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A specific defect in CD3 γ-chain gene transcription results in loss of T-cell receptor/CD3 expression late after human immunodeficiency virus infection of a CD4+ T-cell line

(acquired immunodeficiency syndrome/two-dimensional gel electrophoresis)

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ABSTRACT **Sequential effects on cellular protein expression following human immunodeficiency virus (type 1) infection of a CD4+ T-cell line *in vitro* were investigated. Events in the human interleukin 2-dependent helper T-cell line WE17/10 are similar in several respects to the clinical progression in acquired immunodeficiency syndrome. WE17/10 cell infection is characterized by an extended period during which viral replication occurs without accompanying cytotoxicity and with a maximum 30% decrease in surface CD4. Cellular protein expression generally remains unaffected during this first phase of infection. However, after 2–3 months, a severe defect in the expression of the T-cell receptor/CD3 complex both on the cell surface and inside the cell becomes apparent. Other cell membrane markers, such as CD2 and CD25, remain constant throughout the course of infection; after its initial decrease, CD4 remains at 70% of control values. Lack of surface expression of the TCR/CD3 complex is correlated with a specific defect in transcription of the CD3 γ-chain gene.**

Individuals infected with the human immunodeficiency virus (HIV; refs. 1 and 2) can remain asymptomatic for years before the onset of acquired immunodeficiency syndrome (AIDS), a disease characterized by the gradual loss of CD4+ T-cell-dependent immune responses. Most *in vitro* studies of HIV infection using human CD4+ T-cell lines or normal peripheral blood lymphocytes have demonstrated the development of multinucleated syncytial cells, severe downmodulation of CD4 on the infected cell surface, and cytopathic death (3–7). In some cases, infected cells survive infection, but they no longer express viral proteins or CD4 (8). Initially, these studies suggested that HIV-induced immunodeficiency in AIDS was mainly due to depletion of CD4+ T cells, either via direct cytotoxicity of the virus or through loss of CD4, thereby eliminating the important role of these cells in the immune response. However, these observations have not been shown to parallel completely the course of HIV infection *in vivo* (9).

In addition to a reduction in the CD4+ T-cell population of HIV-infected individuals, clinical studies have demonstrated several functional defects in CD4+ T cells, including their inability to respond to soluble antigen or provide help for antibody production (10–13). A relatively high frequency of HIV-infected T cells still expressing CD4 has been reported for both seropositive individuals and AIDS patients (14, 15), suggesting that HIV may replicate and kill certain CD4+ T cells while maintaining its presence in other viably infected but partially or entirely incapacitated cells. Thus, the clinical progression in AIDS is characterized by both a quantitative reduction and a qualitative defect in the CD4+ T-cell subpopulation, in concert with progressive infection of mononuclear phagocytes (9).

We have observed that *in vitro* HIV-1 infection of a human interleukin 2 (IL-2)-dependent CD4+ T-cell line, WE17/10, mimics some of the clinically observed events. WE17/10 cell infection is characterized by an initial period of productive infection where major alterations in cellular protein expression are not detectable, followed after 2–3 months by impaired expression of the T-cell receptor (TCR)/CD3 complex in the cells. Due to the important immunological role of TCR/CD3 in antigen recognition, we have investigated the nature of this change in HIV-infected WE17/10 cells.

MATERIALS AND METHODS

Cells and Virus. The WE17/10 cell line, established from the blood of a patient with T-cell lymphoma (16), is grown in RPMI 1640 containing 20% fetal bovine serum and 100 units of recombinant human IL-2 per ml. WE17/10 cells are IL-2-dependent and express CD2, CD3, CD4, CD5, CD25, CDw29, TCR, HLA class I and class II antigens, and the transferrin receptor on their surface. WE17/10 cells do not contain integrated proviral sequences from known human retroviruses. The karyotype is abnormal [47,XX,−3, +der(3),t(1;3)(q41;q29),+M] in 100% of the cells. The B-cell line GM607 was obtained from the Human Genetic Mutant Cell Repository, National Institutes of Health.

The LAV-1 strain (1) of HIV-1 was kindly provided by F. Barré-Sinoussi and maintained in CEM cells (5). WE17/10 cells, synchronized by IL-2 deprivation, were infected by resuspension in a 1:1 mixture of virus-containing cell-free culture supernatant and complete medium (5×10^5 cells per ml; reverse transcriptase activity, $\approx 10^5$ cpm/ml). After 4 hr at 37°C, the cells were washed to remove free virus and resuspended in complete medium (5×10^5 cells per ml). Infection of WE17/10 was begun with cells from passages 25 to 35, and uninfected cells were carried in parallel passages for each experiment.

