Differential pathogenesis of primary CCR5-using human immunodeficiency virus type 1 isolates in ex vivo human lymphoid tissue.

Karlsson, Ingrid; Grivel, Jean-Charles; Chen, Silvia Sihui; Karlsson, Anders; Albert, Jan; Fenyö, Eva Maria; Margolis, Leonid B

Published in:
Journal of Virology

DOI:
10.1128/JVI.79.17.11151-11160.2005

2005

Link to publication

Citation for published version (APA):
Karlsson, I., Grivel, J-C., Chen, S. S., Karlsson, A., Albert, J., Fenyö, E. M., & Margolis, L. B. (2005). Differential pathogenesis of primary CCR5-using human immunodeficiency virus type 1 isolates in ex vivo human lymphoid tissue. Journal of Virology, 79(17), 11151-11160. https://doi.org/10.1128/JVI.79.17.11151-11160.2005

Total number of authors:
7

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Differential Pathogenesis of Primary CCR5-Using Human Immunodeficiency Virus Type 1 Isolates in Ex Vivo Human Lymphoid Tissue

Ingrid Karlsson,1* Jean-Charles Grivel,2 Silvia Sihui Chen,2,3 Anders Karlsson,4 Jan Albert,5 Eva Maria Fenyö,1 and Leonid B. Margolis2

Unit of Virology, Division of Medical Microbiology, Department of Laboratory Medicine, Lund University, Lund, Sweden; Laboratory of Cellular and Molecular Biophysics and National Institute of Child Health and Human Development2 and NASA/NIH Center for Three-Dimensional Tissue Culture,3 National Institutes of Health, Bethesda, Maryland; Venhalsan, Karolinska University Hospital, Stockholm, Sweden; and Swedish Institute for Infectious Disease Control and Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, Sweden

Received 23 February 2005/Accepted 25 May 2005

In the course of human immunodeficiency virus (HIV) disease, CCR5-utilizing HIV type 1 (HIV-1) variants (R5), which typically transmit infection and dominate its early stages, persist in approximately half of the infected individuals (nonswitch virus patients), while in the other half (switch virus patients), viruses using CXCR4 (X4 or R5X4) emerge, leading to rapid disease progression. Here, we used a system of ex vivo tonsillar tissue to compare the pathogenesis of sequential primary R5 HIV-1 isolates from patients in these two categories. The absolute replicative capacities of HIV-1 isolates seemed to be controlled by tissue factors. In contrast, the replication level hierarchy among sequential isolates and the levels of CCR5+ CD4+ T-cell depletion caused by the R5 isolates seemed to be controlled by viral factors. R5 viruses isolated from nonswitch virus patients depleted more target cells than R5 viruses isolated from switch virus patients. The high depletion of CCR5+ cells by HIV-1 isolates from nonswitch virus patients may explain the steady decline of CD4+ T cells in patients with continuous dominance of R5 HIV-1. The level of R5 pathogenicity, as measured in ex vivo lymphoid tissue, may have a predictive value reflecting whether, in an infected individual, X4 HIV-1 will eventually dominate.

Transmitted human immunodeficiency virus type 1 (HIV-1) variants almost exclusively use CCR5 for viral entry, and these viruses (R5 variants) also predominate in early stages of HIV-1 infection (2, 3, 58, 61). Later in the course of HIV-1 infection, viruses that use CXCR4 (X4 or R5X4) emerge, leading to rapid disease progression. Here, we used a system of ex vivo tonsillar tissue to compare the pathogeneses of sequential primary R5 HIV-1 isolates from patients in these two categories. The absolute replicative capacities of HIV-1 isolates seemed to be controlled by tissue factors. In contrast, the replication level hierarchy among sequential isolates and the levels of CCR5+ CD4+ T-cell depletion caused by the R5 isolates seemed to be controlled by viral factors. R5 viruses isolated from nonswitch virus patients depleted more target cells than R5 viruses isolated from switch virus patients. The high depletion of CCR5+ cells by HIV-1 isolates from nonswitch virus patients may explain the steady decline of CD4+ T cells in patients with continuous dominance of R5 HIV-1. The level of R5 pathogenicity, as measured in ex vivo lymphoid tissue, may have a predictive value reflecting whether, in an infected individual, X4 HIV-1 will eventually dominate.

* Corresponding author. Mailing address: Division of Medical Microbiology, Department of Laboratory Medicine, Lund University, Solvagatan 23, 223 62 Lund, Sweden. Phone: 46 46 173271. Fax: 46 46 176033. E-mail: Ingrid.Karlsson@mmb.lu.se.

MATERIALS AND METHODS

Patients and virus isolates. The five patients studied here were selected from a cohort of 23 HIV-1-infected individuals described earlier (28–30). The patients were adult homosexual or bisexual men living in Sweden with a median follow-up of 103 months. This follow-up included CD4 counts, viral isolations, and (from 1996) measurement of plasma viral RNA load at South Hospital in Stockholm, Sweden. For the present study, patients and sequential isolates (those taken from the same patient at several time points throughout the course of disease) were selected on the basis of differences in the virus biological phenotypes as assayed by
We prepared virus stocks by infecting 6 studied the evolutionary relationship between virus isolates from the same pa-
infection in both switch virus patients. from patients 2112 and 2242 were obtained between 15 and 64 months postin-
at 23 and 18 months after infection, respectively (Table 1). The studied isolates tively) during the follow-up period, and the first clinical symptom appeared early, within 5 h of excision and was sectioned into 2- to 3-mm blocks. These tissue in culture medium bathing 27 or 54 tissue blocks in three or six wells during the

Nonswitch

Switch

Patient no. 435 1047 1838 2112 2242 3700
Isolate no. 1577 3415 5379 171 1886 64
Time of isolation (mo from infection) 67 41 85 15 45 64
CD4⁺ T-cell count (10⁶ cells/liter) at time of isolation 510 630 410 340 150 213
Antiretroviral therapy at time of isolation -5.7 -4.6 -2.9 -4.8 -5.2 33–84
Loss of CD4⁺ T cells (10⁶ cells/liter/mo) 62–89 48–62 35–140 31–68 33–84
p24 in serum Neg/4 Neg/3 Pos/1 Pos/10 Pos/11
Follow-up (mo from infection) 99 71 131 23 18
Results/no. of tests
First appearance of clinical symptom (mo from infection) a

a Nonswitch, patients with virus that used only CCR5 throughout the study; Switch, patients with a detected switch to R5X4 virus.
b The infection date is calculated as the midpoint between the last negative and the first positive samples.
c AZT, zidovudine.
d HIV-1 antigen enzyme-linked immunosorbent assay (Abbott, Stockholm, Sweden). Neg, negative; Pos, positive.
e The first clinical symptom to appear in patient 435 was perianal herpes infection; in 1838 it was septic arthritis; in 1047 it was oral candidiasis; and in 2242 and 2112 it was persistent generalized lymphanaphody.

