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Downregulation of Cell Surface Molecules during Noncytopathic Infection of T Cells with Human Immunodeficiency Virus

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Noncytopathic infection of human T-lymphoid cell line CR-10 with human immunodeficiency virus (HIV) (CEM-N1T isolate) resulted in a gradual loss of cell surface receptors for OKT4/OKT4A (HIV receptor), OKT8, OKT3, and OKT11 but not for OKT9 (transferrin receptor) within 10 days after infection. Surface receptor decline was accompanied by a rapid increase in HIV antigens and mRNA expression. Multireceptor downregulation was also observed in three T-lymphoid cell lines (MT-4, CEM, and HBD-1) cytopathically infected with the HIV/N1T virus and in HUT-78 cells infected with the HIV/SF-2 isolate. HIV-infected and uninfected CR-10 cells contained similar levels of mRNAs coding for T3, T8, T9, T11, HLA-A2, and HLA-B7 proteins. By densitometry, fully infected CR-10 cells showed approximately 75% reduction in T4 and tubulin (β chain) mRNA levels when compared with uninfected CR-10 cells. No such reduction was detected in HIV-infected MT-4 and HBD-1 cells. A T-cell receptor gene (β chain) rearrangement study revealed that no distinct CR-10 subpopulation was selected out upon infection with HIV. Our results suggest that the reduction in cell surface receptors observed between 1 and 2 weeks postinfection cannot be directly attributed to similar reductions in mRNA levels coding for these receptor proteins. We conclude that HIV infection induces posttranscriptional downregulation of several T-cell surface receptors.

The human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (2, 12, 23), exhibits a profound tropism to human helper-inducer T lymphocytes expressing surface T4 (CD4/Leu3) antigen (7, 21, 22). Infection of $T4^+$ lymphocytes with HIV in vitro causes extensive cytopathic effects (cell fusion, lysis) and cell death within 1 to 2 weeks after virus exposure (2, 12, 21, 23, 31). The affinity of HIV to $T4^+$ cells may, in part, be due to the role of the T4 molecule as a specific viral receptor (7, 26, 28, 29), and the T4 receptor itself may play an important role in the formation of syncytia and cytolysis. For example, expression of the HIV envelope gene has been shown to induce syncytia and cell death in $T4^+$ cells but not in B lymphocytes, presumably owing to the absence or low level of T4 on B cells (24, 25).

In contrast to HIV-induced cytolysis, which has been the focus of intensive research (2, 12, 21, 23–25), relatively little is known about HIV effects on host cells during a persistent noncytopathic infection. This type of HIV-host cell interaction has been reported to occur in vitro in long-term cultures of fresh human T lymphocytes maintained under special conditions (17, 44), in macrophages-monocytes (13), and in some T-lymphoid cell lines (5, 11), and it may represent the principal mechanism responsible for HIV persistence in vivo. Interestingly, the chronically infected cell lines appear to be depleted of surface $T4^+$ receptors (11, 16, 17, 21, 22, 28), perhaps correlating with the reduced capacity of these cells to undergo virus-mediated fusion and lysis. To investigate this phenomenon further and to evaluate whether persistent HIV infection influences the expression of other molecules besides the T4 receptor, we have analyzed surface marker expression in a recently derived subclone of CEM cells which is susceptible to noncytopathic productive infection with HIV (5). Surprisingly, we have found that the decline in the surface $T4^+$ cell population was accompanied by a parallel decline in T3, T8, and T11 surface marker expression (5). The molecular basis for this multireceptor down-
modulation is unclear, but it may be caused by (i) the outgrowth of a T3-, T4-, T8-, and T11-negative cell population, which is resistant to infection and cytolysis; (ii) transcriptional modulation of receptor gene expression; or (iii) posttranscriptional regulation of receptor mRNA or protein processing. Here we describe experiments that examined some of the above possibilities by investigating the effect of a noncytopathic HIV infection on the kinetics of modulation of T4 and other surface receptors, expression of the T3, T4, T8, T9, T11, HLA-A2, and HLA-B7 mRNAs, and clonality of chronically infected cells. Our results suggest that chronic infection of T-lymphoid cells by HIV induces posttranscriptional downregulation of several cytoplasmic and cell surface molecules.

MATERIALS AND METHODS

Cells and virus. CR-10 (5) is a variant of the tumor cell line of T-cell leukemic origin, CEM. MT-4 is a human T-cell lymphotrophic virus type I-transformed human cord blood T-cell line (30). HBD-1 is a OKT4/leu3a-positive human cord blood T-cell line (37). Cells were grown at 37°C in an atmosphere of 5% CO_2 in RPMI 1640 medium (Flow Laboratories, Inc., McLean, Va.) supplemented with 10 to 15% heat-inactivated fetal bovine serum, 7 mM L-glutamine, 2 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), penicillin (100 units/ml), and streptomycin (100 μg/ml). The LAV-N1T strain of HIV was isolated from lymphocytes of a patient with lymphadenopathy (4). The virus was maintained in lytically infected CEM cells periodically fed with fresh CEM cells. For infection studies, cell-free supernatants from virus-producer cultures were filtered through 0.8-μm-pore-size filters (Millipore Corp.,

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Bedford, Mass.); the virus was sedimented by centrifugation at 15,000 × *g* for 2 h and resuspended in medium at 100-fold concentration. HIV preparations typically induced viral antigens in 2 to 10% of CEM cells as determined by immunofluorescence 6 days after infection at 1:10 dilution.

