of Mab's to T4 (Table 1). Cells were metabolically labeled with [^{35}S]cysteine and [^{35}S]methionine, lysates were prepared, and immunoprecipitations were performed with OKT4 as described (Fig. 2). From lysates of uninfected Sup-T1 cells, OKT4 precipitated a 58-kD protein consistent with the size reported for the T4 glycoprotein (*5, 9*) (Fig. 2A, lane 1). No similar band was observed with OKT3 (lane 2), which does not react with Sup-T1 cells. In contrast, when lysates of HTLV-III$_B$–infected Sup-T1 cells were immunoprecipitated with OKT4, a similar, though less intense, 58-kD protein was detected in association with a high molecular weight doublet of approximately 150 kD and 120 kD (Fig. 2A, lane 3). This doublet was not detected by the control antibody, OKT3 (Fig. 2A, lane 4).

To determine whether the 150- and 120-kD proteins that coprecipitated with T4 corresponded to viral products, we immunoprecipitated the labeled lysates using a reference serum from a patient with AIDS-related complex (ARC) which, by Western blot analysis, contained antibodies reactive with the major *env*, *pol*, and *gag* gene products of HTLV-III$_B$. As shown in Fig. 2B, from infected (lane 4) but not uninfected (lane 2) Sup-T1 cells the ARC serum precipitated viral proteins of 24 kD and 53 kD, which represented viral *gag* and *gag* precursor proteins, respectively (*15*). This serum also precipitated a large sized doublet of 150 kD and 120 kD that corresponded precisely to the proteins precipitated by OKT4. Viral products of this size have been shown to represent the HIV envelope precursor molecule and the cleaved externalized portion of the envelope, respectively (*16*). No significant bands were precipitated from infected cells by normal human serum (Fig. 2B, lane 3). In a separate experiment, when [^{35}S]cysteine- and [^{35}S]methionine-labeled lysates of infected Sup-T1 cells were preadsorbed with the ARC serum and then precipitated with OKT4, the 58-kD T4 band was no longer detectable (Fig. 2C, lane 8). A similar reduction in precipitable T4 was not observed when lysates of uninfected Sup-T1 cells were preadsorbed with the ARC serum (Fig. 2C, lane 4).

These data indicated that in Sup-T1 cells infected with HTLV-III$_B$ the T4 molecule occurred as a complex with both the viral envelope (120 kD) and envelope precursor (150 kD) molecules. The finding that preadsorbing lysates with ARC serum removed the T4 molecule suggested that all of the mature T4 protein synthesized by these cells was present as a complex with viral *env* gene products. To confirm this interpretation, we precipitated lysates from infected and uninfected Sup-T1 cells with the OKT4A Mab. This antibody, unlike OKT4, competes with the viral envelope for binding to T4 (*8, 9*). As shown in Fig. 2D, both OKT4 (lane 2) and OKT4A (lane 3) precipitated the 58-kD T4 molecule from uninfected Sup-T1 cells. In the infected Sup-T1 cells, OKT4 again precipitated a less intense 58-kD band corresponding to T4, as well as the 150-kD and 120-kD doublet corresponding to viral *env* gene products; however, neither T4 nor the 150- and 120-kD bands could be detected by OKT4A. The lack of reactivity of OKT4A with T4 in infected but not uninfected cells supports the evidence that in cells infected by HIV, all immunoreactive T4 is complexed to viral envelope and envelope precursor molecules.

To determine whether T4-envelope complexes are present within infected cells or if

these complexes form after lysis of the cells during the immunoprecipitation procedure, we radiolabeled HTLV-III_B–infected Sup-T1 cells (Sup-T1/III_B) as described above and then lysed them in the presence of a tenfold excess of either unlabeled H9/III_B cells (productively infected with HTLV-III_B) or CEM/III_B cells, which produce no virus. No differences were observed in the intensity of the 120- and 150-kD envelope bands that coprecipitated with T4 when these lysates were reacted with the OKT4 Mab. Both Sup-T1/III_B cells and H9/III_B cells produced comparable amounts of envelope as determined by precipitation of cell lysates with ARC serum. Thus, the failure of an excess of unlabeled viral envelope from the H9/III_B cells to compete with the labeled envelope for binding to T4 indicated that the T4-envelope complexes that were precipitated from Sup-T1/III_B cells had formed prior to cell lysis.