Nonswitch 435 1047 1838 Switch 2112 2242
Patient no. 1577 3415 5379 171 1886 64
Isolate no. 1577 3415 5379 171 1886 64
Time of isolation (mo from infection) 67 41 85 15 45 64
CD4⁺ T-cell count (10⁶ cells/liter) at time of isolation 510 630 410 340 150 213
Antiretroviral therapy at time of isolation -5.7 -4.6 -2.9 -4.8 -5.2 33–84
Loss of CD4⁺ T cells (10⁶ cells/liter/mo) 62–89 48–62 35–140 31–68 33–84
p24 in serum Neg/4 Neg/3 Pos/1 Pos/10 Pos/11
Follow-up (mo from infection) 99 71 131 23 18
Results/no. of tests
First appearance of clinical symptom (mo from infection) 

3 days between successive medium changes as a measure of virus replication. We terminated the experiments at day 12 to avoid tissue deterioration, which typically starts after 2 weeks and which may affect viral replication, as well as the quality of flow cytometry analysis.

Flow cytometry. Flow cytometry was performed on cells mechanically isolated from control and infected tissue blocks. Lymphocytes were first identified according to their light-scattering properties and then analyzed for expression of lymphocyte markers. For identification of CD3⁺, CD4⁺, CD8⁺, CD25⁺, CD69⁺, HLA-DR⁺, CCR5⁺, and CXCR4⁺ cells, cells were stained for surface markers with anti-CD3 fluorescein isothiocyanate or phycoerythrin (PE)-Cy7, anti-CD4 allophycocyanin (APC), anti-CD8 TriColor or APC-Cy7, anti-CD25 PE, or anti-

Viruses were isolated from peripheral blood mononuclear cells (PBMC) according to a standard procedure (46) and were passaged only twice in donor PBMC before coreceptor use of sequential isolates was determined (29). We studied the evolutionary relationship between virus isolates from the same patients using phylogenetic analysis of V3 sequences, as previously described (34). We prepared virus stocks by infecting 6 × 10⁶ to 8 × 10⁶ PBMC, which had been obtained from two healthy donors and activated for 3 days with phytohemagglu-
tinin (2.5 μg/ml; Boehrle, Stockholm, Sweden), with 1.5 ml of supernatant from patients’ infected PBMC in the presence of 2 μg/ml Polybrene (Sigma, Stock-
holm, Sweden). The cultures were maintained in RPMI (Invitrogen, Lidingö, Sweden) containing 10% fetal bovine serum (Invitrogen, Lidingö, Sweden), 50 U/ml penicillin (Invitrogen, Lidingö, Sweden), 50 μM streptomycin (Invitrogen, Lidingö, Sweden), and 10 μM interleukin-2 (IL-2; Sigma, Stockholm, Sweden). Supernatants were harvested on day 7 and on day 10 or 11 after infection and stored at −80°C.

HIV infection of human lymphoid tissue ex vivo. Human tonsil tissue removed during routine tonsillectomy and not required for clinical purposes was received within 5 h of excision and was sectioned into 2- to 3-mm blocks. These tissue blocks were placed on collagen sponge gels in culture medium at the air-liquid interface and infected the next day, as described earlier (16). Five microliters of virus, containing at least 10 ng/ml p24, were applied to the top of each tissue block. We assessed productive HIV infection by measuring p24 in the culture medium using an HIV-1 p24 antigen enzyme-linked immunosorbent assay (Beckman-Coulter, Miami, FL); we used the concentration of p24 accumulated in culture medium bathing 27 or 54 tissue blocks in three or six wells during the

| Patient category according to viral phenotype a | Patient no. | Isolate no. | Time of isolation (mo from infection) | CD4⁺ T-cell count (10⁶ cells/liter) at time of isolation | Antiretroviral therapy at time of isolation | Loss of CD4⁺ T cells (10⁶ cells/liter/mo) | p24 in serum | Follow-up (mo from infection) | Results/no. of tests | First appearance of clinical symptom (mo from infection) |
|-----------------------------------------------|-------------|-------------|-------------------------------------|---------------------------------------------------------------|---------------------------------------------|-------------------------------------------|-------------|--------------------------------|-----------------|-----------------------------------------------|
| Nonswitch                                     | 435         | 1577        | 67                                  | 510                                                            | −5.7                                        | 62–89                                      | Neg/4       | 99                             |                               |                                |
| 1047                                          | 3415        | 87          |                                     | 290                                                            | −4.6                                        | 48–62                                      | Neg/3       | 71                             |                               |                                |
| 1838                                          | 5379        | 85          |                                     | 410                                                            | −2.9                                        | 35–140                                     | Neg/6       | 131                           |                               |                                |
| Switch                                        | 2112        | 171         | 15                                  | 340                                                            | −4.8                                        | 31–68                                      | Pos/10      | 23                             |                               |                                |
| 2242                                          | 1886        | 45          |                                     | 180                                                            | −5.2                                        | 33–84                                      | Pos/11      | 18                             |                               |                                |

a Nonswitch, patients with virus that used only CCR5 throughout the study; Switch, patients with a detected switch to R5X4 virus.
b The infection date is calculated as the midpoint between the last negative and the first positive samples.
c AZT, zidovudine.
d HIV-1 antigen enzyme-linked immunosorbent assay (Abbott, Stockholm, Sweden). Neg, negative; Pos, positive.
e The first clinical symptom to appear in patient 435 was perianal herpes infection; in 1838 it was septic arthritis; in 1047 it was oral candidiasis; and in 2242 and 2112 it was persistent generalized lymphadenopathy.

