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Evidence for susceptibility of intrathymic T-cell precursors and their progeny carrying T-cell antigen receptor phenotypes TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ to human immunodeficiency virus infection: A mechanism for CD4$^+$ (T4) lymphocyte depletion

(triple-negative thymocytes/polymerase chain reaction)

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ABSTRACT **Individuals infected by the human immunodeficiency virus type 1 (HIV-1) demonstrate progressive depletion and qualitative dysfunction of the helper T4 (CD4$^+$) cell population. Mechanisms proposed for attrition of CD4$^+$ T cells include direct cytopathicity of these mature cells following infection as well as infection of early T-lymphocyte progenitors. The latter mechanism could lead to failure to regenerate mature functioning CD4$^+$ T cells. The present study determines the susceptibility of thymocytes at various stages of maturity to infection with HIV-1. Various normal thymocyte populations were inoculated with HIV-1, including unfractionated (UF), CD3$^-$ CD4$^-$ CD8$^-$ ["triple negative" (TN)], CD4$^+$ CD8$^+$ ["double positive" (DP)] thymocytes, and thymocyte populations obtained by limited dilution cloning. Cultures were studied for the presence of HIV-1 DNA by polymerase chain reaction in addition to examination for reverse transcriptase activity. We determined that transformed T-cell and thymocyte cell lines completely lacking CD4 were not susceptible to infection by HIV-1, whereas all of the following lines were: UF thymocytes (70–90% CD4hi$^+$); DP thymocytes (99% CD4hi$^+$); TN thymocytes (0% CD4hi$^+$); and TCR$\alpha\beta^+$, TCR$\gamma\delta^+$, or CD16$^+$ CD3$^-$ (natural killer) thymocyte clones expressing variable levels of CD4 and representing the progeny of TN thymocytes. [TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ refer to the chains of the T-cell antigen receptor (TCR), and CD4hi refers to a strong rightward shift (>30 linear channels) of the CD4 curve on flow cytometric analysis compared with control.] Monoclonal antibodies (mAbs) to CD4 (T4a epitope) but not to CD3 (T3) were capable of blocking infection of mature and immature CD4hi$^+$ thymocytes. Moreover, anti-CD4(T4a) mAbs also inhibited infection of CD4hi$^-$ TN thymocytes, indicating that these T-cell precursors — despite their apparent "triple negativity" (CD3$^-$ CD4hi$^-$ CD8$^-$) — expressed sufficient CD4 molecules to become infected. Cell sorter analysis with a panel of CD4 mAbs demonstrated a mean shift of the mean fluorescence channel (MFC) with CD4 mAbs on TN thymocytes of 6 ± 4 MFC units. Thus, intrathymic T-cell precursors and their progeny representing many stages of T-cell ontogeny are susceptible to infection by HIV-1, including early TN thymocytes, which express very low levels of CD4. Infection of multiple stages and multiple subsets of the T-cell lineage in man, mediated via the CD4 molecule, may explain the inability of the T-cell pool to regenerate in the setting of progressive HIV infection.**

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Human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS, selectively infects cells expressing the CD4 molecule on their surface, particularly T lymphocytes and cells of the monocyte/macrophage lineage (1–6). Individuals infected by HIV demonstrate abnormalities of the CD4$^+$ T-cell population that include both progressive depletion and qualitative dysfunction (refs. 7–9; reviewed in ref. 10). Depletion of the CD4$^+$ (T4) (helper/inducer) subset of T lymphocytes is the critical basis for the profound immunosuppression that develops after infection with HIV (7–11). Several mechanisms have been proposed to explain the attrition of CD4$^+$ T cells in HIV-infected individuals. Direct infection of the mature CD4$^+$ T cell with resultant cytopathicity and cell death is one such mechanism (10). However, it is recognized that only a small fraction of mature CD4$^+$ T cells are infected with HIV-1 *in vivo*, particularly early in the course of infection, with an even smaller number of cells expressing virus (12, 13). Although the precise half-life of CD4$^+$ cells in humans is not known, in view of the normal turnover of T lymphocytes in the body, it would seem that the T-cell pool would be able to compensate for such a seemingly low rate of CD4$^+$ cell destruction. Therefore, it has been hypothesized that other mechanisms of CD4$^+$ cell depletion may be operable (14). In particular, infection by HIV of a CD4$^+$ cell precursor or stem cell or infection and depletion of a cell that produces growth factors responsible for regeneration of CD4$^+$ lymphocytes may lead to lack of production of mature CD4$^+$ cells (10).