The immunoprecipitation studies (Fig. 2), in addition to showing complexes of T4 and viral envelope, also showed a reduction in the amount of T4 present in the infected Sup-T1 cells. To further evaluate this alteration in T4 synthesis following infection, we measured steady-state levels of T4 mRNA in the pairs of infected and uninfected T-cell lines shown in Table 1. The mRNA was measured in cells between 1 and 4 months after infection, at which time viability in the cultures was >95%. Total cellular RNA was prepared from T-cell lines (*17*) as described in Fig. 3, and relative levels of T4 mRNA

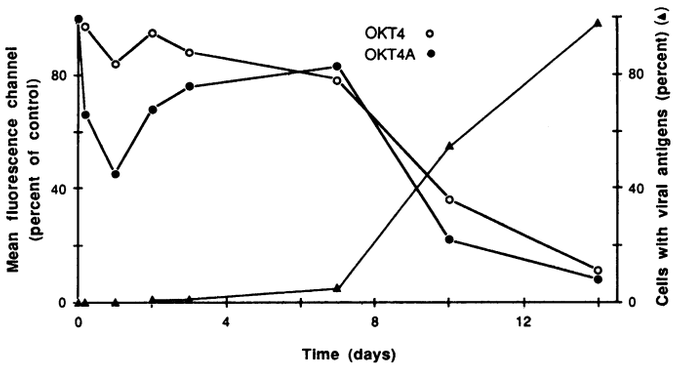

Fig. 1. Alterations in the reactivity of antibodies to T4 with MOLT-4 cells during infection by HIV. MOLT-4 cells (2×10^6) in 0.5 ml of RPMI 1640 containing 20% FCS were cultured at 37°C (5% CO_2) with 0.5 ml of concentrated (100-fold) cell-free supernatant from H9 cells that were either uninfected or productively infected with HTLV-III_B (*28*). Portions containing 5×10^5 cells were removed at the indicated time points and reactivity with OKT4 and OKT4A was determined by flow cytometry (Spectrum III fluorescence cell analyzer, Ortho) as described (*27*). After samples from these cultures were analyzed on day 1, cultures were fed by transferring to 10 ml of fresh medium in a T-25 flask (Corning), and passaged twice weekly. The mean channel of fluorescence intensity on a linear scale was determined at each time point for the antibodies indicated. Results for the cultures incubated with concentrated virus are expressed as a percentage of the control cells that were incubated with concentrated supernatants from uninfected H9 cells. Data from one of three representative experiments are shown. The viability of cells in this culture remained at >80% (trypan blue dye exclusion) throughout the experiment. The percentage of cells positive for HTLV-III antigens in the infected culture was determined by immunofluorescence microscopy on cells fixed in methanol and acetone (1:1) at the indicated time points using serum from a patient with ARC, as described previously (*10*).