Detection of HIV Infection. Infected and uninfected control cells were fixed and stained by indirect immunofluorescence using positive AIDS patient serum followed by fluorescein-conjugated goat anti-human IgG (1), and the virus-positive cells were counted. Reverse transcriptase activity in cell culture supernatants was determined as described (1).

Cell Surface Phenotype. The murine monoclonal antibodies used were OKT3, OKT4, OKT8, and OKT11 (Ortho Pharmaceutical), anti-Tac (a gift from Lorenzo Moretta, Istituto Scientifico Tumori, Genoa, Italy), BMA (a gift from Fatima Faure, Institut Gustave Roussy, Villejuif, France), and anti-

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Abbreviations: HIV, human immunodeficiency virus; IL, interleukin; TCR, T-cell receptor; 2D, two-dimensional; HTLV, human T-cell lymphotropic virus.

HIV p24 (Cellular Products). Cell samples (5×10^5 cells for each antibody) were stained in a two-step process, always using the same dilution of a stock monoclonal antibody solution followed by a saturating concentration of fluorescein-conjugated goat anti-mouse immunoglobulin (17), and cell fluorescence was examined with a cytofluorograph.

Analysis of WE17/10 Protein Expression. Infected and control WE17/10 cells were metabolically labeled as described (17), except that the RPMI 1640 labeling medium contained no cysteine or methionine (Select-Amine kit, GIBCO) and was supplemented with 10% fetal bovine serum, IL-2 (100 units/ml), [^{35}S]cysteine (50 μCi/ml; specific activity, >1000 Ci/mmol; Amersham; 1 μCi = 37 kBq), and [^{35}S]methionine (50 μCi/ml; >1100 Ci/mmol; Amersham). Iodination and immunoprecipitation of cell surface proteins were performed essentially as described (18, 19). Cellular or membrane proteins were extracted and the solubilized proteins or immunoprecipitates were separated by high-resolution two-dimensional (2D) gel electrophoresis (17, 19). The radiolabeled proteins were detected by direct autoradiography after a 1-week exposure.

Analysis of WE17/10 mRNAs. RNA was extracted from cell lines by guanidine hydrochloride/cesium chloride centrifugation (20), and poly(A)$^+$ RNA was selected on oligo(dT)-cellulose columns. mRNA was treated with glyoxal and either 1 μg per sample was dotted onto nitrocellulose membranes (21) or 4 μg per sample was electrophoresed for Northern blot analysis (22). Molecular hybridization was performed using the indicated ^{32}P-labeled cDNA probes.

cDNA Probes. The TCR α- and β-chain (TCR-α and TCR-β) probes (23, 24) were kindly provided by Tak Mak; the CD3-ε probe (25), generated by polymerase chain reaction to the entire ε-chain coding region, by Jason Loveridge (Institute for Molecular Medicine, Oxford, England); the CD3-ζ probe (26) by Allan Weissman; and the CD3-γ probe (27) by M. J. Crumpton. The CD3-δ probe (28) was obtained from the American Type Culture Collection. The cut inserts were labeled with [α-^{32}P]dCTP by the multiprime technique (Amersham).

RESULTS

Kinetics of HIV-1 Infection in WE17/10 Cells. WE17/10 cells are fully susceptible to HIV-1 infection without apparent cytopathic effects, and infected cells can be maintained in culture for >15 months (the furthest point of experiments) without noticeable differences in their morphology or IL-2-dependent growth characteristics. The presence of HIV-1 in WE17/10 cells was monitored by detection of reverse transcriptase activity in culture supernatants and LAV-1 antigens in the cells. Results from one of three similar experiments are shown in Fig. 1. Typically, infection resulted in 50% of the cell population being positive for viral antigen after 3 days, reaching 100% infection between 5 and 7 days (Fig. 1*A*). Reverse transcriptase activity was detectable within 24 hr, rose continuously for ≈1 month, and remained high for several months after infection (Fig. 1*B*). Although reverse transcriptase activity eventually declined to 10% of its peak value, the cells remained 100% viral antigen-positive for >15 months.