The thymus is the principal anatomical site where stem cells migrating from the bone marrow undergo a complex series of genotypic and phenotypic changes that result in mature, immunocompetent T lymphocytes (reviewed in ref. 15). Pathological studies performed on the thymus glands of both adults and children with AIDS have revealed severe involution with depletion of both lymphocytes and epithelial elements (16–20). However, the changes seen are not unlike those in thymus glands from individuals with graft vs. host disease or congenital immunodeficiencies (19, 20). Recently,

Abbreviations: HIV-1, human immunodeficiency virus type 1; PCR, polymerase chain reaction; RT, reverse transcriptase; UF, unfractionated; SP, single positive; TN, triple negative; DP, double positive; TCR, T-cell antigen receptor; NK, natural killer; mAb, monoclonal antibody; phenotypes CD4hi$^+$ and CD4lo$^+$, CD4$^+$ cells with a rightward shift of the CD4 curve on flow-cytometric analysis of respectively >30 and <30 linear channels compared with control; SCL, stem-cell leukemia; MFC, mean fluorescence channel.

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techniques have become available for cloning the progeny of human CD3$^-$ CD4hi$^-$ CD8$^-$ "triple-negative" (TN) thymocytes (21). With these techniques, thymocyte clones that are of the TCR$\alpha\beta^+$, TCR$\gamma\delta^+$, or CD16$^+$ CD3$^-$ natural killer (NK) phenotypes have been derived (22); TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ refer to the chains of the T-cell antigen receptor (TCR). To determine the potential extent of HIV infection of intrathymic precursors of the CD4$^+$ T-cell pool and their progeny, the present study examined the susceptibility to HIV-1 infection of thymocytes at various stages of T-cell development.

MATERIALS AND METHODS

Cell Preparation. Thymic tissue was obtained as discarded tissue from HIV-seronegative subjects undergoing median sternotomy and corrective cardiovascular surgery. Thymocytes were separated, purified, frozen, and stored as described (22). Thymocytes were thawed and the CD3$^-$ CD4hi$^-$ CD8$^-$ population was separated by panning and magnetic bead separation as described (21). Purity of cell separations was monitored by flow cytometry with an EPICS Profile cytometer (Coulter Electronics). Thymocyte clones were prepared as described (21). Cell surface phenotype was determined by flow cytometry (21). For the purpose of this paper CD4hi$^+$ cells are those CD4$^+$ cells in which there was a rightward shift of the CD4 curve on flow cytometric analysis of >30 linear channels (1024-channel full scale) compared to the control curve. CD4lo$^+$ cells are those cells for which there was a slight rightward shift of the CD4 curve of <30 linear channels. CD4$^-$ cells are those cells for which there was no shift from control. In some experiments, CD4$^+$ CD8$^+$ "double-positive" (DP) cells were prepared by cell sorting of unfractionated (UF) thymocytes as described (13). The DU528 stem-cell leukemia (SCL) cell line and fresh leukapheresis SCL no. 5 cells were grown and used as described (23, 24).

***In Vitro* Infections with HIV-1.** Aliquots of thymocyte populations or various cell lines were placed in 10% (vol/vol) fetal calf serum in RPMI 1640 medium [± 10% (vol/vol) T cell-conditioned medium] at 10^6 cells per ml and were inoculated with HIV-1 (strain LAV) at multiplicities of infection of 0.02–0.10 or were mock-infected as controls. As additional controls, cell lines completely lacking CD4 (e.g., A201; ref. 4) were also inoculated with LAV. Virus-inoculated cells and control cells were harvested at 24 and 48 hr after inoculation, washed five times in phosphate-buffered saline (PBS) to remove any contaminating HIV-1 DNA present in the viral supernatants, and centrifuged to a pellet. Cells were then lysed for DNA isolation and prepared for polymerase chain reaction (PCR) as described below. In other experiments, aliquots of the various cell populations that were either inoculated with LAV or mock-infected were placed in culture [10% fetal calf serum and 10% purified interleukin 2 (Electro-Nucleonics) in RPMI 1640 medium] at 10^6 cells per ml. Cultures were examined for syncytia formation, and supernatants were sampled three times per week for reverse transcriptase (RT) activity.