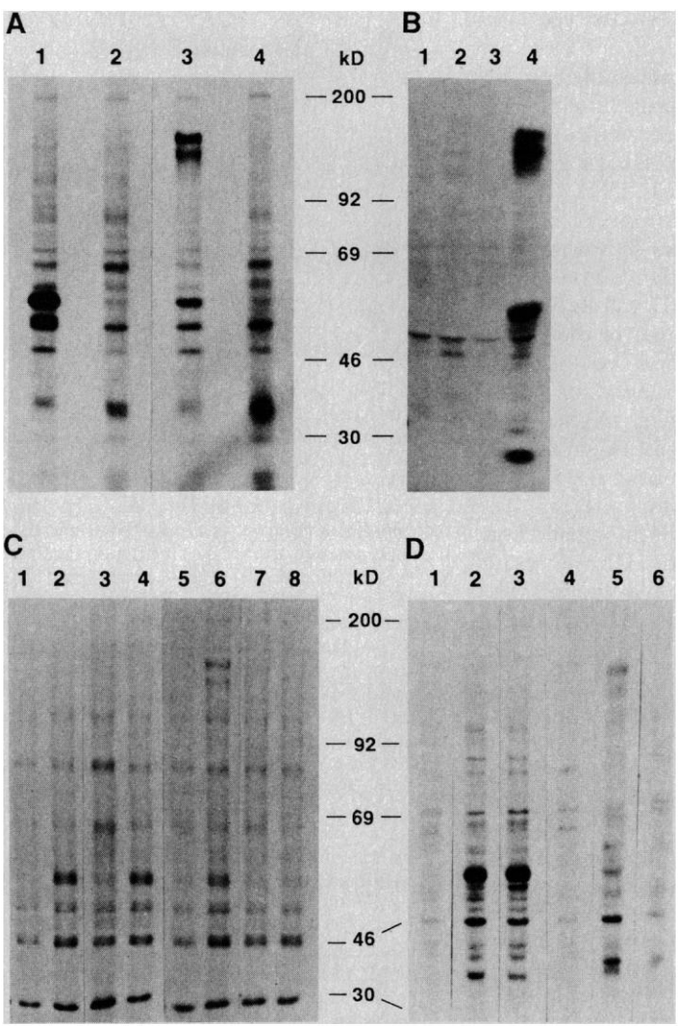

Fig. 2. Coprecipitation of T4 with HIV envelope and envelope precursor molecules. Sup-T1 cells (*14*) were cultured with cell-free supernatant from HTLV-III_B–infected H9 cells. The cultures analyzed 2 months later showed >100,000 cpm of reverse transcriptase in cell-free culture fluids (*10*), and viral antigens in 85 to 98% of cells, as determined by immunofluorescence microscopy of methanol:acetone (1:1) fixed cells with serum from a patient with ARC. Cells were metabolically labeled (*29, 30*) and immunoprecipitated (*31*) essentially as described (*9*). In (A), (B), and (D), lysates were cleared by adding protein A–agarose beads (Boehringer-Mannheim) conjugated to human IgG (*32*) (100 μl of beads per milliliter of lysate) and rotating for 3 hours at 4°C. For all of the immunoprecipitations, protein A–agarose beads were conjugated to OKT3, OKT4, or OKT4A, or to serum from a normal donor or a patient with ARC (*32*). Sup-T1 cells were nonreactive with OKT3. Electrophoresis was performed by layering identical amounts of protein on either 10.0% (A and B) or 7.5% (C and D) polyacrylamide-SDS gels with a 3.5% stacking gel; after electrophoresis, gels were treated with En^{3}hance (Dupont) and developed (Kodak XAR-5). (A) Cleared lysates from uninfected (lane 1 and 2) and infected (lanes 3 and 4) Sup-T1 cell cultures were precipitated with OKT4 (lanes 1 and 3) or OKT3 (lanes 2 and 4). (B) Immunoprecipitation of HIV antigens from lysates of infected Sup-T1 cells by ARC serum. Lysates used in (A) from uninfected (lanes 1 and 2) and infected (lanes 3 and 4) Sup-T1 cells were precipitated with normal human serum (lanes 1 and 3) or ARC serum (lanes 2 and 4). (C) Preadsorption of lysates of infected Sup-T1 cells by ARC serum removes T4. Prior to precipitation, lysates of uninfected (lanes 1, 2, 3, and 4) and infected (lanes 5, 6, 7, and 8) Sup-T1 cells were adsorbed twice with normal human serum (lanes 1, 2, 5, and 6) or ARC serum (lanes 3, 4, 7, and 8) (*33*). Immunoprecipitations were then performed with OKT3 (lanes 1, 3, 5, and 7) or OKT4 (lanes 2, 4, 6, and 8). (D) Precipitation of T4 from infected Sup-T1 cells by OKT4 but not OKT4A. Lysates from uninfected (lanes 1, 2, and 3) and infected (lanes 4, 5, and 6) Sup-T1 cells were precipitated with OKT3 (lanes 1 and 4), OKT4 (lanes 2 and 5), or OKT4A (lanes 3 and 6). In infected cells, both the 58-kD T4 molecule and the 120- and 150-kD viral bands were detected by OKT4 (lane 5) but not OKT4A (lane 6).