Kinetic changes in the amount of CD2, CD3, CD4, and CD8 (negative control) on the surface of infected and control cells were also determined (Fig. 1 *C–F*; the values for 8, 10, 12, and 15 months were the same as indicated for day 160). There was no significant difference in the level of CD2 (Fig. 1*C*) or CD25 (data not shown) on infected and uninfected WE17/10 cells at any time. In addition, the cells were CD8$^-$ and remained negative after infection (Fig. 1*F*). There was a gradual reduction in CD4, reaching a maximum loss of 30% (mean fluorescence from time points 40 days after infection and later were averaged and expressed as percent based on uninfected controls = 100%) (Fig. 1*E*). The histograms in Fig. 2 *A′–C′* indicate that this was due to all cells having less surface CD4 rather than to the appearance of a CD4$^-$ subpopulation. Infected cells remained at only 70% of normal CD4 values after 6 or more months, although a decline in the level of reverse transcriptase activity occurred. This suggests that the slight reduction of CD4 on WE17/10 cells is not related to the formation of complexes with HIV gp120 inside the cell (7).

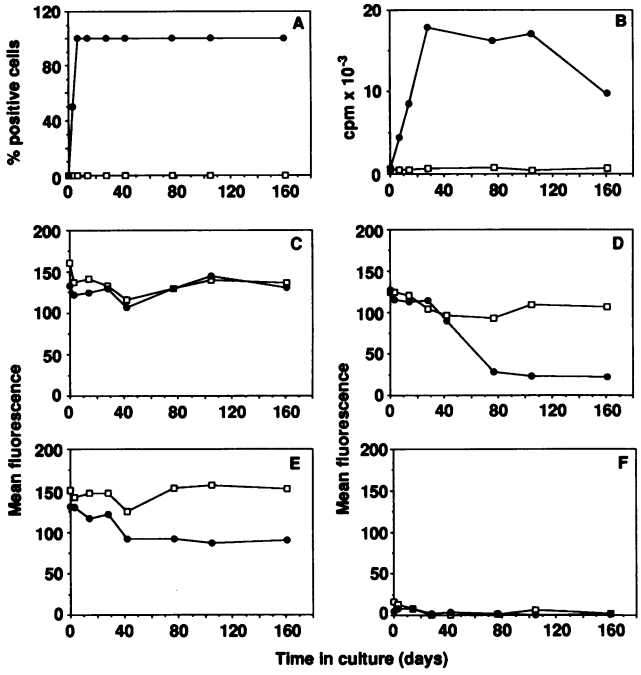

FIG. 1. The evolution, as a function of time after infection, of viral and cellular parameters in HIV-infected WE17/10 cells (●) and in uninfected control WE17/10 cells (□). (*A*) Presence of HIV antigens, expressed as % positive cells. (*B*) Reverse transcriptase activity, expressed as cpm. (*C–F*) CD2, CD3, CD4, and CD8, respectively, expressed as mean fluorescence (background values for normal mouse serum were subtracted) determined by flow cytometry. The figure shows results of one of three similar experiments. For each experiment, when cells frozen at different stages of infection were thawed and cultured again, the decrease in CD3 expression followed approximately the same time course as in the original unfrozen cells.

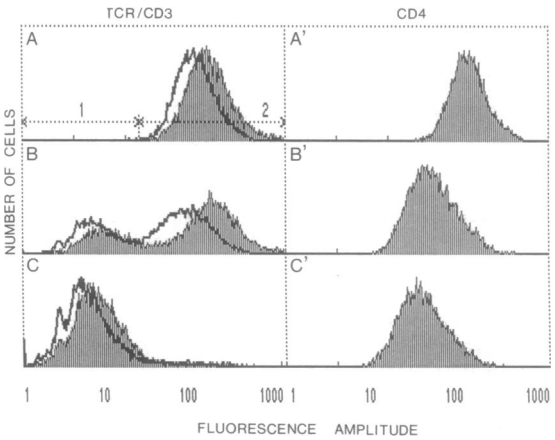

FIG. 2. Histograms showing the distribution of immunofluorescence from anti-CD3 (shaded curve in *A–C*), anti-TCR-β (unshaded curve in *A–C*), and anti-CD4 (shaded curve in *A′–C′*) antibody staining of uninfected (*A* and *A′*) and infected WE17/10 cells, 2.5 months (*B* and *B′*) and 6 months (*C* and *C′*) after infection. Areas 1 and 2 are indicated with arrows in *A*.