Infection of cells with HIV. Cells were harvested at the exponential growth phase, washed two times in phosphate-buffered saline (PBS, pH 7.2), and suspended in virus-containing medium at 1:10 dilution (10^6 cells per ml). After 24 h at 37°C, cells were washed and resuspended in fresh medium at approximately 2×10^5 cells per ml. At designated time intervals, samples of the culture were removed to determine (when indicated) cell count, viability, HIV antigen expression by the immunofluorescence method, expression of surface markers specific for T lymphocytes (OKT series; Ortho Diagnostics Systems, Rahway, N.J.), presence of HIV proviral DNA and expression of HIV mRNA, T-cell receptor gene rearrangement, and the presence of gene transcripts encoding the T3, T4, T8, T9, T11, HLA-A2, and HLA-B7 molecules.

Immunofluorescence staining. Infected cells were washed in PBS, spotted on glass slides, dried, and fixed in acetone at −20°C for 15 min. The fixed cells were reacted with HIV-positive serum from a patient with acquired immunodeficiency syndrome (antibody titer, 1:1,280) at a 1:20 dilution. After a 30-min incubation at 37°C, cells were reacted with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G, and virus antigen-positive cells were counted under an epifluorescence microscope (American Optical Corp., Buffalo, N.Y.).

Cell surface marker analysis. Cell samples (5×10^5 total) were washed with PBS and incubated at 4°C for 30 min in the presence of unlabeled monoclonal T-cell markers OKT3, OKT4, OKT8, OKT9, or OKT11 (Ortho) followed by fluorescein-conjugated second antibody. Cell fluorescence was examined with an Ortho model 50H cytofluorograph.

Preparation of cellular DNA and RNA. Total cellular RNA was prepared by the CsCl-guanidine thiocyanate procedure (6). Cellular RNA, which pelleted through the CsCl cushion, was washed twice with 200 μl of absolute alcohol at room temperature, suspended in 400 μl of diethyl pyrocarbonate-treated H_2O, and precipitated with 2 volumes of absolute ethanol at −20°C in the presence of 0.3 M sodium acetate (pH 5.2). Cellular DNA remaining at the CsCl/guanidium thiocyanate interface was diluted with 2 volumes of Tris-EDTA, extracted once with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol) and once with an equal volume of chloroform-isoamyl alcohol (24:1, vol/vol), and precipitated with 5 volumes of 80% alcohol at −20°C.

Northern and Southern blot analysis. RNA (10 μg per tube) was denatured in 50% formamide–6% formaldehyde–20 mM MOPS (morpholinepropanesulfonic acid; pH 7.0)–5 mM sodium acetate–1 mM EDTA for 15 min at 55°C, chilled on ice, and fractionated by electrophoresis in 1% agarose gels containing 6% formaldehyde, 20 mM MOPS (pH 7.0), 5 mM sodium acetate, and 1 mM EDTA for 16 h at 45 V. The gels were treated in 0.05 N NaOH–0.6 M NaCl for 20 min, followed by soaking in 0.5 M Tris hydrochloride (pH 7.5)–0.6 M NaCl for 45 min. RNA was transferred to Nytran nylon membranes (Schleicher & Schuell, Inc., Keene, N.H.) in 20× SSC (1× SSC is 0.015 M sodium citrate plus 0.15 M NaCl). For Northern (RNA) blot hybridizations, RNA blots were prehybridized for 4 h at 42°C in a solution containing 5× SSC, 50% formamide, 3× Denhardt reagent, 0.25% sodium dodecyl sulfate (SDS), and 100 μg of denatured salmon sperm DNA per ml. Hybridization continued for 24 h in hybridization cocktail (as above) supplemented with 1×10^6 to 2×10^6 cpm of ^{32}P-labeled DNA probe per ml (specific activity, 2×10^8 to 5×10^8 cpm/μg). Hybridized filters were washed with brisk agitation in three changes of 0.2× SSC–0.1% SDS at 55 to 60°C. The washed filters were blotted dry, wrapped in Saran Wrap cling film, and subjected to autoradiography with Kodak X-Omat intensifying screens at −80°C.

Restriction endonuclease-digested DNA (10 μg) was electrophoresed in 0.8% Tris-borate (pH 8.0) agarose gels and transferred to Nytran nylon filters by the procedure of Southern (36). Filters were prehybridized and hybridized at 40°C in a solution containing 5× SSC, 50% formamide, 1% SDS, 3× Denhardt solution, 100 μg of denatured salmon sperm DNA per ml, and 5 mM EDTA (pH 8.0). Blots were washed as described for Northern blots. Bacteriophage λ DNA digested with *Hin*dIII or *Bst*IIE (New England Biolabs, Inc., Beverly, Mass.) and end labeled with DNA polymerase I large fragment was used as size markers.

