

# Preferential infection of CD4<sup>+</sup> memory T cells by human immunodeficiency virus type 1: Evidence for a role in the selective T-cell functional defects observed in infected individuals

(polymerase chain reaction/flow cytometry/CD4<sup>+</sup> T-cell subpopulations/memory and naive T cells)

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**ABSTRACT** CD4<sup>+</sup> T cells of patients with AIDS exhibit a qualitative defect in their ability to respond to soluble antigen while their responses to mitogens remain normal. CD4<sup>+</sup> T cells can be broadly divided phenotypically into “naive” [CD45RA<sup>+</sup> (2H4<sup>+</sup>)] and “memory” [CD29<sup>+</sup> (4B4<sup>+</sup>) or CD45RO<sup>+</sup> (UCHL1<sup>+</sup>)] cell subpopulations, which represent distinct maturation stages. To determine the human immunodeficiency virus type 1 (HIV-1) infectability of memory and naive CD4<sup>+</sup> T-cell subsets *in vitro* and to determine the *in vivo* preference of HIV-1 in these subpopulations, we obtained highly purified CD4<sup>+</sup> T-cell subsets from normal and HIV-1-infected individuals and studied them by viral cultivation, quantitative polymerase chain reaction, and functional assays. Polymerase chain reaction studies demonstrated that the memory cell subset of CD4<sup>+</sup> T cells is preferentially infected (4- to 10-fold more than naive T cells) by HIV-1 *in vitro*, and these memory cells are the principal reservoir for HIV-1 within CD4<sup>+</sup> T cells obtained from infected individuals. Functional abnormalities attributable to CD4<sup>+</sup> T cells in HIV-infected individuals (failure to respond *in vitro* to soluble antigen or to anti-CD3 monoclonal antibodies) were shown to reside primarily within these memory cells. Thus, the present study suggests that the selective functional defects present in the memory CD4<sup>+</sup> T-cell subset of HIV-infected individuals may be a direct result of the preferential infection and consequently greater viral burden within these cells.

The human immunodeficiency virus (HIV), the etiologic agent of AIDS, selectively infects cells expressing the CD4 surface molecule, particularly T lymphocytes as well as cells of the monocyte/macrophage lineage (1–4). As a consequence of infection with HIV-1, CD4<sup>+</sup> T cells demonstrate substantial quantitative and qualitative defects (reviewed in ref. 5). We have previously demonstrated that purified CD4<sup>+</sup> T lymphocytes of patients with AIDS respond normally to mitogen and are capable of proliferating in response to alloantigen; however, they exhibit a selective qualitative defect in the ability to respond to soluble antigen (6). A further delineation of this specific defect would be extremely useful in understanding the immunopathogenesis of HIV infection.

It has been shown that CD4<sup>+</sup> T cells can be broadly divided into “naive” and “memory” cell populations, phenotypically defined by reaction with monoclonal antibodies (mAbs) directed against certain cell surface molecules as the CD45RA<sup>+</sup> (2H4<sup>+</sup>) and CD29 (4B4<sup>+</sup>)/CD45RO<sup>+</sup> (UCHL1<sup>+</sup>) subsets, respectively (refs. 7–11, reviewed in ref. 12). These CD4<sup>+</sup> T-cell subsets demonstrate differential functional capabilities (7–19).

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In particular, the CD45RA<sup>+</sup> (naive) cells respond well to stimulation by mitogens [concanavalin A (Con A) and phytohemagglutinin (PHA)] as well as in autologous mixed lymphocyte reactions; however, they do not respond to recall antigens, but they do induce suppression of B-cell immunoglobulin production. In contrast, the CD29<sup>+</sup>/CD45RO<sup>+</sup> (memory) cell subset of CD4<sup>+</sup> T cells respond well to stimulation by recall antigens and to mAbs to the CD2 and CD3 antigens as well as provide B-cell help for immunoglobulin production. The role of these CD4 subsets in the pathogenesis of HIV infection has not been extensively investigated.