RT Activity. The RT assay used for these experiments was carried out as described (13).

PCR. Cells that were to be examined for HIV-1 DNA by the PCR were washed three times in PBS, and the dry cell pellets were lysed. Details of the PCR procedure have been described (13, 25). Primer pairs used in these experiments included SK68/69 (*env*), SK38/39 (*gag*) or SK145/101 (*gag*), SK29/30 [long terminal repeat (LTR)], and QH 26/27 (*HLA-DQA* control) (Synthetic Genetics, San Diego, CA). Amplified products were hybridized to ^{32}P-labeled ATP end-labeled probes (SK70, SK19, or SK102, and SK31 for the *env*, *gag*, and LTR primers, respectively) and analyzed on 10% polyacrylamide gels. Autoradiograms were obtained at various intervals.

RESULTS

UF Thymocytes and CD4$^+$ CD8$^+$ DP Thymocytes Derived from Normal Individuals Are Susceptible to Infection by HIV-1. To determine the susceptibility of UF thymocytes to infection by HIV-1, suspensions of thymocytes obtained from normal subjects and isolated by density gradient centrifugation were inoculated with HIV-1 (strain LAV) viral supernatant (or control medium) at multiplicities of infection

Table 1. HIV-1 infectability of intrathymic T-cell precursors and their progeny

Cell type (*n*)	Phenotype, %						After HIV inoculation*	
	CD7$^+$	CD3$^+$	CD4$^+$	CD8$^+$	TCR$\alpha\beta^+$	TCR$\gamma\delta^+$	PCR	RT
Fresh unfractionated thymocytes (6)	81	63	72†	54	61	1	+	+
Fresh CD4hi$^+$ CD8$^+$ DP thymocytes (2)	ND	ND	98†	95	ND	ND	+	ND
TCR$\alpha\beta^+$ DP thymocyte clone 6G11	ND	96	100	65	95	0	+	ND
TCR$\alpha\beta^+$ DP thymocyte clone 4F8	ND	96	100	70	94	1	+	ND
CD16$^+$ NK thymocyte clone 1E3	17	0	31†	2	0	0	+	ND
CD16$^+$ NK thymocyte clone 2E11	75	0	1‡	3§	0	0	+	ND
TCR$\gamma\delta^+$ thymocyte clone 1G1	ND	100	0‡	0	1	100	+	ND
TCR$\gamma\delta^+$ thymocyte clone 3E5	46	100	55‡	7	8	90	+	ND
TCR$\gamma\delta^+$ thymocyte clone 5F8	74	100	0‡	0	1	100	+(trace)	ND
Fresh CD3$^-$ CD4hi$^-$ CD8$^-$ TN thymocytes (6)	70	0	0‡	0	0	0	+	+
DU528 SCL TN cell line	100	0	0‡	16	0	0	+	ND
Fresh leukapheresis SCL TN cells no. 5	100	0	0‡	0	0	0	+	−
T-cell lymphoblastic leukemia leukapheresis cells no. 20	100	1.5	96†	1	1	ND	+	ND
Control cells								
A201 T-cell line	ND	100	0†	0	ND	ND	−	−
HSB-2 T-cell line	100	0	0†	0	0	0	−	−

Phenotyping data when more than one specimen were analyzed are mean values. ND, not done.

*Positive PCR means a signal was present in at least two primer pairs; positive RT means RT activity was 4-fold greater than that of control in culture supernatants.

†Numbers represent CD4hi$^+$ cells [i.e., those cells that showed a rightward shift >30 linear channels from control mean fluorescence channel (MFC)]. All remaining cells in the suspension were not shifted and were CD4$^-$.

‡Numbers represent CD4hi$^+$ cells (i.e., those cells that showed a rightward shift >30 linear channels from control MFC). All of the remaining cells in these suspensions were CD4lo$^+$ (i.e., were shifted from the control curve, but the shift was <30 linear channels).