**Table 1. Characteristics of patients and isolates**

| Patient category according to viral phenotype | Patient no. | Isolate no. | Time of isolation (mo from infection) | CD4⁺ T-cell count (10⁶ cells/liter) at time of isolation | Antiretroviral therapy at time of isolation | Loss of CD4⁺ T cells (10⁶ cells/liter/mo) | p24 in serum | Follow-up (mo from infection) | Results/no. of tests | First appearance of clinical symptom (mo from infection) |
|-----------------------------------------------|-------------|-------------|-------------------------------------|---------------------------------------------------------------|---------------------------------------------|-------------------------------------------|-------------|--------------------------------|-----------------|-----------------------------------------------|
| Nonswitch                                     | 435         | 1577        | 67                                  | 510                                                            | −5.7                                        | 62–89                                      | Neg/4       | 99                             |                               |                                |
| 1047                                          | 3415        | 87          |                                     | 290                                                            | −4.6                                        | 48–62                                      | Neg/3       | 71                             |                               |                                |
| 1838                                          | 5379        | 85          |                                     | 410                                                            | −2.9                                        | 35–140                                     | Neg/6       | 131                           |                               |                                |
| Switch                                        | 2112        | 171         | 15                                  | 340                                                            | −4.8                                        | 31–68                                      | Pos/10      | 23                             |                               |                                |
| 2242                                          | 1886        | 45          |                                     | 180                                                            | −5.2                                        | 33–84                                      | Pos/11      | 18                             |                               |                                |

a Nonswitch, patients with virus that used only CCR5 throughout the study; Switch, patients with a detected switch to R5X4 virus.
b The infection date is calculated as the midpoint between the last negative and the first positive samples.
c AZT, zidovudine.
d HIV-1 antigen enzyme-linked immunosorbent assay (Abbott, Stockholm, Sweden). Neg, negative; Pos, positive.
e The first clinical symptom to appear in patient 435 was perianal herpes infection; in 1838 it was septic arthritis; in 1047 it was oral candidiasis; and in 2242 and 2112 it was persistent generalized lymphadenopathy.

RESULTS

Fourteen R5 HIV-1 isolates derived from five patients, including two or three sequential isolates from each patient,
were individually used to infect blocks of human tonsillar tissue obtained from multiple donors. All of these isolates were of R5 phenotype, as evaluated with U87.CD4 and GHOST (3) cell assays (29). The R5 phenotype was confirmed here from inhibition of their replication by the CCR5 ligand RANTES (100 nM) and from lack of inhibition by the CXCR4 ligand AMD3100 (1 μg/ml) (48; also data not shown). In two of the five patients, CXCR4-using HIV-1 evolved after the isolates used for the current study had been collected, whereas later isolates from the other patients remained R5.

In this study, we individually infected human lymphoid tissues ex vivo with all the isolates and monitored viral replication, cell loss, and the activation status of productively infected T cells. Out of the above-mentioned 14 isolates tested, 3 caused an unexplainable loss of tissue lymphocytes of various subsets, in sharp contrast with findings reported earlier (21, 41) regarding selective loss of CD4+ T cells in this ex vivo tissue system. We excluded these 3 isolates from further studies, and we report below on the behavior of 11 isolates tested in lymphoid tissue obtained from five donors.

**HIV-1 replication in human lymphoid tissue.** For tissue inoculation, viruses isolated from a given patient were adjusted to the same concentration of p24. The p24 concentration correlated well with the amount of infectious virus, as determined from 50% tissue culture infective dose titration on PBMC (data not shown). A representative experiment for each HIV-1 isolate is shown in Fig. 1, and the levels of replication by day 12 postinfection in five different tissues are shown in Table 2. Replication of these isolates, as monitored from the release of p24, became evident at day 6 postinfection and continued to increase during the course of the experiment, as reported earlier for other HIV-1 variants (16). The absolute levels of viral replication in tissue samples varied from donor to donor (see also reference 40). Because of the limited amount of material in each tissue sample, systematic comparison of isolates from switch and nonswitch virus patients could not be carried out. However, the replication capacities of isolates from one patient, tested in the same tissue, could be compared. We found, however, that the hierarchy of replication capacities of HIV isolates obtained from any one patient remained constant in lymphoid tissues from various donors. For example, in tissues from all tested donors, isolate 5379 from patient 1838 replicated to a higher level than isolate 8590 from the same patient. Also, from tissues from all tested donors, isolate 3700 from patient 2242 replicated to a higher level than isolate 1886 from the same patient. In both switch and nonswitch virus patients, the levels of CD3+ CD8− CCCR5+ cells of 57.2% ± 5.3%, 58.6% ± 4.1%, and 60.0% ± 6.4%, respectively (n = 8 to 10), R5 isolates from switch virus patients 2112 and 2242 depleted a smaller fraction of CD3+ CD8− CCCR5+ cells in ex vivo-infected lymphoid tissue: the three isolates from patient 2112 depleted on average none (∼4.5% ± 12.6%), 26.5% ± 6.7%, and 30.5% ± 6.4% (n = 4) of these cells relative to the matched uninfected controls, and the two tested isolates from patient 2242 depleted 15.8% ± 3.3% and 33.9% ± 7.2% of CD3+ CD8− CCCR5+ cells relative to matched uninfected controls. On average, infection of tissues from 14 donors with six R5 isolates from the three nonswitch virus patients resulted in the loss of 59.6% ± 2.7% of CD3+ CD8− CCCR5+ cells, whereas infection of tissues from 9 donors with five R5 isolates from the two switch virus patients resulted in a significantly (P < 0.0001; mixed-model analysis) smaller loss of CD3+ CD8− CCCR5+ cells, 20.8% ± 4.2%, relative to matched uninfected controls. Thus, the severity of CD3+ CD8− CCCR5+ cell depletion in R5 HIV-infected tissue blocks seemed to depend on whether the virus was isolated from the switch or the nonswitch virus patients. The level of cell depletion did not correlate with the level of replication in the corresponding tissue (data not shown).

Also, for any given patient, the levels of CD8− CCCR5+ T-cell depletion in infected tissues were different for sequential R5 isolates. Because of the donor-to-donor variability, we restricted comparison of these sequential isolates to matched tissue blocks. In both switch virus patients (2112 and 2242), the depletion of CD3+ CD8− CCCR5+ cells was higher for the last than for the first sequential isolate (Fig. 2), while in nonswitch virus patients, the depletion of CD3+ CD8− CCCR5+ cells was already high with the early isolate and did not increase over time (Fig. 2).