The most dramatic change in a surface antigen occurred for CD3 (Fig. 1*D*). CD3 levels in infected cells remained equal to control values for 2–3 months (the precise length of time depended upon the initial infectious dose, the rate of infection, and the frequency of cell passage). The end of the first phase of productive infection was marked by a significant decrease in CD3 on the surface of infected cells. The amount of TCR-β on the infected cell surface was also reduced, in synchrony with the loss of CD3 (Fig. 2 *A–C*). The loss of TCR/CD3 occurred by individual cells converting from positive to negative, with the majority of the population eventually becoming negative (Fig. 2 *A–C*). The positive cells (area 2) decreased from 98.6% (Fig. 2*A*) to 61.3% (Fig. 2*B*) to 8.2% (Fig. 2*C*). Inversely, the negative cells (area 1) increased from 1.4% (Fig. 2*A*) to 38.7% (Fig. 2*B*) to 91.8% (Fig. 2*C*). Designation of area 1 (negative cells) and area 2 (positive cells) was based on a negative profile obtained with the B-cell line GM607. Once it had occurred, the loss of TCR/CD3 persisted, and the complex was not reexpressed on the surface of infected cells thereafter.

Protein Expression in HIV-1-Infected WE17/10 Cells. Comparison of 2D gel patterns from infected and control cells, analyzed at weekly intervals, revealed specific changes in total cell and membrane protein expression. Surprisingly, there were not many qualitative changes in cellular protein expression immediately after infection, but only quantitative fluctuations of some proteins (data not shown). The major differences in patterns of cells early in infection can be attributed to the appearance of viral proteins, suggesting that initially viral protein synthesis and virion production can occur without significant interference in cellular protein expression. The spot corresponding to the HIV-1 *gag* protein, p24, was identified by immunoprecipitation and is indicated in Fig. 3 *B* and *D*. The incorporation of ^{35}S-labeled amino acids into p24 followed kinetics similar to those shown for immunofluorescent staining (Fig. 1*A*) and was constant with respect to cellular proteins during the conversion from TCR/CD3$^+$ to TCR/CD3$^-$ cells (data not shown).

Major changes in cellular protein expression occurred immediately prior to or coincident with the loss of TCR/CD3 expression in the cells. Human TCR-α is a glycoprotein of 43–49 kDa, TCR-β a glycoprotein of 38–44 kDa, CD3-γ a glycoprotein of 25–28 kDa, CD3-δ a 20-kDa glycoprotein, CD3-ε a 25-kDa nonglycoprotein, and CD3-ζ a 16-kDa nonglycoprotein (23–28). Spots corresponding to the TCR/CD3 chains are indicated with circles in Fig. 3 and were identified by immunoprecipitation (Fig. 3*E*). The intensity of these spots in the ^{35}S patterns is related to the number of methionine and cysteine residues in each protein chain (23–28). TCR/CD3 polypeptides were essentially absent from the infected cell surface (Fig. 3*F*) and were substantially reduced in patterns of cell extracts and cell membrane preparations from infected cells 3 months after infection (TCR/CD3$^-$; Fig. 3 *B* and *D*) by comparison with uninfected control cells (TCR/CD3$^+$; Fig. 3 *A* and *C*) or infected cells 1–2 months after infection (TCR/CD3$^+$; gels not shown). Thus, in addition to the lack of TCR/CD3 expression on the surface of the cell, the TCR/CD3 protein chains disappear intracellularly as well (18). The loss of TCR/CD3 protein spots from 2D gel patterns of HIV-infected WE17/10 cells precisely follows the kinetics shown in Fig. 1*D* for the decrease of surface CD3.

mRNA Expression in HIV-1-Infected Cells. RNA dot and Northern blot hybridization analyses were used to investigate a possible reduction or absence of mRNA for one or more of the TCR/CD3 chains. mRNA was purified from uninfected TCR/CD3$^+$ and infected TCR/CD3$^+$ or TCR/CD3$^-$ WE17/10 cells and the B-cell line GM607. The probes used were derived from cDNAs corresponding to the TCR α and β chains and the CD3 γ, δ, ε, and ζ chains. Dot blots showed that levels of TCR-α, TCR-β, CD3-ζ, CD3-δ, and CD3-ε transcripts did not change during HIV infection (Fig. 4) *A–C*, *E*, and *F*). In contrast, CD3-γ transcripts were progressively reduced 2.5 and 3.5 months after infection and undetectable 13 months after infection (Fig. 4*C*) in cells with 53%, 19%, and <3% of control TCR/CD3 surface values (determined by flow cytometry), respectively. Northern blots from the same mRNA preparations confirmed these findings and demonstrated that transcribed messages were identically spliced in infected and uninfected cells (Fig. 4 *G–L*).