§Number represents CD8hi$^+$ cells (i.e., those cells that showed a rightward shift >30 linear channels from control MFC). All of the remaining cells in this suspension were CD8lo$^+$ (i.e., were shifted out from the control curve but the shift was <30 linear channels).

of 0.02–0.10. The UF thymocytes used in these experiments were 60–90% CD4hi$^+$ (Table 1). In six of six experiments in which UF thymocytes exposed to HIV-1 were harvested at 24 and 48 hr, we documented the presence of HIV-1 DNA in these thymocytes by PCR (Fig. 1). UF thymocyte control cells exposed only to medium were negative for HIV-1. In addition, when suspensions of the A201 T-cell line that does not express CD4 were inoculated with HIV-1, there was no evidence of infection as determined by PCR (Fig. 1 and Table 1). Other aliquots of UF thymocytes and A201 cells were inoculated with either HIV-1 or control medium and placed in long-term tissue culture. In three of three experiments, RT activity was readily detectable in the supernatants of UF thymocytes exposed to HIV-1 (Table 1). In two separate experiments, CD4$^+$ CD8$^+$ DP thymocytes, which are thought to represent an intermediate stage in T-cell development in the inner thymic cortex, were incubated with HIV-1, and the presence of HIV-1 DNA was readily apparent when examined by PCR (Table 1 and Fig. 2). Similarly, clones 6G11 and 4F8, which are CD4$^+$ CD8$^+$ DP TCR$\alpha\beta^+$ thymocyte clones derived from TN thymocytes *in vitro* (21), were also easily infectable with HIV (Table 1, Figs. 2 and 3*C*). Other clones derived *in vitro* from TN thymocytes included clones 1E3 and 2E11 that are CD3$^-$, TCR$\alpha\beta^-$ CD4lo$^+$, and CD16$^+$ (i.e., NK phenotype) and three TCR$\gamma\delta^+$ clones (1G1, 3E5, and 5F8). Both CD16$^+$ NK-phenotype thymocyte clones and two of three TCR$\gamma\delta^+$ thymocyte clones expressed low levels of CD4 and were classified as CD4lo$^+$ (Fig. 3*D*). TCR$\gamma\delta$ clone 3E5 was CD4hi$^+$ (55%). As with other clones derived from TN thymocytes, these two NK-phenotype clones (1E3 and 2E11) and all three TCR$\gamma\delta^+$ thymocyte clones (1G1, 3E5, and 5F8) were also infectable with HIV (Table 1, Fig. 2).

Immature TN Thymocytes (CD7$^+$ CD3$^-$ CD4hi$^-$ CD8$^-$) Are Susceptible to Infection by HIV-1. To determine the susceptibility of early intrathymic T-cell precursors to infection by HIV-1, TN (CD7$^+$ CD3$^-$ CD4hi$^-$ CD8$^-$) thymocytes obtained by negative-selection panning of UF thymocytes were

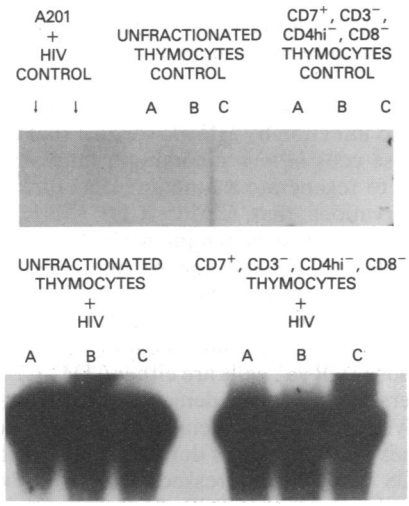

FIG. 1. PCR demonstrates that UF thymocytes (60–90% CD4hi$^+$) and CD7$^+$ CD3$^-$ CD4hi$^-$ CD8$^-$ TN thymocytes (0% CD4hi$^+$), but not a T-cell line lacking CD4 (A201), are susceptible to infection by HIV-1. Hybridization autoradiographs (24-hr exposure) are shown from HIV-1 DNA PCR performed on three sets of UF thymocytes (A, B, and C), three sets of TN thymocytes (A, B, and C), and A201 cells that were inoculated with either medium (*Upper*) or HIV-1 viral supernatant (*Lower*) and harvested at 48 hr. The amplifications shown were performed with the SK101/145 (*gag*) primer pair and probed with SK102. Comparable results were obtained with the SK68/69 (*env*) and SK38/39 (*gag*) primer pairs and in experiments performed on three additional sets of UF and TN thymocytes.