We have recently demonstrated that the CD4<sup>+</sup> T cell is the predominant cell harboring HIV-1 in the peripheral blood of infected individuals (20, 21). This was determined both for cells actively expressing virus as well as cells containing latent HIV-1 proviral DNA. In the present study, we have determined that the memory (CD29<sup>+</sup>/CD45RO<sup>+</sup>) cell subset of CD4<sup>+</sup> T cells is preferentially infected by HIV-1 *in vitro* and that these cells are the principal *in vivo* reservoir for HIV-1 within the CD4<sup>+</sup> T-cell population of infected individuals. Furthermore, we demonstrate that the functional abnormalities attributable to CD4<sup>+</sup> T cells in infected individuals reside within the population of cells preferentially infected by HIV—namely, the memory cell subset of CD4<sup>+</sup> T cells.

## MATERIALS AND METHODS

**Cell Fractionation.** Peripheral blood mononuclear cells (PBMCs) from both normal donors and HIV-1-seropositive individuals were separated by Ficoll/Hypaque centrifugation, washed in 5% fetal calf serum in RPMI 1640, and incubated on ice with fluorescent-labeled mAbs. For two-color cell sorting, PBMCs were stained with anti-CD4 [anti-Leu-3a labeled with fluorescein isothiocyanate (FITC) or phycoerythrin (PE); Becton Dickinson] followed by staining with (i) anti-CD45RO (anti-UCHL1 labeled with FITC; Dako Carpinteria, CA) or anti-CD29 (anti-4B4 labeled with FITC or PE; Coulter), (ii) anti-CD45RA (anti-2H4 labeled with FITC or PE; Coulter), or (iii) anti-CD44 (clone A3D8, a gift from Barton Haynes, Duke University Medical Center, Durham, NC). Fluorescent-labeled PBMCs were sorted using an EPICS C flow cytometer (Coulter) as described (20). Purities for most two-color sortings were 96–99%.

Abbreviations: HIV, human immunodeficiency virus; Con A, concanavalin A; PBMCs, peripheral blood mononuclear cells; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PHA, phytohemagglutinin; RT, reverse transcriptase; SIV, simian immunodeficiency virus; PCR, polymerase chain reaction; mAbs, monoclonal antibodies; IL-2, interleukin 2; LTR, long terminal repeat.

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**Polymerase Chain Reaction (PCR).** Freshly sorted cells that were to be examined for HIV-1 DNA were prepared and assayed by PCR as previously described (20, 21). Primer pairs used in these experiments included SK 68/69 (*env*), SK 38/39 (*gag*) or SK 145/101 (*gag*), SK 29/30 [long terminal repeat (LTR)], and QH 26/27 (HLA-DQa control) (Synthetic Genetics, San Diego). Amplified products were hybridized to <sup>32</sup>P-labeled ATP end-labeled probes (SK 70, SK 19 or SK 102, and SK 31 for the *env*, *gag*, and LTR primers, respectively) and analyzed on 10% polyacrylamide gels. Autoradiograms (Kodak) of the gels were obtained at various intervals.

**Quantification of Virus.** To determine the frequency of either integrated or unintegrated HIV-1 DNA in the sorted CD4<sup>+</sup> T-cell subsets, PCR was performed on serial dilutions of the purified cell populations from the individual donors. Dilutions were performed using Jurkat T cells as fillers, such that every amplification had the DNA equivalent of 10<sup>5</sup> cells. These amplifications were then compared with the PCR performed on serial dilutions of the ACH2 cell line, a chronically infected T-cell line containing one DNA copy of HIV per cell (22). We have previously demonstrated that this cell line could be used as a standard to quantify, by PCR, proviral DNA in cellular suspensions (20, 21).

**In Vitro Infections with HIV-1.** In some experiments, freshly isolated sort-purified CD4<sup>+</sup> T-cell subsets, as well as unfractionated PBMCs (derived from normal donors), were placed in 10% fetal calf serum in RPMI 1640 at 10<sup>6</sup> cells per ml, and aliquots of cells were either inoculated with HIV-1 (strain LAV) at multiplicities of infection of 0.02–0.10 or mock-infected as controls. As additional controls, cell lines lacking CD4 (A201) were also inoculated with LAV. Virus-inoculated cells and control cells were harvested at both 24 and 48 hr after inoculation, washed five times in phosphate-buffered saline, and centrifuged to form a pellet. Cells were then lysed to obtain DNA and prepared for PCR as described above. In other experiments, aliquots of unfractionated PBMCs and sort-purified CD4<sup>+</sup> T-cell subsets that were either inoculated with LAV or mock-infected were placed in culture as described (20), and supernatants were sampled for reverse transcriptase (RT) activity. The RT assay used for these experiments was carried out as described (20).