were measured by Northern blot analysis with the ^{32}P-labeled cDNA probe pT4.B, which contains a full-length T4 complementary DNA (cDNA) (*18*). As expected, the ^{32}P-labeled T4 probe hybridized to a 3-kb mRNA present in all uninfected T4 lines

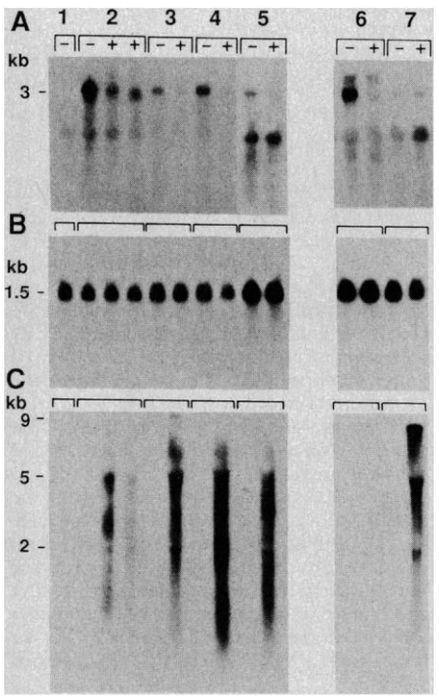

Fig. 3. Northern blot analysis of RNA from human cell lines before and after HIV infection, performed essentially as described (*17*). Briefly, total cellular RNA was isolated by a guanidium isothiocyanate method and electrophoresed (10 μg per lane) in formaldehyde–1% agarose gels, and transferred to GeneScreen Plus filters. All cell lines were >95% viable at the time RNA was prepared. Hybridizations were performed at high stringency with probes labeled with ^{32}P (alpha) labeled deoxycytidine triphosphate by the random primer method. Labeled probes used in hybridizations included pT4.B, specific for T4 mRNA (*18*) (A), He-7, specific for a 1.5-kb mRNA constitutively produced by both proliferating and nonproliferating human cell lines (*19*) (B), and BH-10, a molecular clone of HTLV-III$_B$ (*20*) (C). Filters were initially hybridized with pT4.B, and then sequentially stripped and reprobed with BH-10 and then He-7. Sequential hybridizations from two filters (left and right panels) are shown. The bracketed lanes indicate cell lines before (−) and after (+) infection with HTLV-III$_B$ (Sup-T1, MOLT-4, Jurkat, H9, and CEM) or ARV-2 (HUT-78). Cell lines used were as follows: Ton-B (lanes 1), an EBV-transformed B-cell line; Sup-T1 (lanes 2) (infected cells from two separate experiments are shown); MOLT-4 (lanes 3); HUT-78 (lanes 4); Jurkat (lanes 5); CEM (lanes 6); and H9 (lanes 7). Sizes of mRNA were estimated from the relative positions of the 28*S* and 18*S* RNA bands in ethidium bromide-stained gels prior to transfer. In (A) (left and right panels), which exhibit the 3-kb T4 mRNA, the 18*S* ribosomal band is present in all lanes as a background band. Because of the relatively low levels of T4 mRNA detected in infected and uninfected Jurkat cell lines (A, lane 5), a longer exposure of these lanes (3 days) compared to other lanes (1 day) is presented.