Hybridization probes. The HIV probe (pN1G-G5) contains an almost complete 8.9-kilobase fragment of HIV from a λ phage library of HIV-infected CEM cells (40). The T3 receptor probe was a cDNA complementary to the δ subunit of the T3–T-cell receptor complex (38). The T8 probe was a 1.7-kilobase cDNA insert of the Leu-2 clone complementary to 400 bases of the 3′ coding region and untranslated flanking DNA (20). The T-cell receptor probe was a cDNA complementary to the constant region (β chain) of the T-cell-antigen receptor (43). The T4 probe was a 3-kilobase cDNA containing the entire translated region of the T4 receptor gene (27). The β-tubulin probe Dβ-1 (15) was a cDNA containing 3′ untranslated and translated regions of the human β-tubulin gene. The HLA-A2 probe JYB3.2 (3) was a cloned genomic DNA fragment of the human HLA-A2 locus. The HLA-B6 probe pDp001 was an almost intact cDNA complementary to the human HLA-B7 gene (35). The CD2 (T11) probe 254 (33) was a cDNA containing the entire translated region of the human CD2 receptor gene. The transferrin (T9) probe pTR36 (32) contained the 5′ translated region of the human transferrin gene.

Immunoprecipitation analysis. HIV-infected and uninfected cell lines were washed twice with PBS and labeled for 6 h in RPMI medium (1×10^6 to 2×10^6 cells per ml) containing 5% dialyzed calf serum and 50 to 100 μCi each of L-[^{35}S]methionine and cysteine per ml (1,000 μCi/mmol; New England Nuclear Corp., Boston, Mass.). At the end of the labeling period, cells were washed with PBS containing 0.2 mM phenylmethylsulfonyl fluoride at 4°C. Cell pellets (10^7 cells) were incubated in 1 ml of immunoprecipitation buffer for 30 min at 4°C and centrifuged at 3,000 × *g* for 20 min to remove nuclei. Immunoprecipitation buffer contained 0.02 M Tris hydrochloride, 0.12 M NaCl (pH 8.0), with 0.2 mM phenylmethylsulfonyl fluoride, 5 μg of aprotinin per ml, 0.2 mM EGTA, 0.2 mM sodium fluoride, 0.2% sodium deoxycholate, and 0.5% (vol/vol) Nonidet P-40. Cell lysates were preabsorbed with protein A-Sepharose beads (Pharmacia, Inc., Piscataway, N.J.) overnight at 4°C and then incubated with monoclonal antibody OKT4A-treated beads with gentle rotation at 4°C for 12 h. Typically, 100 μg of Sepharose beads was absorbed with 100 to 200 μl of monoclonal antibodies and conjugated with extract of 10^7 cells. Conjugated beads were washed once with immunoprecipitation buffer, once with buffer containing 0.5 M NaCl, and once in buffer containing 0.1% SDS. Washed beads were suspended in 50 μl of electrophoresis sample buffer (0.01 M