**Culturing of Virus.** Aliquots of unfractionated PBMCs and sort-purified CD4<sup>+</sup> T-cell subsets derived from HIV-infected individuals were cocultivated with 3-day PHA-stimulated PBMCs derived from normal donors, and supernatants were sampled for RT activity.

**Functional Studies of CD4<sup>+</sup> T-Cell Subsets.** Aliquots of freshly isolated unfractionated PBMCs and sort-purified CD4<sup>+</sup> T-cell subsets derived from both normal controls and HIV-infected individuals were placed in culture media (15% human AB serum in RPMI 1640) in triplicate in 96-well plates at 10<sup>5</sup> cells per well to which one of the following was added: PHA (final concentration of 2 μg/ml; Wellcome), Con A (final concentration of 100 μg/ml; Calbiochem), tetanus toxoid (final concentration of 10 μg/ml; Eli Lilly), anti-CD3 monoclonal antibody (final concentration of 10 ng/ml, Ortho Diagnostics), or medium as a control. All wells contained 10<sup>5</sup> autologous feeder PBMCs irradiated with 4000 rad (40 grays). All cultures were performed in a final volume of 200 μl. At the end of 3 days (for wells containing PHA, Con A, and anti-CD3) or 6 days (for wells containing tetanus toxoid), each well was pulsed with 0.44 μCi (1 Ci = 31 GBq) of [<sup>3</sup>H]thymidine (New England Nuclear), incubated for 4 hr at 37°C in 5% CO<sub>2</sub>, and harvested onto glass-filter fiber strips, and the strips were assayed after placement in a liquid scintillation vial.

## RESULTS

### HIV-1 Preferentially Infects the Memory (CD29<sup>+</sup>/CD45-RO<sup>+</sup>) Cell Subset of CD4<sup>+</sup> T Cells *in Vitro*. Highly purified

subsets of CD4<sup>+</sup> T lymphocytes from normal donors, specifically cells of the memory (CD29<sup>+</sup> or CD45RO<sup>+</sup>) and naive (CD45RA<sup>+</sup>) phenotypes, were obtained by cell sorting. We then employed quantitative PCR to determine the presence of HIV-1 DNA in these subsets following *in vitro* infection. We determined that CD4<sup>+</sup> memory T cells contain more HIV DNA than equal numbers of CD4<sup>+</sup> naive T cells 24 and 48 hr after *in vitro* infection with HIV-1 (strain LAV) (Fig. 1A). The higher levels of DNA found in the *in vitro*-infected CD4<sup>+</sup> memory T cells compared to the CD4<sup>+</sup> naive T cells represents preferential infection and not preferential viral replication as there is no evidence of viral replication in these cells at 24 and 48 hr when examined for RT activity or cytopathic effects. As controls, A201 cells (a T-cell line lacking CD4) were exposed to LAV with no evidence of infection by PCR. To quantitate the differences in HIV DNA more precisely, we performed PCR on serial dilutions of the purified cell populations. We determined that following *in vitro* infection CD4<sup>+</sup> memory T cells contain 4- to 10-fold more HIV DNA than CD4<sup>+</sup> naive T cells (Fig. 1B). The ACH2 cell line, which contains a single proviral copy of HIV per cell, was used as a standard for serial dilutions (Fig. 1C). Similar results were obtained with purified cells obtained by sorting normal PBMCs with each of several mAbs that generally distinguish between memory and naive CD4<sup>+</sup> T cells, including CD29, CD45RA, and CD45RO (data not shown). However, when the purified subsets of CD4<sup>+</sup> T cells were infected with HIV-1 and placed in culture with PHA and interleukin 2 (IL-2) for up to 7 days, no significant difference in viral expression (as measured by RT activity) was seen among the populations (data not shown). The comparable levels of viral expression observed in the CD4<sup>+</sup> T-cell subsets were not surprising as it