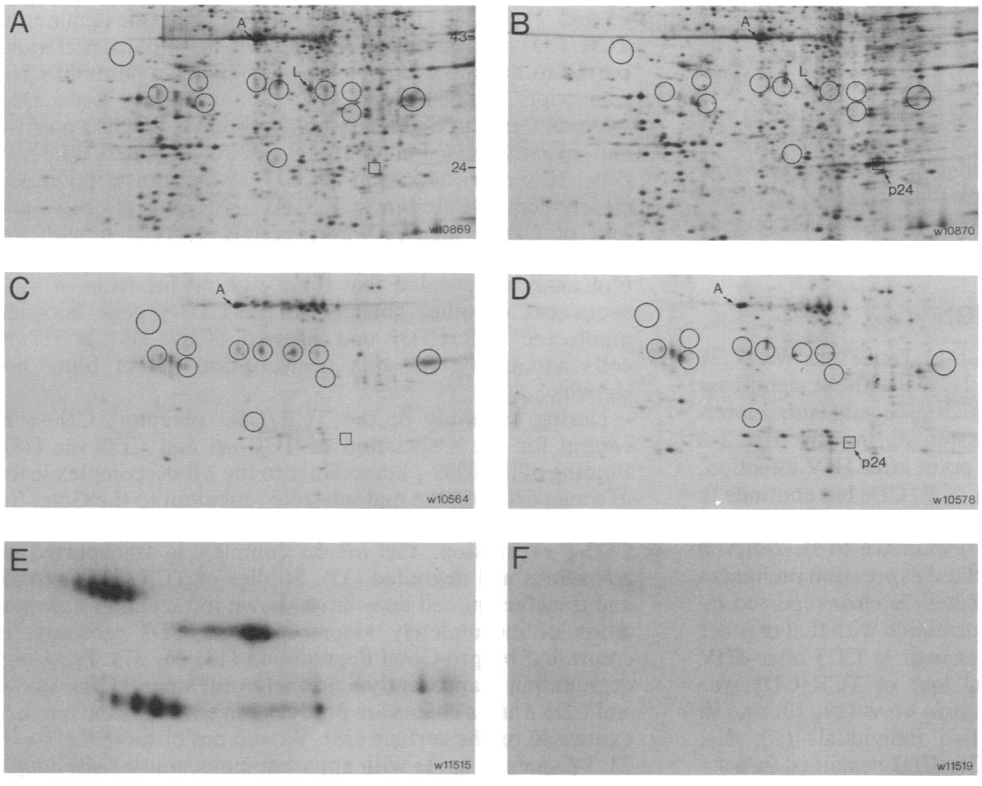

FIG. 3. Portions of autoradiographs of 2D electrophoretic patterns from WE17/10 cells. (*A–D*) Metabolic labeling, ^{35}S autoradiographs: total protein extract from uninfected TCR/CD3$^+$ cells (*A*) and infected TCR/CD3$^-$ cells 3 months after infection (*B*); membrane protein extract from uninfected TCR/CD3$^+$ cells (*C*), and infected TCR/CD3$^-$ cells 3 months after infection (*D*). (*E* and *F*) Surface labeling, ^{125}I autoradiographs: CD3 immunoprecipitates from uninfected TCR/CD3$^+$ cells (*E*) and infected TCR/CD3$^-$ cells 4 months after infection (*F*). The HIV *gag* protein, p24, is indicated with squares; TCR/CD3 proteins with circles; and actin (A), lactate dehydrogenase, B form (L), and the molecular mass (kDa) scale are identified for reference. The gels are oriented with the basic end to the right and the acidic end to the left.