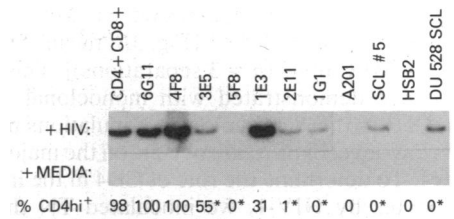

FIG. 2. Susceptibility to infection by HIV-1 of other intrathymic T-cell precursors. Forty-eight hours after inoculation with HIV-1 or control medium, PCR was performed on thymocytes representing various stages in T-cell development, including CD4$^+$ CD8$^+$ DP thymocytes; TN thymocyte-derived DP clones 6G11 and 4F8 (CD4$^+$ CD8$^+$); NK phenotype clones 1E3 and 2E11 (CD3$^-$, CD4lo$^+$, CD16$^+$); TCR$\gamma\delta^+$ clones 3E5, 5F8, and 1G1; TN cell line DU528 SCL; SCL no. 5 leukopheresis cells; a mature T-cell line lacking CD4 (A201); and an immature T-cell precursor line (HSB2) lacking CD4. Amplifications were performed with SK145/101 (*gag*) primers and probed with SK102. Hybridization autoradiographs are shown for 6-hr exposure. The % CD4hi$^+$ for each cell type is shown in the bottom row, where asterisks signify remaining cells were CD4lo$^+$. Complete phenotypes are shown in Table 1.

inoculated with HIV-1 viral supernatant (or control medium) as described. Phenotypic analysis of the TN thymocytes demonstrated that they were strongly CD7$^+$ (>60–90%), CD3$^-$, CD4hi$^-$, and CD8$^-$ (Table 1, Fig. 3*B*) and remain CD4hi$^-$ during the 48 hr of culture. In six of six experiments in which the TN thymocytes exposed to HIV-1 were harvested at 24 and 48 hr, we demonstrated the presence of HIV-1 DNA by PCR (Fig. 1). TN thymocyte control cells exposed only to medium were negative for HIV-1. Other aliquots of TN thymocytes were inoculated with either HIV-1 or control medium and placed in long-term tissue culture. In three of three experiments, RT activity was detected in the TN thymocytes exposed to HIV-1 (Table 1).