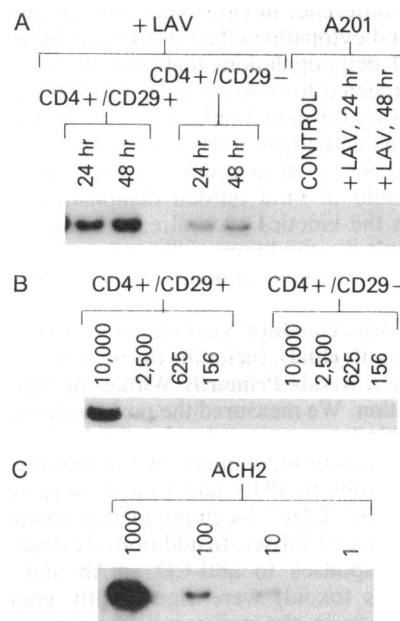


FIG. 1. Oligomer hybridization autoradiographs of amplified HIV-1 (LAV) DNA from *in vitro*-infected memory (CD29<sup>+</sup>) and naive (CD29<sup>-</sup>) CD4<sup>+</sup> T cells. (A) Depiction of the greater intensity of the PCR signals in 10<sup>5</sup> CD4<sup>+</sup>/CD29<sup>+</sup> cells compared to 10<sup>5</sup> CD4<sup>+</sup>/CD29<sup>-</sup> cells at both 24 and 48 hr after infection (utilizing SK 68/69 *env* primers with the SK 70 probe). A201 control cells (lacking CD4) exposed to LAV show no evidence of infection by PCR. (B) Comparison of the viral burden in *in vitro*-infected CD4<sup>+</sup>/CD29<sup>+</sup> cells and CD4<sup>+</sup>/CD29<sup>-</sup> cells analyzed by PCR of serial dilutions of the purified cell populations. These signals are compared to the signals obtained in serial dilutions of the ACH2 standard (cell line containing a single proviral copy of HIV per cell) shown in C. Comparable results were obtained utilizing *gag* and LTR primer pairs and probe.

has been previously demonstrated that stimulation of naive cells (e.g., by PHA) in culture will activate and irreversibly convert them to memory cells (23, 24). In addition, the culture conditions maximize viral expression in any infected cells. Thus, determination and quantitation of HIV-1 DNA by use of PCR shortly after infection has the advantage of stable cell phenotypes that are not affected by activation.

**The Principal Reservoir for HIV-1 Within CD4<sup>+</sup> T Cells Obtained from Infected Individuals Is the Memory Cell Subpopulation.** We obtained highly purified memory and naive cell subsets of CD4<sup>+</sup> T lymphocytes from nine HIV-infected individuals (two with AIDS and seven seropositives) by cell sorting. We performed PCR on equal numbers of their CD4<sup>+</sup> memory and naive T cells and determined that CD4<sup>+</sup> memory T cells were preferentially infected (Fig. 2A). The higher levels of HIV DNA found in the CD4<sup>+</sup> memory T cells compared to the CD4<sup>+</sup> naive T cells from infected individuals most likely represents preferential infection rather than preferential viral replication for several reasons. First, viral replication in CD4<sup>+</sup> T cells is closely associated with cell death (reviewed in ref. 5). In addition, we and others have previously shown that peripheral blood CD4<sup>+</sup> T cells contain one or at most a few HIV DNA copies per infected cell (20, 25). We quantitated the differences in HIV DNA in CD4<sup>+</sup> T-cell subsets by performing PCR on serial dilutions of the purified cell subsets and detected from 4- to >10-fold more HIV-1 DNA within the CD4<sup>+</sup> memory T cells as compared to the CD4<sup>+</sup> naive T cells (Fig. 2B). Similar results were obtained with purified cells obtained by sorting with each of several mAbs that distinguish between memory and naive CD4<sup>+</sup> T cells (CD29, CD45RA, and CD45RO). However, when the purified subsets of CD4<sup>+</sup> T cells obtained from infected individuals were placed in coculture in the presence of normal PHA blasts and IL-2, we noted no significant difference in viral expression (as determined by RT activity and cytopathic effects) between the memory and naive CD4<sup>+</sup> T-cell populations (data not shown). Again, this was not unexpected for two reasons. First, the culture conditions necessary for induction of viral expression (PHA and IL-2) cause cell activation maximizing viral expression in all infected cells, such that in coculture, even cell populations differing 100-fold in viral burden demonstrate only minor differences in the kinetics of viral expression (20). Second, these same culture conditions will activate naive cells and irreversibly convert them to memory cells within 7–14 days (23, 24).

