HIV-1 pathogenesis differs in rectosigmoid and tonsillar tissues infected ex vivo with CCR5- and CXCR4-tropic HIV-1

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Gut-associated lymphoid tissue (GALT) has been identified as the primary target of HIV-1 infection. To investigate why GALT is especially vulnerable to HIV-1, and to determine whether the selective transmission of CCR5-using viral variants (R5) in vivo is the result of a greater susceptibility of GALT to this viral variant, we performed comparative studies of CXCR4-using (X4) and R5 HIV-1 infections of human lymphoid (tonsillar) and rectosigmoid tissues ex vivo under controlled laboratory conditions. We found that the relative level of R5 replication in rectosigmoid tissue is much greater than in tonsillar tissue. This difference is associated with the expression of the CCR5 co-receptor on approximately 70% of CD4 T cells in rectosigmoid tissue, whereas in tonsillar tissue it is expressed on fewer than 15% of CD4 T cells. Furthermore, tonsillar tissue responds to X4 HIV-1 infection by upregulating the secretion of CC-chemokines, providing a potential CCR5 blockade and further resistance to R5 infection, whereas gut tissue failed to increase such innate immune responses. Our results show that rectosigmoid tissue is more prone than tonsillar tissue to R5 HIV-1 infection, primarily because of the high prevalence and availability of R5 cell targets and reduced chemokine blockade. The majority of CD4 T cells express CXCR4, however, and X4 HIV-1 readily replicates in both tissues, suggesting that although the differential expression of co-receptors contributes to the GALT vulnerability to R5 HIV-1, it alone cannot account for the selective R5 infection of the rectal mucosa in vivo.

Introduction

It has been well established that critical events in HIV-1 infection occur in lymphoid tissue [1–8]. More recently, gut-associated lymphoid tissue (GALT) has been identified as the primary target of HIV-1 infection independent of the route of transmission [9–12], probably because gut-associated lymphocytes are predominantly activated memory cells [13]. Typically, infection is transmitted by R5, although both X4 and R5 HIV-1 are often present in seminal fluid [14,15]. To determine whether the selective R5 transmission stems from a greater susceptibility of GALT to this viral variant, we performed comparative studies of X4 and R5 infections of lymphoid (tonsillar) and rectosigmoid tissues ex vivo [16].

Here, we show that in rectosigmoid tissue ex vivo, R5 virus replicates more efficiently than in tonsillar tissue in association with the expression of the CCR5 co–receptor on the majority of CD4 T cells in rectosigmoid but not in tonsillar tissue. Furthermore, whereas both tissues express significant levels of CXCR4, tonsillar tissue responds to...
X4 HIV-1 infection by upregulating the secretion of CC-chemokines, whereas gut tissue failed to mount such an innate immune response. Rectosigmoid tissue is thus more prone to R5 HIV-1 infection than tonsillar lymphoid tissue; however, both tissues ex vivo readily replicate X4 HIV-1. Therefore, the differential expression of co-receptors alone cannot account for the selective R5 transmission across the rectal mucosa in vivo.

Materials and methods

Viral stocks

R5SF162 and X4LAI.04 HIV-1 isolates were obtained through the National Institutes of Health (NIH) AIDS Research and Reference Program and expanded in activated human peripheral blood mononuclear cells to provide the following viral stocks X4LAI.04 166 ng/ml of p24 and 4 × 10^7 T-cell infectious dose (TCID50) per ml; for R5SF162 76 ng/ml of p24 and 10^7 TCID50 per ml.

Tissues

Colonic biopsies were obtained at the University of California at Los Angeles (UCLA) from healthy, HIV-1-seronegative individuals recruited from the UCLA Clinical Trial Registry (internal review board approved). Up to 20 biopsies were acquired as previously reported at 30 cm from the anus [16]. Briefly, the biopsies (8 × 2 × 1 mm) were washed twice in RPMI and mounted on a 1 cm² gelfoam raft (Wyeth Pharmaceuticals, Madison, New Jersey, USA) at the medium–air interface in a 24-well tissue culture plate in the presence of 500 μl RPMI plus 10% fetal calf serum supplemented with HEPES (1 mmol; Invitrogen Life Technologies, Carlsbad, California, USA), and a mixture of antibiotics. Each experimental condition was made up of four wells of one biopsy per well; the supernatants from four biopsies per culture were pooled for analysis.

Human tonsils obtained from routine tonsillectomies were dissected into 2 mm blocks and cultured atop gelfoam with a mixture of antibodies at the medium–air interface as previously described [17, 18]. Nine individual blocks were placed on 12 × 4 mm gelfoam in a well of a six-well plate. Each experimental condition was composed of three wells for a total of 27 blocks whose culture media were pooled. The size of the blocks of both tissues was optimized for culture conditions [16, 18].