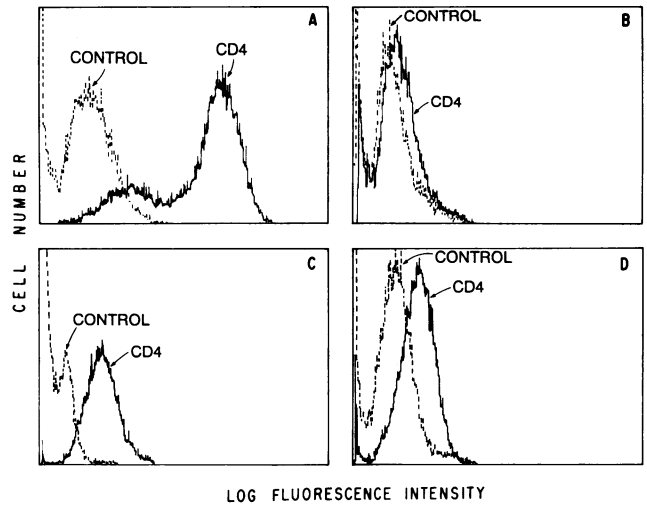

FIG. 3. Flow cytometric analysis of total unfractionated thymocytes and CD3$^-$ CD4hi$^-$ CD8$^-$ TN thymocytes and TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ progeny of TN thymocytes to determine expression of CD4. (*A* and *B*) Total unfractionated thymocytes (*A*) and CD3$^-$ CD4hi$^-$ CD8$^-$ TN thymocytes (*B*) were prepared as described and analyzed for cell surface CD4 expression. Clones of progeny of TN thymocytes were grown as described. (*C* and *D*) CD4 expression of the TCR$\alpha\beta^+$ DP clone 4F8 (*C*) and CD4lo$^+$ expression of CD4 on the TCR$\gamma\delta^+$ T-cell clone 5F8 (*D*). Cells were stained with isotype control or CD4 (Leu-3a) and analyzed by flow cytometry (10,000 cells counted). In each panel, the solid line is CD4 expression, while the broken line is the background control. The small rightward shift in MCF with anti-CD4 compared with control indicates the low level of CD4 expression present on TN thymocytes (*B*) and on the TCR$\gamma\delta^+$ T-cell clone 5F8 (*D*).

Although 100% of TN thymocytes were CD4hi$^-$, a majority of TN thymocytes were CD4lo$^+$ [Fig. 3*B*, mean shift ± SD in MFC = 6 ± 4 MFC units (n = 3 separations)]. This low level of fluorescence demonstrated with monoclonal antibodies (mAbs) to CD4 in the TN thymocyte populations most likely represents low-level expression of CD4 on the majority of TN thymocytes. To determine the role of CD4 in the infection of TN thymocytes by HIV-1, we inoculated TN thymocytes with HIV-1 viral supernatant following preincubation with either control medium, anti-CD3 (Leu-4) mAb, or anti-CD4(T4a) (Leu-3a) mAb. In three experiments, TN thymocytes that were incubated with either control medium or anti-CD3 mAb prior to exposure to HIV-1 demonstrated the expected positive PCR signals when harvested at 48 hr (Fig. 4). In contrast, TN thymocytes that were incubated with anti-CD4(T4a) mAb demonstrated a greatly diminished-to-absent PCR signal. These experiments, which show that anti-CD4(T4a) mAb is capable of blocking HIV-1 infection of TN thymocytes, strongly suggest that HIV-1 infection of TN thymocytes is mediated via the CD4 molecule.

Ability to Infect Malignant TN T-Cell Precursor Populations with HIV. A TN cell line is available (DU528 SCL line) that is clonal and is multipotent with T, B, myeloid, and erythroid lineage differentiation capabilities (23, 26). The DU528 cell line was derived from a patient with SCL whose malignancy arose from TN thymocytes (23, 26). In addition, leukopheresis cells from a second patient with SCL (SCL no. 5) that were TN were also available for study (24). Similar to the TN thymocytes described above, these TN SCL cells demonstrated a small shift in MFC by flow cytometry, consistent with low-level expression of CD4 (Table 1). Thus, we determined if these CD4lo$^+$ SCL cells could be infected with HIV. As shown by PCR in Table 1 and Fig. 2, both the DU528 and SCL no. 5 leukophoresis fresh cells could be infected with HIV. However, when supernatant RT activity was determined on SCL no. 5 cells, none was detected, undoubtedly because of the higher sensitivity of PCR compared with virus isolation procedures. As with cocultures of HIV and normal TN thymocytes, HIV infectivity of the DU528 SCL cell line was inhibited by anti-CD4(T4a) mAb (data not shown). Thus, these malignant TN precursors of the T lineage could indeed be infected by HIV via the CD4 molecule.