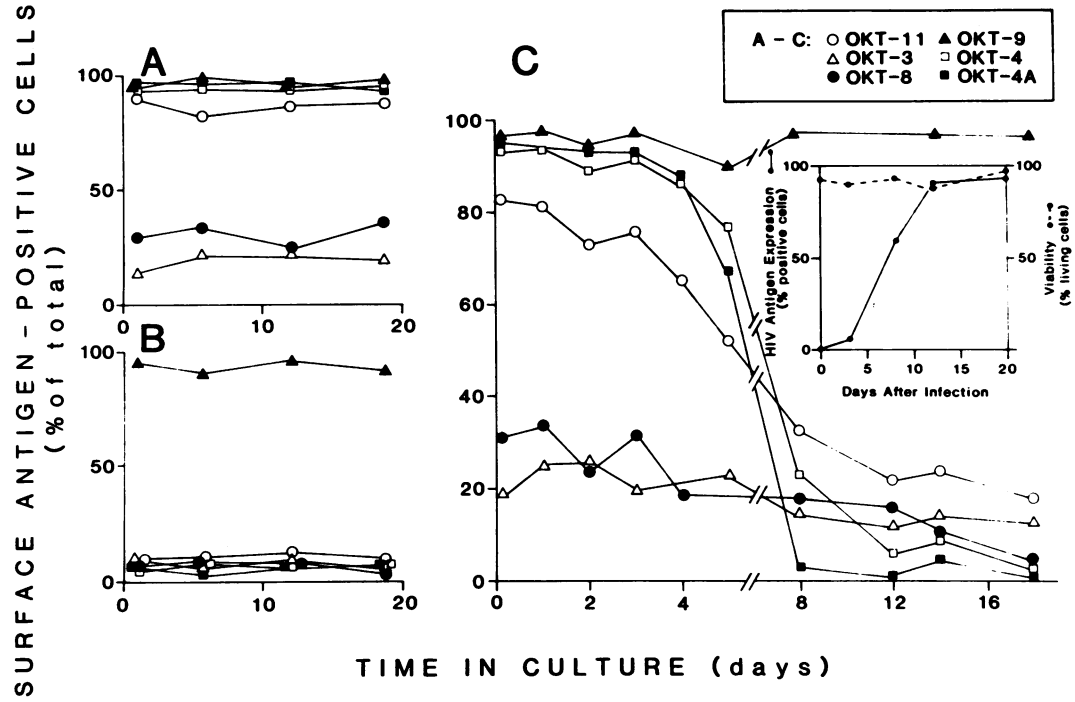

FIG. 1. Kinetics of surface antigen modulation in CR-10 cells in response to noncytopathic HIV infection. (A) Control uninfected CR-10 cells; (B) CR-10/HIV$_{LT}$ cells, 10 months in continuous culture after infection with N1T strain of HIV (4); (C) CR-10 cells after exposure to HIV/N1T. At the indicated times after infection, surface antigens were analyzed by using fluorescein-conjugated monoclonal antibodies and an Ortho flow cytofluorograph. The same samples were used to determine cell viability and HIV antigen positivity (inset in Fig. 1C) and to analyze cellular DNA and RNA (see Fig. 2 and 3). The figure shows results of one of four similar experiments.

Tris hydrochloride [pH 8.0], 2% SDS, 5% 2-mercaptoethanol, 25 μg of bromophenol blue per ml, 10% glycerol. Adsorbed material was eluted by heating beads at 65°C for 30 min. Electrophoresis was performed in a 10% polyacrylamide gel with a 3.5% stacking gel. Gels were fixed, dried, and autoradiographed with Kodak X-Omat intensifying screens. Protein molecular weight markers were myosin (200,000), phosphorylase *b* (97,400), bovine serum albumin (68,000), ovalbumin (43,000), α-chymotrypsin (25,700), β-lactoglobulin (18,400), and lysozyme (14,300) (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

RESULTS

Kinetics of cell surface receptor modulation during noncytopathic infection of CR-10 cells with HIV. CR-10 cells are characterized by a full susceptibility to HIV infection in the absence of cytopathic effects of the virus, such as syncytium formation and lysis (5). These characteristics made the CR-10 cells particularly suitable for kinetic studies of protein and gene expression in relation to HIV genome expression. The modulation of cell surface receptors in CR-10 cells during the first 10 days after infection, as detected by flow cytometry, is depicted in Fig. 1. Uninfected CR-10 cells expressed high levels (80 to 95%) of OKT4, OKT4A, OKT11, and OKT9 receptors, while 15 to 30% of the cells expressed OKT3 and OKT8 receptors (Fig. 1A). A chronically infected cell line, CR-20/HIV$_{LT}$ (10 months in culture) (5) did not express any of the surface markers tested except for OKT9 (Fig. 1B). Exposure of CR-10 cells to HIV did not induce significant marker modulation for the first 3 to 4 days after infection (Fig. 1C); this was followed by a rapid decline in surface expression of OKT4, OKT4A, and OKT11 between day 4 and day 8 after infection. The decline in OKT3 and OKT8 expression was more gradual, reaching background levels within 21 days postinfection (Fig. 1C). The decline in surface OKT4, -4A, -8, and -11 expression was accompanied by a rapid increase in HIV expression in the infected cells, 90% of which became HIV antigen positive by day 12 postinfection (Fig. 1C, inset). As described before (5), the viability of CR-10 cells was not affected by HIV infection (Fig. 1C, inset), suggesting that the loss of surface receptor expression was a direct result of establishing a virus-carrier state in living CR-10 cells rather than cytolysis of receptor-carrying cells.

Modulation of surface molecules in other T4$^+$ cells. To determine whether the multireceptor downmodulation observed in HIV-infected CR-10 cells is a general phenomenon related to HIV infection, we studied expression of surface protein in three T4$^+$ T-cell lines exposed to CEM-N1T virus and in HUT-78 T-lymphoid cells infected with the HIV/SF-2 strain (23) of HIV (Table 1). As with CR-10 cells, the levels of all surface markers studied were significantly reduced within 1 week of infection with HIV (Table 1). The extent of modulation for each marker differed among various cell lines, perhaps in correlation with the kinetics of virus infection (not tested in this experiment). For example, the downregulation of T11 was more pronounced in HBD-1 and HUT-78 cells than in CEM or MT-4 cells. T9 did not appear to be downregulated in MT-4 cells but underwent significant modulation in HBD-1 and HUT-78 cells. Surface T10 was downmodulated in N1T-infected CEM cells but not in SF-2-infected HUT-78 cells. The N1T (4) and SF-2 (23) strains of HIV seemed to have similar modulatory effects on the expression of most of the surface proteins studied with the exception of T10 (Table 1). Unlike CR-10 cells, MT-2 and

TABLE 1. Modulation of surface receptors in HIV-infected cells

Cells[a]	Origin	Surface marker	Surface marker expression in:		HIV antigen expression in infected cells (% of total)
			Uninfected cells	Infected cells	
HBD-1*	Human T-lymphoblastoid cell line, Epstein-Barr virus genome positive	T3	9.0	5.0	80
		T11	95.5	28.7	
		T4	93.1	2.0	
		T8	32.0	2.9	
		T9	85.5	55.0	
HUT-78**	Human T-lymphoid tumor cell line	T3	76.6	6.3	90
		T11	26.1	6.3	
		T4	89.8	16.0	
		T8	4.2	4.9	
		T9	96.6	57.0	
		T10	97.2	95.2	
CEM*	Human T-cell leukemia	T3	48.5	3.0	50
		T11	52.9	35.1	
		T4	82.9	25.1	
		T8	27.0	3.2	
		T9	72.7	ND[b]	
		T10	87.0	59.4	
MT-4*	HTLV-I-positive human T-lymphoblastoid cell line[c]	T4	88.0	7.0	90
		T9	90.0	93.0	
		T11	90.3	62.0	

[a] Cells were infected with CEM/N1T(*) or HIV/SF-2(**) virus isolate and evaluated for the specified surface markers 1 week after infection as described in Materials and Methods.
[b] ND, Not determined.
[c] HTLV-I, Human T-cell lymphotrophic virus type I.