(Fig. 3A), and varied in intensity in patterns that were consistent with the expression of T4 on the cell surface (Table 1). In contrast, in five of six cell lines infected with HTLV-III$_B$ or ARV-2 the levels of T4 mRNA were markedly reduced or absent. In H9 cells, which prior to infection showed less T4 mRNA compared to other lines, no change in the level of message was apparent after infection. When we eluted the T4 cDNA probe from these blots and reprobed with the ^{32}P-labeled probe He-7, which reacts with a constitutively expressed gene in human cells (*19*), we found 1.5-kb bands of comparable intensity in pairs of infected and uninfected cells. Similarly, we found no differences in levels of mRNA between infected and uninfected cells when we used probes for β-actin and phosphoglycerate kinase. Thus, differences in the level of T4 mRNA were not due to variations in amounts of RNA loaded onto gels or to a failure of RNA from the infected cells to transfer during blotting. Rehybridization of these blots with the ^{32}P-labeled probe BH-10 (*20*), to detect HIV-specific mRNA's, revealed multiple RNA bands (9.8, 4.7 to 5.0, and 2.1 kb) characteristic of viral mRNA (*21*) in all the infected cell lines except CEM.

In view of the quantitative differences in levels of T4 protein and mRNA after HIV infection, we determined whether alterations in the structure of the T4 gene occurred in HIV-infected cells. DNA was prepared from five of the six pairs of infected and uninfected T-cell lines shown in Table 1, digested with restriction endonucleases Sst I, Bam HI and Hind III, and analyzed by Southern blot with the use of the pT4.B probe (Fig. 4). Differences were not observed in the multiple fragments of the T4 gene generated by Sst-I (Fig. 4A), Bam HI, or Hind III, indicating that no gross deletions or rearrangements within the T4 gene had occurred after infection.

In the CEM line, which initially was productively infected with HTLV-III$_B$ and expressed viral antigens on >95% of cells, neither viral mRNA nor T4 mRNA could be detected. This suggested that an outgrowth of cells that did not produce T4 occurred, and that these cells were probably resistant to HIV infection. When Southern blot hybridization of DNA from the infected CEM cells was performed with a full-length HTLV-III$_B$ probe (*20*), no viral bands could be identified (Fig. 4B), indicating that at the level of sensitivity of this method, latent infection had not occurred. Similar results were reported by Folks *et al.* (*13*), who described the transient infection of the CEM subclone A3.01, and Lifson *et al.* (*22*), who demonstrated the outgrowth of uninfected, T4-negative cells from cultures of T-cell lines inoculated with HIV.

Our results show that the decrease in expression of T4 protein on the cell surface after HIV infection is associated with a reduction in T4 mRNA. In the Sup-T1 cell line infected with HTLV-III$_B$, T4 protein is both reduced in amount and detectable as a complex with the externalized viral envelope and envelope precursor molecules. McDougal *et al.* (*9*) showed that when exogenous HIV is added to T4$^+$ cell lines, a complex forms between the T4 glycoprotein and the