DISCUSSION

In the present study, we have demonstrated that intrathymic T-cell precursors representing many stages and subsets of the T-cell lineage are susceptible to infection by HIV-1. Not surprisingly, precursors representing stages of T-cell differentiation that abundantly express the CD4 molecule on their surface, such as DP (CD4$^+$ CD8$^+$) thymocytes and the more mature single-positive (SP) (CD4$^+$ and CD8$^-$) thymocytes, were readily infected by HIV. Of interest is that we have determined that cells within the immature TN (CD7$^+$ CD3$^-$ CD4hi$^-$ CD8$^-$) thymocyte pool express sufficient CD4 molecules to become infected by HIV. That this infection was mediated via the CD4 molecule was supported by the ability of mAb directed against the HIV binding site on CD4 (T4a) to block infection of TN thymocytes. Because of the high affinity of the HIV envelope protein gp120 for the CD4 molecule (K_d, 10^{-9} M) (27, 28), the ability of HIV to bind to and infect a given cell is an extremely sensitive means of detecting CD4 on the cell surface. Combining this avid interaction with a highly sensitive method for detecting HIV infection—namely, PCR—one can detect the presence of very small numbers of infected cells (1 in 10^5–10^6) that express very low levels of CD4 (13, 25, 29, 30). To provide additional evidence that precursors of the T lineage are capable of being infected with HIV, we determined if HIV could infect CD7$^+$ TN malignant cells that arose from the earliest stages of human T-cell development (refs. 23, 24, 26; reviewed in ref. 31). Although they are CD4hi$^-$, these two clonal SCL populations could also be infected with HIV. Finally, NK-phenotype and TCR$\gamma\delta^+$ clones derived from CD7$^+$ TN thymocytes *in vitro* expressed variable levels of CD4 and were infectable with HIV.

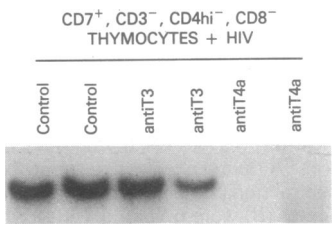

FIG. 4. Anti-CD4(T4a) mAb is capable of blocking infection of CD3$^-$ CD4hi$^-$ CD8$^-$ TN thymocytes by HIV-1. PCR was performed on TN thymocytes that were inoculated with HIV-1 after preincubation with either control medium, anti-CD3 (Leu-4) mAb, or anti-CD4 (T4a; Leu-3a) mAb. Anti-CD4(T4a), but not anti-CD3(T3) or control medium, was capable of blocking HIV-1 infection of the TN thymocytes as shown by the significantly diminished PCR signal with anti-CD4(T4a). The amplifications were performed with SK145/101 (*gag*) primers and SK102 probe.

These findings are of particular interest in delineating the mechanisms of immunopathogenesis of HIV infection. The hallmark of infection by HIV is depletion of the CD4$^+$ T lymphocyte (7–11). Despite the clear-cut association between HIV-1 infection and the development of AIDS, the precise mechanisms whereby HIV-1 contributes to the pathogenesis of HIV-1-associated immunodeficiency are unclear at present (10). Given the relatively low frequency of HIV-1-infected cells, particularly early in the course of infection as determined by *in situ* hybridization, immunofluorescence, and PCR (12, 13, 25), a variety of hypotheses other than the direct effects of HIV-1 infection of mature CD4$^+$ T cells have been proposed to explain the profound immunodeficiency these patients ultimately develop (14). One mechanism proposed for attrition of CD4$^+$ T cells involves the infection of early T-lymphocyte progenitors, which could lead to failure to regenerate mature functioning CD4$^+$ T cells (10). Indeed, the findings in the present study, which show that intrathymic T-cell precursors at many stages of T-cell ontogeny are susceptible to infection by HIV-1, suggest that infection and death of these cells *in vivo* could contribute significantly to the inability to regenerate a functional, mature T-cell pool.

The observations that CD16$^+$ CD3$^-$ (NK) and CD3$^+$ TCR$\gamma\delta^+$ clones derived from human TN thymocytes express variable levels of CD4 and can be infected with HIV are noteworthy. In a separate study, 32 of 33 TCR$\gamma\delta^+$ clones generated by limiting dilution from TN thymocytes were CD4lo$^+$, and 1 was a CD4hi$^+$ TCR$\gamma\delta^+$ T-cell clone (22). Although most TCR$\gamma\delta^+$ cells are either CD4$^-$ CD8$^+$ or CD4$^-$ CD8$^-$, others have recently demonstrated CD4hi$^+$ TCR$\gamma\delta^+$ clones derived from human fetal liver (32). In contrast, we found that all TCR$\gamma\delta^+$ clones derived from TN thymocytes, whether CD8$^+$ or CD8$^-$, expressed at least low levels of CD4. We show in this paper that TCR$\gamma\delta^+$ thymocytes and CD16$^+$ thymocytes are infectable with HIV. While the significance of these observations for T-cell maturation in HIV infection is as yet unknown, the recent suggestion that TCR$\gamma\delta^+$ thymocytes might play an inductive role in TCR$\alpha\beta^+$ lineage development in the thymus may be relevant to this issue (reviewed in ref. 33). HIV infection of certain TCR$\gamma\delta^+$ cells could prevent the normal inductive effects of TCR$\gamma\delta^+$ thymocytes for the development of TCR$\alpha\beta^+$ T cells. Moreover, infection with HIV of NK cells and TCR$\gamma\delta^+$ cells that mediate non-major histocompatibility complex-restricted killing could lead to further deficiencies of normal immune surveillance mechanisms.