**Functional Abnormalities Attributable to CD4<sup>+</sup> T Cells in HIV-Infected Individuals, Including the Inability to Respond to Soluble Antigen, Reside Primarily Within the Memory CD4<sup>+</sup> T-Cell Population.** We measured the proliferative capabilities of highly purified memory and naive CD4<sup>+</sup> T cells derived from normal, healthy individuals. We determined that proliferative responses to PHA and Con A were significantly greater in the naive CD4<sup>+</sup> T-cell population compared to the memory CD4<sup>+</sup> T-cell subset. In addition, we determined that proliferative responses to anti-CD3 mAb and to soluble antigen (tetanus toxoid) were significantly greater in the memory cell than in the naive cell population (Fig. 3A). Similar results were seen for three additional normal individuals whose PBMCs were sorted with either CD29, CD45RA, or CD45RO as the secondary antibody. These observations essentially confirm the findings of other investigators (7–19). In highly purified CD4<sup>+</sup> T-cell subpopulations derived from HIV-infected individuals, we determined that the naive CD4<sup>+</sup> T-cell subset proliferated normally in response to PHA and Con A. However, proliferation of the memory CD4<sup>+</sup> T-cell subset in response to anti-CD3 mAb and to tetanus toxoid was severely impaired (Fig. 3B). Comparable results were seen in three additional seropositive individuals as well as in two AIDS patients, sorted with either CD29, CD45RA, or CD45RO as the secondary antibody. This