HBD-1 cells are sensitive to HIV-induced cytolysis, and analysis of cells beyond 1 week postinfection was difficult owing to the high percentage of dead or dying cells in the infected cell cultures.

Configuration of T-cell receptor gene. The CEM cell line from which the CR-10 line was derived contains subclones with unique phenotypic responses to HIV infection such as the A3.01 clone with high sensitivity to complete lysis by HIV (10) and the A2.01 clone that harbors a latent iododeoxyuridine-inducible HIV genome (11). To examine whether or not HIV infection of CR-10 cells resulted in a rapid selection of genetically distinct subclones, we examined the configuration of the β chain of the T-cell receptor gene in infected ($T4^-$) and uninfected ($T4^+$) CR-10 cultures (Fig. 2). The rearranged pattern of the T-cell receptor gene was identical in infected and uninfected CR-10 cells when analyzed with three separate restriction enzymes. Taken together with data on cell viability (Fig. 1C) and the demonstration of HIV antigen expression in the majority of infected cells (Fig. 1), these results demonstrate that the loss of T4 expression in chronically infected CR-10 cultures was not caused by an outgrowth of a distinct receptor-negative subpopulation that preexisted in the CR-10 cell line.

Receptor mRNA levels during HIV infection. We next sought to determine at which intracellular level the loss of surface receptor expression in CR-10/HIV cells was manifest by testing the relative levels of T4, T3, T8, T9, and T11 mRNAs at various time points after infection (Fig. 3). Use of a cDNA probe complementary to the 5′ end of the T4 receptor (27) identified a 3.0-kilobase mRNA in Northern blot extracts from CR-10 cells (Fig. 3A). The amount of this mRNA did not appear to change significantly (when compared with tubulin mRNA) at any point after infection. Occasional examinations of the long-term chronically infected CR-10/HIV cells for the expression of T4 mRNA revealed a steady-state level of the message comparable to that in uninfected controls during 10 months of continuous

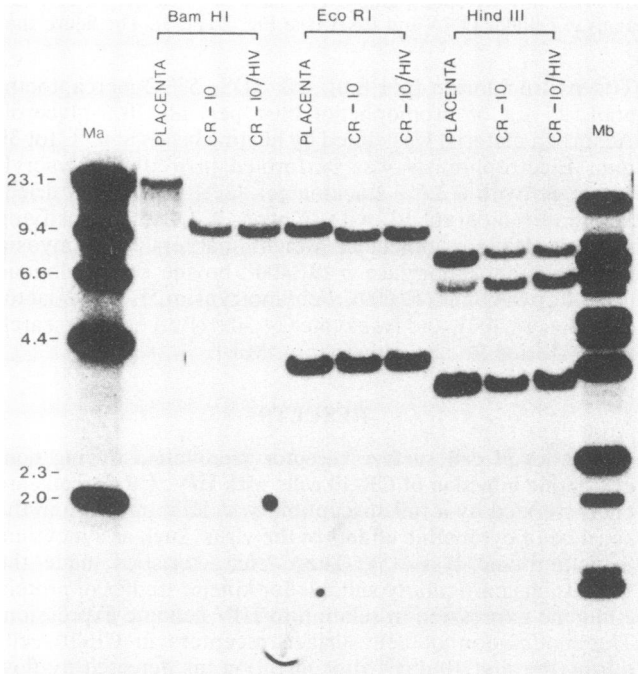

FIG. 2. Analysis of T-cell-antigen receptor gene (β chain) rearrangement pattern 14 days after infection of CR-10 cells with HIV. Genomic DNA was digested with the indicated restriction enzymes and blotted onto nylon filters as described in Materials and Methods. Placental DNA provides the unrearranged (germ line) T-cell receptor configuration. [^{32}P]dCTP-end-labeled *Hin*dIII digests of DNA (Ma) or *Bst*EII digests of DNA (Mb) were used as molecular weight markers. The T-cell receptor gene probe was a 770-base-pair Jurkat cDNA (43) covering the constant region of the T-cell receptor gene.