Considerable progress has been made in the last few years in understanding the developmental biology of T lymphocytes (34–38). In humans, two distinct lineages of mature T cells have been described, those bearing TCR$\alpha\beta^+$ and those bearing TCR$\gamma\delta^+$, with expression of TCR$\gamma\delta^+$ cells preceding the expression of TCR$\alpha\beta^+$ cells (reviewed in ref. 15). While it is generally believed that the earliest, most immature intrathymic T cell is of the phenotype CD7$^+$ CD3$^-$ CD4hi$^-$ CD8$^-$ (TN), the developmental pathway from this early stage of the mature T cell has been difficult to delineate (39). It is believed (reviewed in refs. 15 and 31) that CD7$^+$ stem cells begin to express CD3 (initially in the cytoplasm, eventually on the surface) close to the time of migration to the thymus. In the next step, the CD7$^+$ intrathymic T-cell precursors express CD2, and the CD7$^+$ CD2$^+$ CD3$^+$ cells give rise to either TCR$\gamma\delta^+$ or TCR$\alpha\beta^+$ T cells. The CD7$^+$ CD2$^+$ CD3$^+$ cells of the TCR$\alpha\beta^+$ lineage give rise to an intermediate DP CD4$^+$ CD8$^+$ stage that will eventually mature into either the SP CD4$^+$ CD8$^-$ or CD4$^-$ CD8$^+$ T-cell subset. The findings of our present study show that intrathymic T-cell precursors are susceptible to a CD4-mediated infection by HIV at various stages of development, and it appears there is sufficient expression of CD4 even on the TN thymocyte, to permit infection of some of these cells by HIV. This obviously has potentially important implications for understanding the immunopathogenesis of HIV infection. If a broad pool of T-cell precursors is capable of being infected by HIV, then it would be possible that such cells would die before giving rise to healthy, mature CD4$^+$ cell progeny.

Based on the T-cell maturation scheme described above, infection at the TN or DP thymocyte stage should result in depletion of both CD4$^+$ and CD8$^+$ SP T cells. Yet, mature CD8$^+$ T cells are relatively resistant to depletion in HIV-infected individuals. One explanation is that both CD4$^+$ and CD8$^+$ intrathymic T-cell precursors are susceptible to HIV, wheras only mature CD4$^+$ T cells are susceptible in the peripheral blood. This results in double jeopardy for CD4$^+$ T cells. Any explanation of these phenomena is confounded by the fact that the majority of DP thymocytes die *in vivo* even in the absence of HIV infection (reviewed in refs. 15 and 31). Furthermore, the above T-cell maturation pathway is still only hypothetical, particularly in view of the fact that CD4$^+$ CD3$^-$ as well as CD8$^+$ CD3$^-$ early thymocytes have recently been demonstrated to be progeny of TN thymocytes in man (ref. 22; reviewed in ref. 31). Clearly, further and more precise delineation of this maturational pathway is necessary before the full implications of early thymocyte infection with HIV can be fully appreciated. Certainly, our data as presented do not preclude the possibility that infection of T-cell precursors by HIV prior to the SP thymocyte stage could lead to selective inhibition only of CD4$^+$ T-cell development, due perhaps to a greater sensitivity of precursors of mature CD4$^+$ T cells to HIV infection. If this were the case, then mature CD4$^+$ T cells lacking self-regenerative capacities in either adults or children would gradually be depleted by natural attrition as well as by direct infection with HIV. The net effect of such a pathogenic process would be the progressive immunodeficiency characteristic of HIV infection.

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