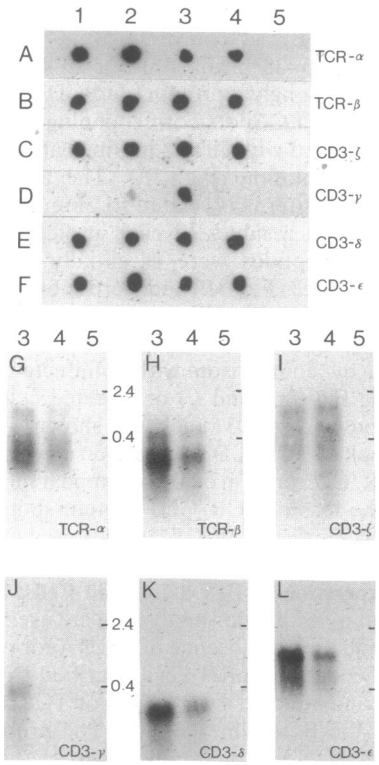

FIG. 4. Expression of messages for TCR-α (*A* and *G*), TCR-β (*B* and *H*), CD3-ζ (*C* and *I*), CD3-γ (*D* and *J*), CD3-δ (*E* and *K*), and CD3-ε (*F* and *L*) determined by dot (*A–F*) or Northern (*G–L*) blots of mRNA from uninfected WE17/10 cells (column 3 and lanes 3); WE17/10 cells 3.5 months (column 1), 2.5 months (column 2), and 13 months (column 4 and lanes 4) after HIV infection; and the B-cell line GM607 (column 5 and lanes 5). The filters were exposed for 24 hr (*A–C*, *E–H*, *K*, and *L*) or 48 hr (*D*, *I*, and *J*). For the Northern blots, positions of size standards are shown (2.4 and 0.4 kilobases).

In uninfected WE17/10, radioactivity bound to the dot blots using the CD3-γ probe was only one-third of that obtained with the other TCR/CD3 probes (determined by bound cpm), suggesting that CD3-γ may be a limiting factor for surface expression of the complex. The lower level of radioactivity bound to the Northern blots for TCR-α, TCR-β, CD3-δ, and CD3-ε infected-cell mRNA (Fig. 4, lanes 4) was most likely due to the RNA preparation; Northern analysis of a different TCR/CD3$^-$ infected-cell mRNA extract was similar to controls except for the consistent absence of CD3-γ (data not shown). mRNA for the six chains was not present in the GM607 B-cell line (lanes 5).

DISCUSSION

We have shown that the human CD4$^+$ T-cell line WE17/10 can be productively infected with HIV-1 without significant reduction of CD4, cellular cytotoxicity, or substantial interference in cellular protein expression during the first 2–3 months of infection. At a specific point after HIV infection, WE17/10 cells become deficient in TCR/CD3 but continue to express CD2 and CD4 (70%) and the IL-2 receptor (CD25) on the cell surface and remain fully responsive to IL-2-driven growth. Earlier studies of CD3 surface expression on human peripheral blood lymphocytes infected *in vitro* reported either a loss of CD3 (4), a CD3 loss correlated with that of other markers such as CD2 (6), or no change in CD3 after HIV infection (3). Recently, functional loss of TCR/CD3 was found both in lymphocytes infected *in vitro* (29, 30) and in lymphocytes from HIV-seropositive individuals (10, 41), although surface expression of TCR/CD3 remained intact.

Decreased surface expression of CD3 can also accompany infection of CD4$^+$ T cells by another human retrovirus, human T-cell lymphotropic virus type I (HTLV-I; refs. 31 and 32). Yssel *et al.* (31) found that HTLV-I transformation of T-cell clones *in vitro* occurred in two phases; initially there was loss of functional activity and the need for antigenic stimulation, followed 200 days postinfection by loss of TCR/CD3 on the cell surface. However, as with HIV, conflicting results on TCR/CD3 expression after infection have been reported. TCR/CD3$^-$ cells were demonstrated in some HTLV-I-infected patients (33), but many patient samples or cell lines derived from them, as well as cells infected *in vitro*, have normal levels of CD3. Changes in TCR/CD3 on HTLV-I- or HIV-infected CD4$^+$ T cells may be a function of time and/or changes in viral gene expression. Although TCR/CD3$^-$ cells possibly exist in HIV-infected individuals, previous clinical studies were not focused on a search for CD4$^+$CD3$^-$ T cells, and this remains to be carefully investigated. The unusual character of infection in WE17/10 cells potentially provides a means for detecting events occurring in a subpopulation of HIV-infected CD4$^+$ T cells otherwise not apparent due to the heterogeneous mixture of lymphocytes present in the peripheral blood.