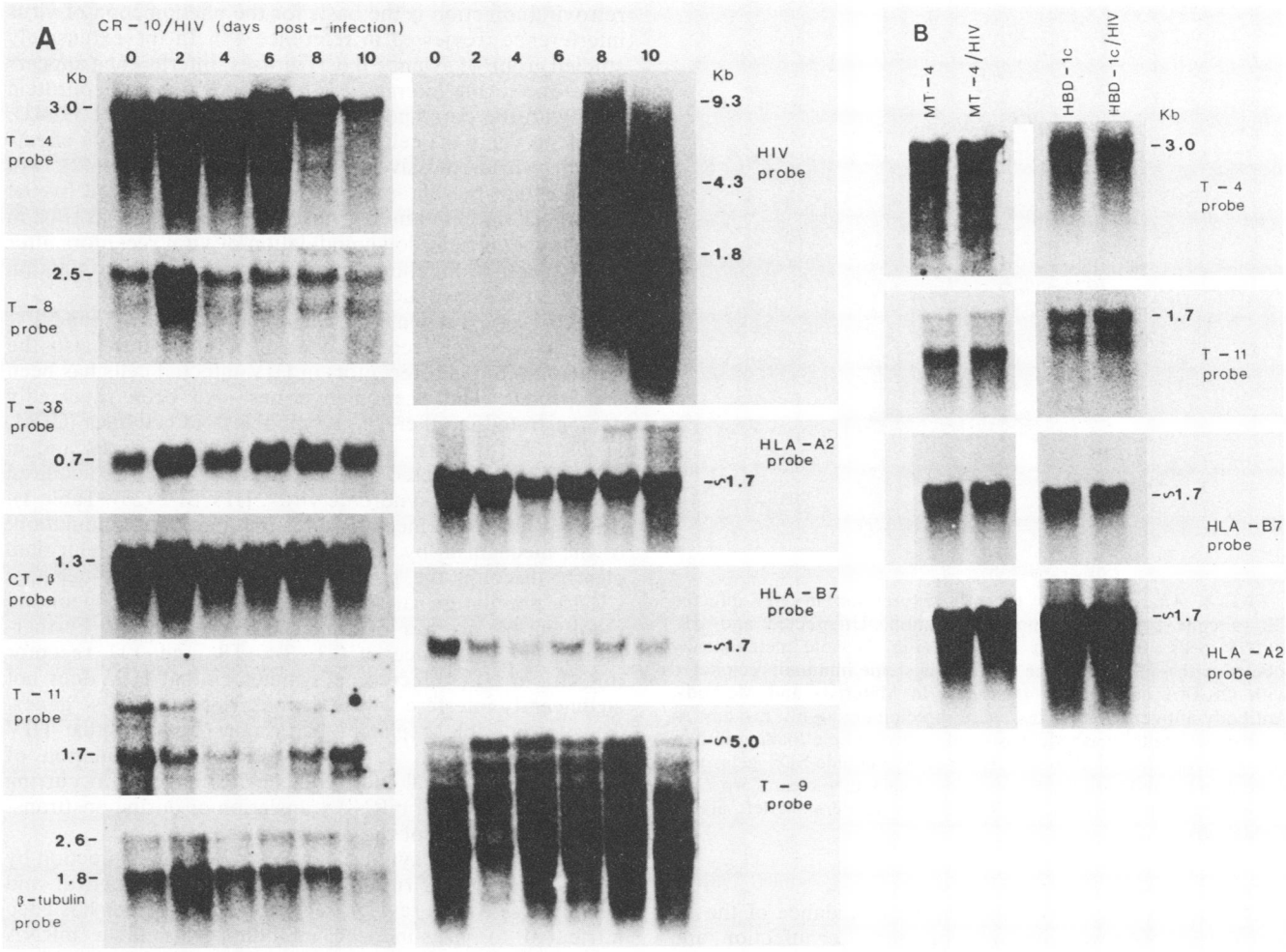

FIG. 3. Analysis of T3, T4, T8, and T11 receptor mRNAs and HIV RNA in HIV-infected CR-10 cells (A) and HBD-1 and MT-4 cells (B). Total cellular RNA was isolated from infected CR-10 cells by the guanidine thiocyanate method (6) at various times after infection and analyzed by Northern blotting with the indicated ^{32}P-labeled DNA probes as described in Materials and Methods. The relative sizes of the major hybridizing mRNA species are indicated in kilobases (kb). Initial hybridizations were performed with T4, T8, T3 δ, CT β, β-tubulin, and HIV probes. The T4, T8, T3 δ, and β-tubulin blots were then stripped by washing for 1 h in 5 mM Tris hydrochloride (pH 8.0)–0.2 mM EDTA–0.05% PP_i–0.1 × Denhardt reagent and rehybridized with HLA-A2, T9, T11, and HLA-B7 probes, respectively.

culture (data not shown; see flow cytometric data in Fig. 1B). The relative levels of T3, T8, T9, and T11 mRNAs also seemed unperturbed as compared with tubulin mRNA over the course of establishing chronic HIV infection. However, the levels of the tubulin mRNA itself seemed to fluctuate (over the 10 days of observation) more than expected for a housekeeping gene, and it was unclear whether this represented differences in the quality of mRNA at each sampling time or an HIV-induced modulation. We therefore evaluated mRNA levels of several other genes that should be relatively independent of the cell cycle, such as the β chain of the T-cell-antigen receptor (43) and HLA-A2 and HLA-B7 (3, 35). This involved stripping the signal of the T4, T8, tubulin, and T3 blots and reprobing with the CT β, HLA-A2, and HLA-B7 cDNA probes. The levels of these mRNAs were relatively constant during the period of observation (Fig. 3A), confirming that the mRNAs tested were of comparable quality and amounts and indicating that the differences observed in tubulin mRNA were probably due to fluctuations in tubulin gene expression. When T4 mRNA levels in HIV-infected cells were compared by scanning densitometry with CT β and HLA mRNAs rather than with β-tubulin mRNA, 75% reduction in the T4 mRNA was detected at day 10. It should be emphasized, however, that these cells still contained clearly detectable T4 transcripts (Fig. 3A), but no surface T4 antigen (Fig. 1).