To test to what extent the decrease in the numbers of CD3+ CD8− CCCR5+ cells in infected tissues is due to cell depletion and to what extent it is due to downregulation of CCR5 following HIV-1 infection of tissues, we compared the decrease in the number of CD3+ CD8− CCCR5+ cells with that in the total number of CD3+ CD8− cells. We assumed that the death of a CD3+ CD8− CCCR5+ cell should be reflected by the loss of a CD3+ CD8− cell, whereas a decrease in the number of CD3+ CD8− CCCR5+ cells due to downregulation would not be re-
lected in a decrease in the total number of CD3⁺ CD8⁻ cells. However, the levels of depletion in the R5-infected tissues were too small to make this comparison statistically sound. Nevertheless, gating on productively infected cells, we found that on average only 5.3% ± 0.8% of the CD3⁺ CD8⁻ p24⁺ cells expressed CCR5 (19 infections by 10 different isolates in tissues from 10 different donors), indicating that in productively infected cells CCR5 has been downregulated (Table 3). Therefore, CD3⁺ CD8⁻ p24⁺ cells are the counterparts of CCR5⁺ CD3⁺ CD4⁺ cells in the noninfected population.

T cells of different activation status support productive infection of R5 HIV-1 isolates. We investigated whether the tested HIV isolates differentially infect and deplete activated and nonactivated cells. There were no differences between the two patient categories, switch and nonswitch virus patients (data not shown), and the data below are therefore pooled. In this study, we defined activation as expression of CD69 and HLA-DR. The former is considered an early activation marker, and the latter is considered a late one (11, 24). First, we compared the distribution of activation markers among

FIG. 1. Replication of primary R5 HIV-1 isolates in human lymphoid tissue ex vivo. Shown are the replication kinetics of 11 primary R5 HIV-1 isolates from five different patients in ex vivo-infected human lymphoid tissue. One representative experiment (out of five) is shown for each patient. Indicated are the patient numbers and the isolate numbers. Each point represents the p24 concentration accumulated in pooled medium bathing 27 or 54 tissue blocks (9 blocks per 4-ml well) from a single donor over a period of 3 days between medium changes.
CD3<sup>+</sup> CD8<sup>+</sup> cells with that among cells of the CD3<sup>+</sup> CD8<sup>−</sup> CCR5<sup>−</sup> subset (Fig. 3A shows a representative experiment). On average, 82% ± 1% (n = 6) of the CD3<sup>+</sup> CD8<sup>−</sup> cells were CD69<sup>+</sup> HLA-DR−, thus exhibiting a nonactivated phenotype. Single-positive CD69<sup>+</sup> cells, single-positive HLA-DR<sup>+</sup> cells, and double-positive CD69<sup>+</sup> HLA-DR<sup>+</sup> cells constituted on average 13% ± 1%, 4% ± 0.4%, and 1% ± 0.1% of the CD3<sup>+</sup> CD8<sup>−</sup> cells, respectively. In the CCR5-expressing subsets of CD3<sup>+</sup> CD8<sup>+</sup> cells, CD69<sup>+</sup> HLA-DR<sup>−</sup>, CD69<sup>+</sup> HLA-DR<sup>−</sup>, CD69<sup>+</sup> HLA-DR<sup>−</sup>, and CD69<sup>+</sup> HLA-DR<sup>+</sup> cells constituted 71% ± 5%, 20% ± 4%, 6% ± 1%, and 3% ± 0.8% of the CD3<sup>+</sup> CD8<sup>−</sup> CCR5<sup>+</sup> cells, respectively, thus representing a significant increase (P = 0.004; Mann-Whitney test) in the frequency of double-positive activated cells in this population. Does HIV-1 infection of lymphoid tissue result in activation of the general T-lymphocyte population and/or of infected T cells? Analysis of tissues from six different donors infected with 11 isolates revealed no significant difference between the distributions of activation markers among CD3<sup>+</sup> CD8<sup>−</sup> cells and in matched uninfected tissues: the proportions of CD69<sup>−</sup> HLA-DR<sup>−</sup>, CD69<sup>+</sup> HLA-DR<sup>−</sup>, CD69<sup>−</sup> HLA-DR<sup>+</sup>, and CD69<sup>+</sup> HLA-DR<sup>+</sup> cells were 83% ± 1%, 12% ± 0.9%, 4% ± 0.3%, and 1% ± 0.1%, respectively (n = 14; P = 0.937; Mann-Whitney test) (Fig. 3 shows a representative experiment). In contrast, in productively infected T cells (CD3<sup>+</sup> CD8<sup>+</sup> p24<sup>+</sup>), activation marker expression was significantly increased compared with that in the total population of CD3<sup>+</sup> CD8<sup>−</sup> cells: CD69<sup>+</sup> HLA-DR<sup>−</sup>, CD69<sup>+</sup> HLA-DR<sup>+</sup>, CD69<sup>−</sup> HLA-DR<sup>−</sup>, and CD69<sup>−</sup> HLA-DR<sup>+</sup> cells in the CD8<sup>−</sup> p24<sup>+</sup> T-cell subset constituted 66% ± 3%, 19% ± 3%, 10% ± 1%, and 5% ± 1% of the total number of cells, respectively (n = 13; P = 0.002; Mann-Whitney test) (Fig. 3B shows a representative experiment). However, the frequencies of activated cells among p24<sup>+</sup> CD8<sup>−</sup> T lymphocytes did not significantly (P = 0.078; Mann-Whitney test) exceed that in the general population of CCR5<sup>+</sup> CD8<sup>−</sup> T lymphocytes, which are potential targets for R5 HIV-1.

In summary, in tissues, the proportion of cells with an activated phenotype was higher among CD8<sup>−</sup> T lymphocytes expressing CCR5 than in the total CD8<sup>−</sup> T-lymphocyte population. However, the activation status of the host cell does not seem to be a determinant for productive HIV-1 infection.