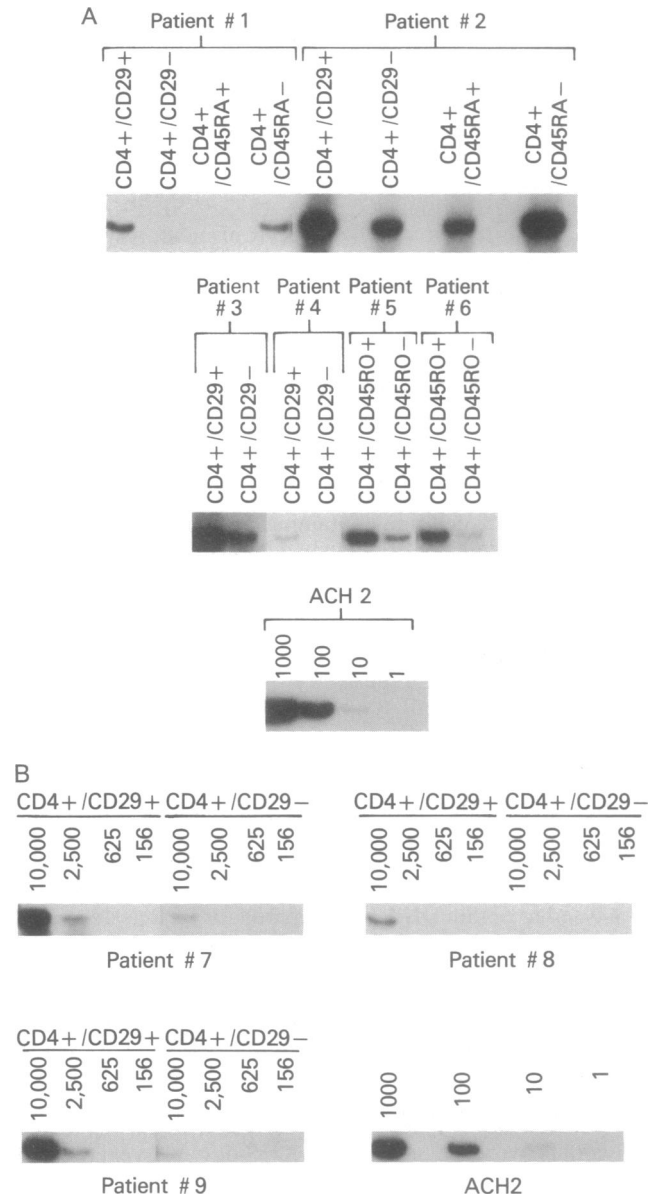


FIG. 2. (A) Oligomer hybridization autoradiographs of amplified HIV-1 DNA from sort-purified memory (CD29<sup>+</sup>, CD45RA<sup>-</sup>, or CD45RO<sup>+</sup>) and naive (CD29<sup>-</sup>, CD45RA<sup>+</sup>, or CD45RO<sup>-</sup>) CD4<sup>+</sup> T cells in six HIV-infected individuals. The results for patients 1–6 demonstrate the greater intensity of the PCR signals in memory compared to naive CD4<sup>+</sup> T cells in the infected individuals (utilizing SK 68/69 *env* primers with the SK 70 probe). These signals may be compared to the signals obtained in serial dilutions of the ACH2 standard. Similar results were obtained utilizing the *gag* and LTR primer pairs and probe. (B) Oligomer hybridization autoradiographs of amplified HIV-1 DNA from serial dilutions of sort-purified CD4<sup>+</sup>/CD29<sup>+</sup> and CD4<sup>+</sup>/CD29<sup>-</sup> cells obtained from three additional infected individuals. These signals may be compared to the signals obtained from the ACH2 standard dilution.

inability of memory CD4<sup>+</sup> T cells derived from HIV-infected individuals to proliferate in response to soluble antigen is reflected in the inability of the total CD4<sup>+</sup> T-helper cell population to proliferate to antigen as has been demonstrated (6). It is noteworthy that the observed proliferative defects seen reside within the subset of CD4<sup>+</sup> T cells (memory cells) that carries the bulk of the HIV viral burden.

**Human CD4<sup>+</sup> T Cells Have a Unimodal Distribution of the CD44 Adhesion Glycoprotein, and These Cells Uniformly Contain HIV-1 DNA in Infected Individuals.** It has been demonstrated recently that macaques have a bimodal distri-

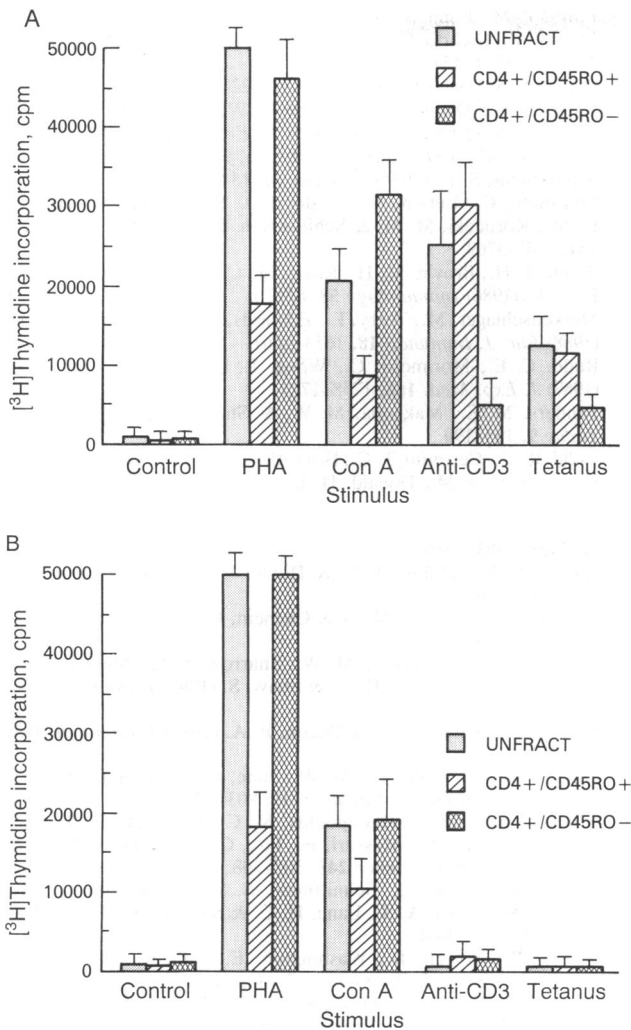


FIG. 3. CD4<sup>+</sup> T cells from HIV-infected individuals display functional abnormalities that reside primarily within the memory cell subpopulation. (A) In experiments using cells derived from normal individuals, the graph displays proliferative responses of unfractionated PBMCs (UNFRACT) and CD4<sup>+</sup>/CD45RO<sup>+</sup> and CD4<sup>+</sup>/CD45RO<sup>-</sup> cells as the [<sup>3</sup>H]thymidine incorporation (in cpm) per 100,000 cells for each of the stimuli (PHA, Con A, anti-CD3, and tetanus). (B) The proliferative responses of unfractionated PBMCs and CD4<sup>+</sup>/CD45RO<sup>+</sup> and CD4<sup>+</sup>/CD45RO<sup>-</sup> cells derived from HIV-1-infected individuals are shown as the [<sup>3</sup>H]thymidine incorporation (in cpm) per 100,000 cells for each of the stimuli.