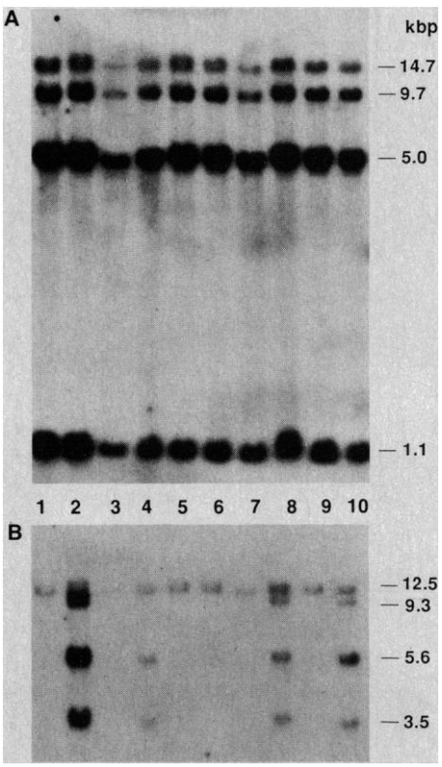

Fig. 4. The T4 locus is intact in cell lines surviving HIV infection. (A) DNA (approximately 10 μg per lane) prepared from cell lines shown in Table 1, MOLT-4 (lane 1), MOLT-4/III$_B$ (lane 2), Sup-T1 (lane 3); Sup-T1/III$_B$ (lane 4), CEM (lane 5), CEM/III$_B$ (lane 6), H9 (lane 7), H9/III$_B$ (lane 8), Jurkat (lane 9), and Jurkat/III$_B$ (lane 10) was digested with an excess of restriction enzyme Sst I, fractionated on agarose, transferred to GeneScreen Plus filters (New England Nuclear) and hybridized with radiolabeled pT4.B, the full-length T4 cDNA clone (*18*). (B) The filter in (A) was stripped of the T4 probe and rehybridized to the radiolabeled pHXB2 plasmid containing a full-length HTLV-III$_B$ proviral DNA and cellular flanking sequences (*20*). Only the HTLV-III$_B$–infected cells, with the exception of CEM/III$_B$ (lane 7) show HTLV-III$_B$ Sst I fragments of expected sizes (*20*). All lanes in (B) show a band at 12.5 kb which represents hybridization to the human sequences flanking the proviral genome within the pHXB2 plasmid. Conditions of hybridization used were 6× SSC (standard saline citrate), *p*H 7.0, 1× Denhardt's, 0.2 μg/ml of denatured salmon sperm DNA, 68°C for 16 hours. After hybridizations, blots were washed in 0.1× SSC containing 1.0% SDS at 68°C for several hours.

110-kD to 120-kD externalized portion of viral envelope (*9*). Our finding, that in virus-producing cells T4 is diminished on the cell surface yet detectable as a complex not only with the 120-kD viral envelope but also with the viral envelope precursor molecule, suggests that T4–viral envelope complexes may form within infected cells. Thus, the decrease or loss of T4 from the cell surface after infection results, in part, from an alteration by viral envelope molecules in the post-translational processing or stability of the T4 glycoprotein, or its transport to the cell surface. These findings may have implications for the more general mechanism by which retroviruses modulate their specific cellular receptors after infection, since the down-regulation of viral receptors is also induced by other retroviruses including avian leukosis virus, human T-lymphotropic virus types I and II, and bovine leukemia virus (*23*).

The molecular basis for the reduced T4 mRNA in most of the HIV-infected T-cell lines is not known, but could result from a decrease in T4 gene transcription or a reduction in the stability of T4 mRNA. Although this decrease may reflect a direct effect of viral products on T4 gene transcription, it is also possible that in these cultures a selection occurred that favored the growth of cells that produced less T4 protein. Such selection could occur if the co-expression of viral envelope and T4 within infected cells and the resulting formation of T4-envelope complexes were cytopathic, thereby conferring a growth advantage to cells that produced less T4. The binding of T4 to the HIV envelope could conceivably expose fusigenic determinants on the envelope that could mediate cytopathic effects between cells, as shown by others (*22, 24*), or within an infected cell. Recent studies have correlated the expression of the viral *env* gene with cytopathic effects on T4 cells (*24, 25*). Moreover the finding that an isolate of HIV that was highly cytotoxic for T4 cells produced no cytopathic effects after productive infection of T8 cells has suggested that, in addition to HIV *env* gene products, cellular factors unique to T4 cells (perhaps the expression of the T4 molecule itself) could be relevant to the mechanism by which HIV kills T4 cells (*26*).