HIV-1 infects CD25<sup>+</sup> T cells. We investigated whether the R5 HIV-1 isolates used in this study infect CD4<sup>+</sup> T cells that express CD25, a marker which is present on both activated and regulatory CD4<sup>+</sup> T cells (53, 54, 56). As in our studies of activation markers, we have pooled the data from switch and nonswitch virus patients. In the tonsillar tissues from six donors used for these experiments, CD25<sup>+</sup> cells constituted 19% ± 1% of the CD3<sup>+</sup> CD8<sup>−</sup> cells. Of these CD25<sup>+</sup> cells, 16% ± 4% were CD69<sup>+</sup>, 14% ± 4% were HLA-DR<sup>+</sup>, and 14% ± 0.3% were CD69<sup>−</sup> HLA-DR<sup>−</sup>. Further analysis showed that the CD3<sup>+</sup> CD8<sup>−</sup> CCR5<sup>+</sup> subset was significantly enriched in CD25<sup>+</sup> cells, which constituted 30% ± 2% (n = 6; P = 0.004; Mann-Whitney test) of this subset (Fig. 4A shows a representative experiment). Infection of tissues with HIV-1 did not change the fraction of CD25<sup>+</sup> CD3<sup>+</sup> CD8<sup>−</sup> cells (17% ± 1% in infected tissues versus 19% ± 1% in matched controls; P =

---

**TABLE 2. Virus replication in tissues of five different donors 12 days postinfection**

| Patient no. | Isolate no. | Viral replication between days 9 and 12 (p24 ng/ml) for donor no.: |
|-------------|-------------|---------------------------------------------------------------|
| 2112        | 171         | 10 14 13 8 7                                                  |
| 1156        | 6           | 13 11 15 6                                                   |
| 3502        | 6           | 6 14 9 8                                                   |
| 1838<sup>a</sup> | 5379       | 11 16 28 13 82                                               |
| 8590        | 2           | 6 4 3 15                                                  |
| 435         | 1577        | 21 9 73 4 40                                                  |
| 1047        | 314         | 6 30 15 3 39                                                  |
| 4223        | 3415        | 10 5 56 4 40                                                  |
| 2242<sup>a</sup> | 1886       | 14 5 1 1 15                                                  |
| 3700        | 73          | 74 28 7 3 25                                                  |

<sup>a</sup> The relative replication capacities of the isolates from patients 1838 and 2242 were significantly different (P = 0.04, Wilcoxon signed rank test).

---

**TABLE 3. Distributions of cell populations in lymphoid tissues infected with different primary R5 HIV-1 isolates**

| Patient no. | Isolate | Percent |
|-------------|---------|---------|
|             |         | CD8<sup>+</sup> of CD3<sup>+</sup> | CCR5<sup>+</sup> of CD3<sup>+</sup> CD8<sup>+</sup> | p24<sup>+</sup> of CD3<sup>+</sup> CD8<sup>+</sup> | CCR5<sup>+</sup> of CD3<sup>+</sup> CD8<sup>−</sup> p24<sup>+</sup> |
| 435         | Uninfected | 86.1 | 5.8 | 0.4 | NA<sup>b</sup> |
| 1577        | 84.9      | 3.6 | 1.9 | 4.6 | NA |
| 3415        | 84.0      | 3.0 | 2.1 | 3.1 | NA |
| 1838<sup>a</sup> | Uninfected | 82.5 | 5.0 | 0.3 | NA |
| 5379        | 78.9      | 1.9 | 3.6 | 5.3 | NA |
| 8590        | 74.1      | 1.9 | 1.8 | 17.9 | NA |
| 1047        | Uninfected | 81.0 | 6.0 | 0.6 | NA<sup>b</sup> |
| 314         | 80.2      | 2.1 | 2.7 | 3.0 | NA |
| 4223        | 78.5      | 2.3 | 3.5 | 3.2 | NA |
| 2112<sup>a</sup> | Uninfected | 82.5 | 6.3 | 0.5 | NA<sup>b</sup> |
| 171         | 82.7      | 5.2 | 2.4 | 1.9 | NA |
| 1156        | 82.5      | 4.2 | 2.1 | 0.4 | NA |
| 3502        | 81.1      | 4.0 | 2.7 | 1.2 | NA |
| 2242<sup>a</sup> | Uninfected | 80.6 | 5.6 | 0.4 | NA |
| 1886        | 79.2      | 5.4 | 0.2 | NA | NA |
| 3700        | 78.7      | 4.4 | 1.4 | 7.0 | NA |

<sup>a</sup> See the text for details. One representative experiment is shown for each patient, corresponding to Fig. 1.

<sup>b</sup> NA, not applicable.
In contrast, the fraction of productively infected (CD3^+ CD8^+ p24^+) T cells was significantly enriched in CD25^+ cells relative to that in the total CD3^+ CD8^- subset (Fig. 4B shows a representative experiment): CD25^+ CD3^+ CD8^- p24^+ cells constituted 48% ± 3% of the CD3^+ CD8^- p24^+ T cells. In summary, CD4^+ CD25^+ T cells efficiently support productive infection by HIV-1 of the R5 phenotype.

DISCUSSION

Recent studies of HIV pathogenesis in vivo have emphasized that critical events in HIV disease occur in lymphoid tissue and are not necessarily reflected by changes in blood (6, 35). Here, we used a system of ex vivo tonsillar tissue to study the tissue pathogeneses of 11 HIV primary isolates, all of which utilized CCR5 for cell entry but which were obtained from patients at different stages of disease progression. We showed that these patients’ HIV-1 isolates efficiently replicate in ex vivo-infected human lymphoid tissue. These isolates deplete their natural targets, i.e., CCR5^+ CD4^+ T cells, and such depletion seems to be related to the modes of disease progression in the patients that harbored them.

It is widely accepted that HIV disease progression is determined by a complex and as yet poorly understood combination...
of host and viral factors (15). By infecting lymphoid tissue from one donor with a panel of different isolates, and by infecting a panel of lymphoid tissues from different donors with one particular HIV-1 isolate, we were able to separate which parameters are controlled by host and viral factors in HIV tissue pathogenesis. We have found that although the absolute levels of viral replication varied as much as 30-fold between tissues obtained from different donors and were different for different isolates, the viral hierarchy among sequential isolates remained constant, emphasizing viral factors as major determinants of the relative replication capacities of these isolates in human lymphoid tissues ex vivo. In contrast, the absolute replicative capacity of HIV-1 isolates used in the present work seemed to be largely controlled by viral factors, whereas the absolute replication levels were greatly affected by a tissue (host) factor(s).