tribution of the CD44 adhesion receptor on their CD4<sup>+</sup> T cells (26). Animals infected with simian immunodeficiency virus (SIV; strain Mne) demonstrate a selective loss of CD4<sup>+</sup> T cells expressing a high density of CD44 *in vivo*, and this subset of cells expresses significantly more virus in culture. In addition, macaque high-density CD44/CD4<sup>+</sup> T cells are much more susceptible to productive infection after exposure to SIV *in vitro* (27, 28). To determine whether this property of CD4<sup>+</sup> T cells is also present in humans, we obtained PBMCs from several normal and HIV-infected individuals, labeled the cells with fluorescent mAbs to CD4 and CD44, and performed flow cytometry. We observed that human CD4<sup>+</sup> T cells, unlike what is described for macaque cells, have a unimodal distribution of CD44 (analyzed by both logarithmic and linear scales). Despite this, we empirically sort-purified the cells into two portions, representing CD4<sup>+</sup>/CD44 bright and CD4<sup>+</sup>/CD44 dull subsets, and prepared the cells for PCR. We determined that the PCR signal intensities in the two subpopulations did not differ significantly (data not

shown), suggesting that in humans, there is uniform distribution of HIV-1 DNA in CD4<sup>+</sup>/CD44 T cells.

## DISCUSSION

In the present study, we have demonstrated that CD4<sup>+</sup> T cells, which are the reservoir for HIV in the peripheral blood of infected individuals (20, 21), are not uniformly infected. We have determined that within the CD4<sup>+</sup> T-cell population, the subset of cells phenotypically defined as the memory cell [CD29 (4B4<sup>+</sup>) or CD45RO<sup>+</sup> (UCHL1<sup>+</sup>)] population is the subset that is preferentially infected with HIV *in vivo*. We have also shown that this memory CD4<sup>+</sup> T-cell subset is likewise preferentially infected by HIV-1 *in vitro*. In addition, we have demonstrated that the selective qualitative defect in the ability to respond to soluble antigen seen in patients with HIV infection resides within the population of cells preferentially infected by HIV, specifically the memory cell subset of CD4<sup>+</sup> T cells.

The subsets of human peripheral blood CD4<sup>+</sup> T cells identified by mAbs to CD45RA (2H4<sup>+</sup>) and to CD29 (4B4<sup>+</sup>)/CD45RO (UCHL1<sup>+</sup>) have become the object of considerable research in both basic and clinical immunology. Initially described as "suppressor/inducer" and "helper/inducer" T-cell subsets (7, 8), recent observations suggest that this may be an oversimplification. It is now theorized that these subsets do not represent distinct lineages but in fact reflect different maturational stages (16). It has been shown that the CD45RA<sup>+</sup> subset of T cells converts to the phenotype of the CD29<sup>+</sup>/CD45RO<sup>+</sup> subset when activated with PHA (23, 24). This conversion is unidirectional and is associated with the increased expression of CD2 and lymphocyte function-associated antigens 1 and 3 (9, 17, 19, 23, 29, 30) surface molecules involved in intercellular adhesion. In addition, there are fundamental differences in the functional capabilities of these subsets, which are reflective of whether there has been prior exposure to antigen (7–19). Consequently, these subsets are now designated as either naive (CD45RA<sup>+</sup>) or memory (CD29<sup>+</sup> or CD45RO<sup>+</sup>) T cells.

Naive T cells have been shown to proliferate preferentially in response to nonspecific immunologic stimuli such as PHA and Con A, and to autologous non-T cells (7–19). Naive cells induce the suppression of B-cell immunoglobulin production and fail to respond to recall antigens such as tetanus toxoid. In contrast, memory T cells proliferate well in response to recall antigens and respond much better than naive cells to activation by mAbs to CD2 or CD3 receptors. Memory cells do provide B-cell help for immunoglobulin production. In addition, while both subsets produce significant quantities of IL-2 in response to PHA, the memory cells produce significant quantities of IL-3 and interferon  $\gamma$ .