We investigated possible mechanisms that might explain the loss of TCR/CD3 expression in HIV-infected WE17/10 cells. An accumulation of unintegrated viral DNA or RNA could interfere with host protein synthesis (34). We have observed that the amounts of unintegrated (or integrated) viral DNA forms in infected WE17/10 cells were similar at TCR/CD3-positive and -negative stages after infection and therefore could not explain the changes in receptor expression (data not shown). Furthermore, we did not observe changes in reverse transcriptase activity or synthesis of the viral *gag* protein, p24, occurring in conjunction with the loss of TCR/CD3.

Interference with the normal synthesis, degradation, transport, or processing of gene transcripts coding for the various TCR/CD3 chains would prohibit expression of the antigen-receptor complex (35, 36). Mature TCR/CD3 complexes on the surface of human cells are composed of two different TCR chains, α and β, in combination with four CD3 chains, γ, δ, ε, and ζ (23–28). In the absence of any one chain, the TCR/CD3 complex cannot be correctly assembled and transported to the plasma membrane (18). The amounts of RNA transcripts for TCR-α, TCR-β, CD3-δ, CD3-ε, and CD3-ζ were all found to be comparable in both TCR/CD3-positive and -negative HIV-infected cells and in uninfected WE17/10 cells. However, inhibition of CD3-γ gene transcription was clearly demonstrated in TCR/CD3$^-$ HIV-infected cells, and loss of CD3-γ mRNA was precisely correlated with decreased expression of receptors on the cell surface. Southern blot analysis revealed that there was no insertion of viral sequences or other changes in the CD3-γ gene between uninfected TCR/CD3$^+$ and infected TCR/CD3$^-$ WE17/10 cells accounting for this transcription defect (data not shown).

During assembly of the TCR/CD3 receptor, CD3-γ is central for the association of TCR-$\alpha\beta$ and CD3-$\gamma\delta\varepsilon$ (18). Binding of the CD3-ζ homodimer to the $\alpha\beta\gamma\delta\varepsilon$ complex leads to transport from the endoplasmic reticulum to the Golgi for glycosylation prior to surface expression (36). In cells lacking CD3-ζ expression, the $\alpha\beta\gamma\delta\varepsilon$ complex is transported to lysosomes and degraded (35). Studies of TCR/CD3 mutant and transfectant cell lines have shown intracellular accumulation of incompletely assembled TCR/CD3 receptors is controlled by pre-Golgi degradation (18, 36, 37). Pre-Golgi degradation is apparently a normal event since TCR α and β and CD3 δ and ε chains are produced in excess of the number expressed on the surface (38). We did not observe CD3-ε or CD3-ζ spots or spots with apparent molecular weight equiv-

alent to that of unglycosylated precursors of TCR-α or -β or CD3-δ, suggesting that intracellular accumulation of these chains in WE17/10 cells lacking CD3-γ is controlled by pre-Golgi degradation. Thus, in the later stages of HIV infection in WE17/10 cells, the absence of CD3-γ leads to the TCR/CD3$^-$ phenotype. The lack of CD3-$\gamma\delta\varepsilon\zeta$ and CD3-$\delta\varepsilon$ expression in two HTLV-I-transformed T-cell lines (31, 32) supports the data presented here, suggesting that human retroviruses may have mechanisms for specifically interfering with normal regulation of the closely linked CD3 gene family.

Changes in the growth characteristics of WE17/10 cells, their TCR/CD3 genes, or integrated and unintegrated HIV forms were not apparent during HIV infection. Uninfected WE17/10 cells had stable expression of TCR/CD3 when cultured in parallel with infected cells or for longer periods of time. It is unlikely that identical variant viruses or mutant cell clones arose in three independent experiments and successfully outgrew the entire population with repeatedly similar kinetics. Simultaneous or sequential alterations in viral and/or cellular gene regulation could account for the dramatic change in phenotype. Thus, the defect in CD3-γ gene transcription possibly results from expression of a viral gene product(s) that interferes with normal regulation of the TCR/CD3 complex.

Functionally, the loss of TCR/CD3 in HIV-infected T cells would significantly inhibit their ability to respond immunologically. A rare case of a familial defect in CD3 expression is clinically characterized by increased susceptibility to infection (39). Patients with chronic fatigue syndrome also have T cells deficient in CD3 expression leading to abnormal T-cell function (40). The CD4$^+$ T-cell defect observed in AIDS patients may be partially attributable to loss of the TCR/CD3 antigen-activation pathway (10, 41). Further study of HIV infection in WE17/10 cells should provide new insight into the mechanism(s) causing the CD4$^+$ T-cell immune dysfunction characterizing AIDS.

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