In contrast to T4 and tubulin mRNAs, the relative levels of T3, T8, T9, and T11 mRNAs were unperturbed over the course of establishing chronic HIV infection (Fig. 3A). HIV mRNA synthesis peaked between days 8 and 10 after infection (Fig. 3A), in parallel with the establishment of a full viral infectious cell cycle in the CR-10/HIV line (Fig. 1C, inset). The relative amount of viral RNA remained stable thereafter, at about 3.5×10^3 HIV genomic RNA equivalents per cell (D. J. Volsky, unpublished data).

The relative levels of T4, T11, HLA-B7, and HLA-A2 mRNAs were also evaluated in MT-4 and HBD-1 cells 1 week after HIV infection, after significant downmodulation of T4 and T11 (Table 1). The reduction in surface antigen expression could not be accounted for by a reduction in

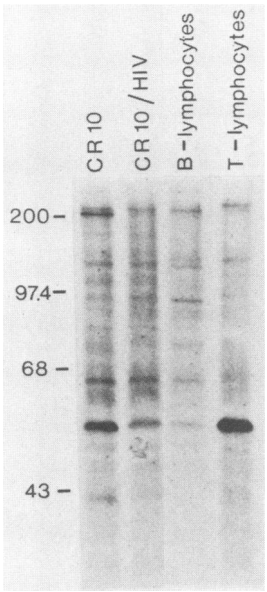

FIG. 4. Analysis of T4 receptor expression in HIV-infected CR-10 cells by radioimmunoprecipitation. Uninfected and CR-10/HIV cells 10 days after infection (Fig. 1) were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine immunoprecipitated with OKT4A antibody as described in Materials and Methods. Antibody-antigen complexes were precipitated with protein A-Sepharose beads, washed, dissolved in the sample loading buffer, and electrophoresed through 10% polyacrylamide-SDS gels; gels were then treated with En^{3}Hance (New England Nuclear Corp.) and autoradiographed (Kodak XAR-5 film). Numbers on left are in kilodaltons.

mRNA levels (Fig. 3B). The relative abundance of the T4 and T11 mRNAs was similar before and after infection, and the HLA-A2 and HLA-B7 probes demonstrated that equivalent amounts of RNA were being analyzed.

Immunoprecipitation of T4 receptor in HIV-infected cells. Since the CR-10/HIV cells expressed high levels of T4 mRNA but showed no T4 molecules on the cell surface, it was possible that the cells contained a detectable pool of intracellular nonutilized T4 molecules. This possibility was evaluated by radioimmunoprecipitation of whole-cell lysates with an OKT4A monoclonal antibody (Fig. 4). This antibody competes with the viral envelope for binding to T4 (28, 29). Thus, we did not attempt to show the amount of intracellular T4 that is complexed with the viral envelope glycoprotein (16) but rather attempted to determine how much T4 was available for localization on the cell membrane. The antibody reacted with a protein of approximately 55 kilodaltons in human T lymphocytes and very weakly with B lymphocytes (Fig. 4). Protein of a similar electrophoretic mobility was observed in uninfected CR-10 cells but was greatly reduced in HIV-infected cells (Fig. 4). At this time, the proportion of cells expressing HIV was approximately 98%.

DISCUSSION

The experiments reported here demonstrate that the establishment of a chronic HIV carrier state under noncytopathic and cytopathic conditions results in a modulation of several cell surface antigens, including the T4 receptor for the virus. The downregulation of the HIV receptor itself is not without precedent. Blocking of the cell receptor after retroviral infection is the basis for the phenomenon of virus interference (reviewed in reference 41). In the extensively studied group of avian leukosis viruses, interference appears to be due to the interaction of viral envelope glycoprotein gp85 with the corresponding subgroup receptor (8, 39, 41), which prevents the cells from undergoing cytopathic effects resulting from massive second-round infection (42). We propose that the cytopathicity resistance displayed by the CR-10 cell line used in the present studies is due, at least in part, to an efficient downmodulation of viral receptors after the first-round infection, thereby preventing superinfection with the newly produced virus.

The observed downregulation of surface T3, T8, and T11 (and, in the most recent series of experiments, also the interleukin-2 [IL-2] receptor) in HIV-infected cells has been unexpected. The phenomenon has also been repeatedly demonstrated in other T4$^+$ lymphoblastoid cell lines (CEM, HBD-1, MT-4 cells) infected with HIV/N1T virus and in HUT-78 cells infected with a distinct variant of acquired immunodeficiency syndrome virus, HIV/SF2 (23) (Table 1). The role of HIV in mediating this multireceptor modulation, or the mechanism by which it occurs, is unclear. Our data clearly discount the possibility that the receptor-negative CR-10 population arises as a result of HIV-mediated cytolytic elimination of receptor-positive cells. Furthermore, the abundance of T3, T4, T8, and T11 receptor mRNAs in HIV-infected cells indicates that HIV does not significantly interfere with the transcription of these genes. Thus, a possible explanation for our results is that HIV infection results in a posttranscriptional modulation of expression of several endogenous genes, possibly occurring either at the level of mRNA translation or during posttranslational processing of the receptor protein.