We made attempts to identify host factors by measuring tissue production of cytokines and chemokines, since these host factors are known to affect HIV-1 pathogenesis (1). However, in our ex vivo tissues, no infection with any of the primary R5 HIV-1 isolates affected the levels of the 16 measured cy-

of host and viral factors (15). By infecting lymphoid tissue from one donor with a panel of different isolates, and by infecting a panel of lymphoid tissues from different donors with one particular HIV-1 isolate, we were able to separate which parameters are controlled by host and viral factors in HIV tissue pathogenesis. We have found that although the absolute levels of viral replication varied as much as 30-fold between tissues obtained from different donors and were different for different isolates, the viral hierarchy among sequential isolates remained constant, emphasizing viral factors as major determinants of the relative replication capacities of these isolates in human lymphoid tissues ex vivo. In contrast, the absolute replicative capacity of HIV-1 isolates is controlled by host (tissue) factors that seem to enhance or suppress all replicating HIV-1 variants.

Replication of HIV isolates in this ex vivo system resulted in depletion of CD4+ T cells, but only those expressing CCR5 (see also reference 22). These T cells constitute a minority of CD4+ T cells (5, 22), and therefore, the 50% depletion of the cells observed in our experiments did not translate into a significant depletion of the total numbers of CD4+ T lymphocytes. We found that depletion of CD4+ CCR5+ cells was accompanied not only by downregulation of CD4, observed earlier in other systems (26, 45), but also by downregulation of CCR5. Coreceptor downregulation was reported earlier for CXCR4 (14, 57, 59) and has recently been reported for CCR5 (8, 59) also.

It should be pointed out that the levels of CCR5+ CD4+ T-cell depletion caused by a given isolate in tissues from different donors were similar in spite of the large variation in the levels of replication. Thus, together with relative replicative capacity, the absolute levels of CD4+ T-cell depletion by the R5 isolates used in the present work seemed to be largely controlled by viral factors, whereas the absolute replication levels were greatly affected by a tissue (host) factor(s).

We made attempts to identify host factors by measuring tissue production of cytokines and chemokines, since these host factors are known to affect HIV-1 pathogenesis (1). However, in our ex vivo tissues, no infection with any of the primary R5 HIV-1 isolates affected the levels of the 16 measured cy-
tokines/chemokines. Earlier, similar results were reported for an R5 laboratory strain and for recombinant viruses carrying R5 Envs, whereas an X4 strain significantly changed chemokine release (9, 27). However, one recent study (8) has suggested that R5 HIV-1 infection of fetal thymic organ cultures induces IL-10 and transforming growth factor β, cytokines not studied here, and thereby upregulates the expression of CCR5. 

Another tissue factor that may control the absolute level of viral replication is the activation status of cell targets. The use of ex vivo human lymphoid tissues, which do not require exogenous stimulation to support productive HIV-1 infection, allowed us to address this question. In vitro infection of PBMC requires activated or mature cells (7, 25, 42, 47, 52). In contrast, in the context of lymphoid tissue, nonactivated CD4⁺ T cells support productive infection as well (13, 19, 20). Recently, Kinter et al. (31) provided further evidence for the role of the lymphoid tissue microenvironment in controlling HIV infection by demonstrating that HIV-1 productively infected nonactivated CD4⁺ T cells in tissue ex vivo, while the same cells could not be infected if isolated from this tissue. Our present results confirm that in tissues the majority of the productively infected cells are of the nonactivated phenotype, as evidenced by the lack of CD69 and HLA-DR expression. These results reflect the situation in vivo, where HIV-1 gene expression is detected in nonactivated and naïve cells (4, 37, 60).

To further characterize tissue cell targets for primary R5 isolates, we analyzed the expression of CD25, a marker of activated CD4⁺ T cells (56) that is also expressed on regulatory CD4⁺ CD25⁺ T cells (53, 54). Tonsils are thought to harbor a larger proportion of regulatory CD4⁺ CD25⁺ T cells than peripheral blood, because of constant antigen exposure and the need to control inflammation and tissue destruction (50). We found that only a small number of T cells coexpress the activation markers CD69, HLA-DR, and CD25. This finding supports the notion that a fraction of CD4⁺ CD25⁺ T cells in tonsil tissue have a regulatory function and may not be activated. This may be an important host factor affecting HIV infection, in view of a recently reported suppression of HIV-specific responses in vitro by CD25⁺ regulatory T cells isolated from HIV-infected donors (32). Infection and depletion of T-cell subsets, as shown

![FIG. 4. Expression of CD25 on T cells in HIV-1-infected human lymphoid tissue ex vivo; comparison of the distributions of CD25 among different cell populations in uninfected and ex vivo-infected lymphoid tissue after 12 days in culture. The data were obtained in experiments using 54 pooled tissue blocks per condition from a single donor. (A) Distributions of CD25 among CD8⁻ CD3⁺ lymphocytes (left) and among CCR5⁺ CD8⁺ CD3⁺ lymphocytes (right) in uninfected tissue. The contour plots are at log 50% probability. Presented is one representative experiment out of six. (B) The distributions of CD25 among CD8⁻ CD3⁺ lymphocytes (left) and among p24⁺ CD8⁻ CD3⁺ lymphocytes in tissue infected with isolates 314 and 4223 from patient 1047; one representative experiment out of six matching that presented in panel A is shown.](image-url)
by our experiments, may include the regulatory T cells and could be an important host factor affecting the efficiency of HIV replication ex vivo.

As discussed above, the relative replication level and the ability to deplete CD4+ T cells are largely determined by viral factors. Although several viral gene products, including Nef, Vpu, Vpr, and Vif, have been reported to determine viral replication capacity in various systems, including the one used for the current study (17, 18, 36, 44, 51), most of our knowledge regarding differential pathogenesis of HIV-1 in tissues is related to coreceptor usage. Rapid progression of HIV-1 disease has been shown to be associated with evolution of virus coreceptor use from CCR5 to CXCR4. One explanation for the higher virulence of X4 viruses is the abundance of their target cells (CD4+ CXCR4+) in lymphoid tissue (22). However, it has been an enigma that about 50% of patients progress to AIDS without apparent R5-to-X4 evolution (12, 28).