The Sup-T1 cell line, which showed the highest level of surface T4 and T4 mRNA, has, in our experience, been exquisitely sensitive to the cytotoxic effects of acute HIV infection, showing fewer than 1% viable cells 14 days after inoculation with virus. Decreased viability, as determined by trypan blue dye exclusion, was observed in single cells as well as syncytial forms, indicating that the cytotoxic effects were not exclusively due to cell-to-cell fusion. This high degree of cytopathology compared to the other cell lines studied could result from the increased amounts of intracellular T4–viral envelope complexes that would form during the production of viral envelope. By contrast, H9 cells, which showed no change in T4 mRNA level after infection, had a comparatively low level of surface T4 and T4 mRNA prior to infection and showed less than 20% cell death during infection.

The direct interaction of the HIV envelope with the T4 molecule mediates the initial binding of HIV to T4 cells and may be important in determining the specificity of these viruses for $T4^+$ cells or other cells which express the T4 molecule. However, our data indicate that during viral production, additional interactions occur between the viral envelope and T4 that are associated with alterations in T4 synthesis at the protein and RNA level. These effects may have relevance for the mechanisms by which viral gene products interacting with cellular structures produce alterations in cellular function and cytopathic effects.

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28. Supernatants of HTLV-III_B–infected and uninfected H9 cells were centrifuged (5,000*g* for 10 minutes) to remove cells, filtered (0.45 μm), and concentrated 100-fold by centrifugation (100,000*g* for 60 minutes; Beckman Ti-50 rotor). The pellet was resuspended in 1 ml of RPMI 1640 with 20% fetal calf serum (FCS). This virus preparation was biologically active as determined by its ability to infect H9 cells at dilutions $>1:10^6$.
29. Uninfected and infected Sup-T1 cells (50×10^6) were cultured for 1 hour in 10 ml of cysteine- and methionine-free RPMI 1640 containing 10% dialyzed FCS, and then [^{35}S]cysteine (100 μCi/ml) and [^{35}S]methionine (100 μCi/ml) were added. Cells were cultured for 16 hours, washed three times, and incubated in 1 ml of lysing buffer (LB) (*30*), for 30 minutes at 4°C, and centrifuged at 3000*g* for 20 minutes to remove nuclei.
30. Lysing buffer contains 0.02*M* tris and 0.12*M* NaCl, *p*H 8.0, with 0.2 m*M* phenylethylsulfonyl fluoride, 0.2 m*M* EGTA, 0.2 m*M* NaF, 5 μg/ml of aprotinin, 0.2% sodium deoxycholate, and 0.5% Nonidet P-40 (by volume).
31. Portions (200 μl) of cleared lysates were added to 20 μl of protein A–agarose beads conjugated to monoclonal antibodies and rotated for 16 hours at 4°C. Beads were washed sequentially in LB; LB containing 0.5*M* NaCl; and LB with 0.1% sodium dodecyl sulfate (SDS). The adsorbed material was eluted by heating at 65°C for 30 minutes in 50 μl of sample buffer [0.01*M* tris, *p*H 8.0, containing 2% SDS, 5% 2-mercaptoethanol (by volume), bromophenol blue (25 μg/ml), and 10% glycerol (by volume)].
32. Protein A–Sepharose beads used to clear lysates were conjugated to human immunoglobulin G (IgG) by incubating 5 mg of purified human IgG per milliliter of agarose for 16 hours at 4°C and washed in phosphate-buffered saline (PBS). For immunoadsorptions, 200 μl of OKT4, OKT4A, or OKT3 (Ortho), or undiluted normal serum or ARC serum was incubated with 100 μl of protein A–Sepharose and prepared as described above for conjugates with human IgG.
33. Portions of lysates (300 μl) were adsorbed twice by adding 50 μl of protein A–Sepharose beads conjugated to either normal human serum or ARC serum for 1 hour at room temperature and then adsorbed with 50 μl of unconjugated protein A–Sepharose.

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