In the present study, we have demonstrated that in individuals infected with HIV, the CD4<sup>+</sup> naive T cells generally function normally while the memory cells fail to respond to tetanus toxoid and anti-CD3 mAb. Therefore, it appears that the defect in the ability of CD4<sup>+</sup> T cells of AIDS patients to respond to soluble antigen resides within the memory cell population. It is significant that the memory subset of CD4<sup>+</sup> T cells is also the subset that is predominantly infected by HIV-1 *in vivo*. We have previously determined that the CD4<sup>+</sup> T cell is the reservoir for HIV-1 in the peripheral blood of infected individuals (20, 21). In addition, we have recently found that an increasing level of viral burden within CD4<sup>+</sup> T cells (as determined by quantitative PCR) coincided with a significant decline in CD4<sup>+</sup> T-cell number that was seen in patients with clinical deterioration (S.M.S., A.S.F., and H.C.L., unpublished data). Therefore, our present findings suggest that the functional defects present in the CD4<sup>+</sup> memory T-cell subset of infected individuals are due directly to the presence of a greater HIV viral burden within these cells.

The qualitative dysfunction and depletion of the CD4<sup>+</sup> memory T-cell population in HIV-infected individuals may also contribute directly to the increasing viral burden. Helper T cells play an important role in the induction of major histocompatibility complex-restricted cytotoxicity (reviewed in ref. 31), a function that clearly declines with progressive HIV disease (32–34). Therefore, an important consequence of CD4<sup>+</sup> memory T-cell failure in HIV infection may be the inability to induce cytotoxic T cells to kill virally infected targets.

Our present findings are also of note in light of recently reported experiments (26–28) in which it was shown that macaques infected with SIV selectively lose their high-density CD44/CD4<sup>+</sup> T-cell subset. In addition, it was demonstrated that these cells were markedly more susceptible to productive infection after exposure to SIV *in vitro*, compared to the low-density CD44/CD4<sup>+</sup> T-cell subset. It is apparent that the distribution of CD44 in humans differs greatly from macaques, and, not unexpectedly, we have demonstrated that HIV-1 infects human CD44 cells uniformly. Nonetheless, the separation of CD4<sup>+</sup> T cells in humans into the distinct functional and maturational subsets of naive and memory cells appears to have considerable relevance to understanding the pathogenesis of HIV-1 infection.

The increased susceptibility to HIV-1 of memory compared to naive CD4<sup>+</sup> T cells may have a physiologic explanation. Both the CD4 and CD45R molecules have been shown to possess enzymatic activity located on intracellular domains that can regulate levels of activation of T cells. Specifically, it has been shown that CD45RA can activate CD4 p56<sup>lck</sup> by dephosphorylation (35). It is also hypothesized that CD4 p56<sup>lck</sup> can phosphorylate CD3/T-cell receptor and CD45RO, leading to activation of memory T cells, whereas CD45RA may dephosphorylate CD3/T-cell receptor leading to down-regulation of naive T cells (reviewed in ref. 36). One may speculate that binding of HIV to CD4 on memory CD4<sup>+</sup> T cells can trigger CD4 p56<sup>lck</sup>, facilitating either enhanced viral uptake and/or cellular activation, resulting in the observed increased infectability of the memory cells.

Clinical studies of CD4<sup>+</sup> T-cell subpopulations have consistently failed to reveal any preferential loss of a given subset as the total CD4<sup>+</sup> T-cell number declines with progressive disease (37–39). One way our present findings can fit with these observations is that the high level of nonspecific activation seen in cells of HIV-infected individuals (40) may result in continuous conversion of naive CD4<sup>+</sup> T cells into memory CD4<sup>+</sup> T cells. In addition, any naive CD4<sup>+</sup> T cells that do become infected may also convert to memory CD4<sup>+</sup> T cells. This would then result in a gradual depletion of the naive cells. Concomitantly, depletion of HIV-infected memory CD4<sup>+</sup> T cells may occur following exposure to antigen, with subsequent activation, viral replication, and cell death. Ultimately, when a threshold level of memory CD4<sup>+</sup> T cells is destroyed, one would expect to see, as has been observed, a loss of ability to respond to recall antigens, resulting in the relentless progression of immune deficiency that characterizes HIV infection.

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