HIV-1 infection, with early clinical symptoms, and in low viral isolate may have predictive value and reflects whether the dominance of R5 HIV-1.

The less cytopathic R5 virus from switch virus patients eliminates the abundance of their target cells (CD4+ CXCR4+) in lymphoid tissue (22). However, it has been an enigma that about 50% of patients progress to AIDS without apparent R5-to-X4 evolution (12, 28).

Here, we studied viral isolates, all of which were of the R5 phenotype. Whatever the viral factors that determine differential pathogenesis of these R5 isolates in tissues are, we provide here the first published evidence that the evolution of these factors is consistent with the pattern of disease progression. The degree of depletion of CCR5+ CD4+ T cells by a given viral isolate may have predictive value and reflects whether the individual from whom the virus was obtained eventually became a switch virus patient. Indeed, R5 viruses isolated from nonswitch patients depleted more target cells than isolates from switch virus patients. Conversely, the patients’ viral load, expressed as the level of HIV-1 p24 antigen in serum, was undetectable in nonswitch virus patients, while it was detectable in switch virus patients. It is tempting to speculate that the highly cytopathic R5 virus in nonswitch patients eliminates the CD4+ CCR5+ target cells and thereby limits its own replication. The less cytopathic R5 virus from switch virus patients leaves more target cells intact and therefore replicates to higher titers in vivo. A high viral load in vivo, in combination with the eventual appearance of CXCR4-using virus in the switch virus patient, results in an increased severity of HIV-1 infection, with early clinical symptoms, and in low CD4+ T-cell counts. Also, the appearance of X4 HIV-1 in switch virus patients seems to be preceded by an evolution of R5 HIV-1, since our experiments demonstrated that sequential isolates from such patients increase their ability to deplete CCR5+ CD4+ T cells during the course of the patient’s infection.

In conclusion, various host factors seem to enhance or inhibit replication of all viral isolates, whereas viral factors determine which isolate has a higher or lower relative capacity to replicate. R5 isolates from patients with progressive HIV-1 disease can efficiently infect, replicate, and deplete CCR5+ CD4+ T cells in human lymphoid tissue ex vivo. In the course of disease progression leading to the switch to X4 dominance, R5 HIV-1 variants appear to undergo evolution associated with an increase of their cytopathicity. R5 HIV-1 isolates from nonswitch virus patients are more cytopathic than R5 variants from switch virus patients, and this difference may explain the steady decline of CD4+ T cells in patients with continuous dominance of R5 HIV-1.

ACKNOWLEDGMENTS

We thank M. R. Santi and the Department of Pathology of Children’s Hospital (Washington, D.C.) for their kind assistance in providing tonsillar tissue and Bengt Johansson-Lindblom, William Agace, and Håkan Lövkvist for expert advice.

Grants were received from the Swedish Research Council, the Swedish International Development Cooperation Agency/Department for Research Cooperation (SIDA/SAREC), and the Crafoord Foundation.

REFERENCES

1. Alfano, M., and G. Poli. 2002. The cytokine network in HIV infection. Curr. Mol. Med. 2:677–689.
2. Berger, E. A., R. W. Doms, E. M. Fenyo, B. T. Korber, D. R. Littman, J. P. Moore, Q. J. Sattentau, H. Schuitemaker, J. Sodroski, and R. A. Weiss. 1998.
3. Bjorndal, A., H. Deng, M. Jansson, J. R. Fiore, C. Colognesi, A. Karlsson, J. Albert, G. Scarlatt, D. R. Littman, and E. M. Fenyo. 1997. Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype.
4. Blaak, H. A., A. R. van’t Wout, M. Brouwer, B. Hovenkamp, and H. Schuitemaker. 2000. In vivo HIV-1 infection of CD45RA+CD4+ T cells is established primarily by syncytium-inducing variants and correlates with the rate of CD4+ T cell decline. Proc. Natl. Acad. Sci. USA 97:1269–1274.
5. Bleuel, C. C., L. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay. 1997. The HIV coreceptors CCRX4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc. Natl. Acad. Sci. USA 94:1925–1930.
6. Bronowicki, J. P., T. W. Schacker, L. E. Reff, D. A. Price, J. H. Taylor, G. Bliment, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek. 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J. Exp. Med. 200:749–759.
7. Chou, C. S., O. Ramilo, and E. S. Vitetta. 1997. Highly purified CD25− testing cells cannot be infected de novo with HIV-1. Proc. Natl. Acad. Sci. USA 94:1361–1365.
8. Choudhary, S. K., N. R. Choudhary, C. K. Kimbrerly, J. Colasanti, A. Ziqgas, D. Kwa, H. Schuitemaker, and D. Camerini. 2005. R5 human immunodeficiency virus type 1 infection of fetal thymic organ culture induces cytokine and CCRX5 expression. J. Virol. 79:458–471.
9. Cunfi, A., G. Bleiber, M. Munoz, R. Martinez, C. Loeuillette, M. Rehr, M. Fischer, H. F. Gunthard, D. A. Price, J. H. Taylor, G. Bliment, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek. 2004. CD4 + T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J. Exp. Med. 200:749–759.
10. Connor, R. I., K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau. 1997. Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. J. Exp. Med. 185:621–628.
11. Cotner, T., J. M. Williams, L. Christenson, H. M. Shapiro, T. B. Strom, and J. Strominger. 1983. Simultaneous flow cytometric analysis of human T cell activation antigen expression and DNA content. J. Exp. Med. 157:461–472.
12. de Roda Husman, A. M., R. P. van Rij, H. Blaak, S. Broersen, and H. Schuitemaker. 1999. Adaptation to promiscuous usage of chemokine receptors is not a prerequisite for human immunodeficiency virus type 1 disease progression. J. Infect. Dis. 180:1106–1115.
13. Eckstein, D. A., M. L. Penn, Y. D. Korin, D. D. Scripture-Adams, J. A. Zack, J. F. Kreisberg, M. Roederer, M. P. Sherman, P. S. Chin, and M. A. Goldsmith. 2001. HIV-1 actively replicates in naive CD4+ T cells residing within human lymphoid tissues. Immunity 15:671–682.
14. Endres, M. J., P. R. Clapham, M. Marsh, M. Ahuja, J. D. Turner, A. McKnight, J. F. Thomas, B. Stoebenau-Haggarty, S. Choe, P. J. Vance, T. N. Wells, C. A. Power, S. S. Sutterwala, R. W. Doms, N. R. Landau, and J. A. Hoxie. 1996. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. Cell 87:745–756.
15. Fauci, A. S. 1996. Host factors in the pathogenesis of HIV disease. Antibiot. Chemother. 48:4–12.
16. Glashakova, S., B. Bajbakov, L. B. Margolis, and J. Zimmerman. 1995. Infection of human tonsil histocultures: a model for HIV pathogenesis. Nat. Med. 1:1320–1322.
17. Glashakova, S., J. C. Grevil, K. Suryanarayana, P. Meylan, J. D. Lifson, R. I. Endres, M. J., P. R. Clapham, M. Marsh, M. Ahuja, J. D. Turner, A. McKnight, J. F. Thomas, B. Stoebenau-Haggarty, S. Choe, P. J. Vance, T. N. Wells, C. A. Power, S. S. Sutterwala, R. W. Doms, N. R. Landau, and J. A. Hoxie. 1996. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. Cell 87:745–756.
18. Fauci, A. S. 1996. Host factors in the pathogenesis of HIV disease. Antibiot. Chemother. 48:4–12.
19. Glashakova, S., B. Bajbakov, L. B. Margolis, and J. Zimmerman. 1995. Infection of human tonsil histocultures: a model for HIV pathogenesis. Nat. Med. 1:1320–1322.
Kinter, A. L., M. Hennessey, A. Bell, S. Kern, Y. Lin, M. Daucher, M. Planta, 2000. Human immunodeficiency virus type 1 induces apoptosis in CD4+ but not in CD8+ T cells in ex vivo-infected human lymphoid tissue. J. Virol. 74:8077–8084.