Host cell protein synthesis is shut down after infection by many eucaryotic viruses, including polio, influenza, and adenoviruses (for a review, see reference 18). This has been attributed to virus-induced inhibition of cellular mRNA translation at both initiation and elongation (9, 19), since such cells contain high levels of cellular gene transcripts that are fully functional in in vitro translational assays (1). Although HIV-infected CR-10 cells contain high levels of T4, T8, and T3 mRNA (Fig. 3), the present data do not allow the determination of the functionality of these messages. The detection of immunoprecipitable 55-kilodalton polypeptide in extracts of CR-10/HIV cells (Fig. 4) indicates that at least a fraction of the T4 mRNA in CR-10/HIV cells is amenable to translation. However, if a translational inhibition similar to that postulated for influenza or adenoviruses (1, 9, 18) would take place after HIV infection, it would have to be somehow restricted to the transcriptional products of certain genes (T3, T4, T8, T11, IL-2) but not the others (T9, HLA-A1, HLA-131, and HLA-B60) (Fig. 1C) (5).

The downregulation of the T4 HIV receptor itself could also occur via a posttranslational mechanism owing to an interference with the transport or membrane localization of the receptor, or both. In analogy to the avian leukosis virus (8, 39, 40), this could include formation of an intracellular complex between the cytoplasmic T4 precursor protein and a 160-kilodalton viral envelope protein precursor, thereby effectively limiting the localization of T4 molecules on the infected cell surface and rendering the cell receptor negative and superinfection resistant. A variant of this possibility could be formation of a membrane complex between T4 and viral *env* gene product, followed by the internalization of the complex, similar to hormone-receptor ligands (14). However, formation of complexes with viral *env* protein precur-

sors cannot possibly account for the observed downmodulation of T3, T8, T11, and IL-2 receptors. It is clear that the perturbation in cell surface receptor expression observed after noncytopathic HIV infection involves a combination of several events resulting from an interference of the virus with cellular gene expression.

During preparation of this manuscript, Hoxie et al. (16) published an article describing alterations in the T4 protein and mRNA synthesis in cells infected by HIV. They demonstrated that chronically infected cells show greatly decreased amounts of T4 mRNA. In CR-10 cells, we observed up to 75% reduction in T4 mRNA, and the cells were 100% virus antigen positive and were negative for OKT4 receptor molecules. In HBD-1 and MT-2 cells, there was no significant decrease in T4 mRNA levels despite an absence of surface T4 antigen expression. However, in contrast to the work of Hoxie et al. (16), the experiments described here were based on HIV-infected cells over a 1 to 2-week period. We were unable to latently infect MT-2 or HBD-1 cells since the cell cultures were lysed at approximately 10 days postinfection, and this suggests that the LAV-N1T virus strain used in our studies was more cytopathic than the HIV type III B strain used by Hoxie et al. (16). Our experiments thus demonstrate that the dramatic downregulation in cell surface T4, T3, T8, and T11 receptors during the first 2 weeks after infection cannot be directly attributable to similar reductions in mRNA levels coding for these receptors. The experiments described by Hoxie et al. (16) also suggested modulation of T4 receptor expression owing to intracellular complexes forming between viral envelope glycoproteins and the T4 receptor molecules. Our data demonstrate that the level of T4 receptor molecules is greatly reduced after HIV infection (Fig. 4). In HIV-infected CR-10 cells, T4 receptor molecules which had not bound HIV envelope (as determined by the OKT4A antibody) were detectable by immunoprecipitation (Fig. 4), although by fluorescence-activated cell sorter analysis these cells were T4 receptor negative (Fig. 1). Thus, an interference with T4 receptor protein localization on the cell surface appears to be the main mechanism by which HIV modulates this cell receptor. This mechanism, however, cannot account for the downregulation of the other surface receptors (T3, T8, T11) observed during HIV infec tion. The abundance of RNA molecules encoding these proteins in infected cells again indicates a posttranscriptional mechanism of downmodulation. We have extended our studies to investigate the expression of the IL-2 receptor in certain cell lines after HIV infection (M. Stevenson, C. Meier, and D. J. Volsky, submitted for publication). As with the cell receptors described in this report, the IL-2 receptor is dramatically downregulated after HIV infection, despite the abundance of full-length mRNA encoding this receptor.

We demonstrated that the establishment of a chronic, noncytopathic HIV carrier state in human T lymphoid cells results in a persistent downregulation of several cell surface polypeptides belonging to the T-cell receptor supergene family. Whatever the mechanism of the observed downregulation, our results indicate that a noncytopathic infection of T cells by HIV may have as yet unexpected effects on the T-cell functions mediated by the affected receptors.

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