Grivel, J. C., L. A. Ehler, S. B. Mizell, C. W. Hallahan, and A. S. Fauci. 2001. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Rapid induction of a phosphorylated 28 kD/32 kD disulfide-linked early activation antigen (EA 1) by 12-O-tetradecanoylphorbol-13-acetate, mitogens, and antigens. J. Exp. Med. 196:1988–2000.

Hoxie, J. A., J. D. Alpers, J. L. Rackowski, K. Huebner, B. S. Haggarty, A. J. Miller, M. D., M. T. Warmerdam, I. Gaston, W. C. Greene, and M. B. 2000. The human immunodeficiency virus-1 nef gene product: a role in the selective depletion of CD4+ T cells. Proc. Natl. Acad. Sci. USA 97:5555–5559.

Karlsson, I., L. Antonsson, Y. Shi, A. Karlsson, J. Albert, T. Leitner, B. Olde, M. C. Cowman, and E. M. Fenyos. 2003. HIV biological variability unveiled: frequent isolations and chimeric receptors reveal unprecedented variation of coreceptor usage. J. Virol. 77:2561–2569.

Kinter, L. L., L. Antonsson, Y. Shi, A. Karlsson, J. Albert, B. Olde, M. C. Cowman, and E. M. Fenyos. 2004. Coevolution of RANTES sensitivity and mode of CCR5 receptor usage by human immunodeficiency virus type 1 variants in coinfected human lymphoid tissues. J. Clin. Microbiol. 42:2126–2131.

Kinter, J. C., F. Santoro, S. Chen, G. Faga, M. S. Malnati, Y. Ito, L. Karlsson, and P. Russo. 2003. Pathogenic effects of human herpesvirus 6 in human lymphoid tissue. J. Virol. 77:2805–2829.

Hara, T., L. K. Jung, J. M. Bjornjard, and S. M. Fu. 1986. Human T cell activation. III. Rapid induction of a phosphorylated 28 kD/32 kD disulfide-linked early activation antigen (EA 1) by 12-O-tetradecanoylphorbol-13-acetate, mitogens, and antigens. J. Exp. Med. 196:1408–2000.

Grivel, J. C., and L. B. Margolis. 1997. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CCR4. J. Exp. Med. 186:1383–1388.

Shankarappa, R., J. B. Margolick, S. J. Gange, A. G. Rodrigues, D. Ubelherr, H. B. Madigan, P. Gange, C. B. Huang, G. H. Hultin, X. L. Hu, B. M., and J. J. Mullins. 1999. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. J. Virol. 73:10489–10502.

Hoxie, J. A., and J. D. Alpers. 1996. The human immunodeficiency virus-1 nef gene product: a role in the selective T-cell functional defects observed in infected individuals. Proc. Natl. Acad. Sci. USA 93:6705–6709.

Penn, M. L., A. C. Fauci, H. C. Lane, J. S. Justement, M. Baseler, and A. S. Fauci. 1990. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc. Natl. Acad. Sci. USA 87:3958–3962.

Mehandru, S., M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Bucceri, E. M. Fenyos, H. Wiggel, P. Rossi, et al. 1991. Polymerase chain reaction, virus isolation and antigen assay in HIV-1-antibody-positive mothers and their children. AIDS 5:1173–1178.

Rucker, E., J. Munch, S. Wildum, B. BRENNER, J. Liesmann, L. Margolis, and A. Kirchhoff. 2004. A naturally occurring variation in the proline-rich region does not attenuate human immunodeficiency virus type 1 nef function. J. Virol. 78:1917–1920.

Schnittman, S. M., H. C. Lane, J. Greenhouse, J. S. Justement, M. Baseler, and A. S. Fauci. 1990. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc. Natl. Acad. Sci. USA 87:3958–3962.

Kool, M. M., J. WALTER, J. L’AGE, and P. C. Beverley. 1997. HIV infection of CD45RA+ CD40+ CD4+ T cells. Clin. Exp. Immunol. 107:300–305.

Sentman, T. C., M. H. Cane, J. Greenhouse, J. S. Justement, M. Baseler, and A. S. Fauci. 1990. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc. Natl. Acad. Sci. USA 87:3958–3962.

Gallo, R. C., M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Bucceri, E. M. Fenyos, H. Wiggel, P. Rossi, et al. 1991. Polymerase chain reaction, virus isolation and antigen assay in HIV-1-antibody-positive mothers and their children. AIDS 5:1173–1178.