Infection of explants

Within 5 h of excision, tissue explants were infected by the topical application of 3–6 μl of a stock of X4LAI.04 (0.5–1.0 ng of p24 or 120 or 240 TCID50) of a clarified viral stock, and in the case of R5SF162, by applying 3.0–6.5 μl of a stock of R5SF162 (0.25–0.54 ng of p24 or 32–65 TCID50). For rectal biopsies, viruses were incubated overnight and washed away to prevent contamination. In the case of tonsillar explants, washing was postponed to day 3 to avoid a massive loss of lymphocytes. The residual inoculum constituted less than 7% of the cumulative p24 production. For both tissues culture medium was collected and replaced every 3 days.

Flow cytometry

Single cell suspensions from rectosigmoid biopsies [19] and from tonsillar tissue [20, 21] were prepared as described and subjected to flow cytometry [20, 21]. Samples were analysed on a FACSCalibur (BD Biosciences, San Jose, California, USA) using CellQuest software. Lymphocyte numbers were determined by gating on CD45 cells. The absolute numbers of lymphocytes stained for CD45/CD3/CD4, CD45/CD3/CD8, CD45/CD3/CD16/CD56, and CD45/CD3/CD19 with SimulTest (BDIS) were evaluated by TruCount tubes. Cell suspensions of tonsillar tissue were stained with a combination of anti-CD3-Cy7-PE, anti-CD4-Cy5.5-PE, anti-CD8-Tricolor (Caltag, Burlingame, California, USA), anti-CCR5-APC-Cy7, and anti-CXCR4-APC (BDIS); before cell surface staining, counting beads (Caltag) were added to each tube to quantify cell depletion. The samples were acquired on a BD LSR.II equipped with the 355, 407, 488, 532 and 638 nm laser lines. Data were acquired with DIVA 4.1.2 (Becton-Dickinson) and analyzed with Flow Jo 6.8 (TreeStar Inc., Ashland, Oregon, USA).

For intracellular p24, cell suspensions from the tissue blocks and biopsies were stained for cell surface markers, were permeabilized with Fix and Perm (Caltag) and stained with 2 μl of the anti-p24 antibody KC57-RD1 or isotype control (Coulter, Miami, Florida, USA).

Cytokine assay (Luminex) of explant supernatants

Macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation normally T-cell expressed and secreted (RANTES), stromal-derived factor (SDF)-1α, IFN-γ-inducible protein (IP)-10, IFN-γ, tumor necrosis factor (TNF)-α, IL-1β, IL-2, IL-4, IL-10, IL-12, IL-16 and mononoke induced by IFN-γ (MIG) were evaluated in culture medium by a multiplexed fluorescent microsphere immunoassay using the Luminex 100 Systems (Luminex, Austin, Texas, USA). Cytokine capture antibodies (R&D Systems, Minneapolis, Minnesota, USA) were coupled to the assay beads. Bead sets coupled with capture antibodies (1250 of each specificity) were mixed with 50 μl standard or culture medium, incubated overnight at 4°C. Bound cytokines were detected with biotinylated antibodies (R&D Systems) and streptavidin–phycoerythrin (Molecular Probes, Eugene, Oregon, USA). Data were analysed with Biorad Bioplex Manager software using a five-parameter fitting algorithm.

Real-time polymerase chain reaction of viral RNA

Viral RNA was extracted from 100 μl culture medium using QiaAmp viral RNA isolation kits [22]. RNA was
eluted in 50 μl water, and 10 μl were reverse transcribed in 50 μl reactions using GeneAmp real-time polymerase chain reaction (PCR) kits (Applied Biosystems, Foster City, California, USA). Primers used for the amplification of viral complementary DNA were designed to amplify segments of the V3–V5 region of the gp120 gene selectively and have been described previously [22]. Real-time PCR assay was performed on the ABI-PRISM 7000 Sequence Detector (Applied Biosystems) using a SYBR Green PCR master mix (Applied Biosystems).

**P24 measurement**

HIV-1 replication was measured in tissue culture supernatant harvested at days 0, 1, 4, 7, 9 and 12 using the reliance enzyme-linked immunosorbent assay kit (Perkin Elmer, Wellesley, Massachusetts, USA). P24 concentration data were acquired and analysed using delta soft software (Biometallics, Princeton, New Jersey, USA).

**Results**

**R5 and X4 replication in rectosigmoid and tonsillar tissue**

The inoculation of rectosigmoid and tonsillar tissue blocks with R5 or X4 variants resulted in productive HIV-1 infection. To account for differences in cellularity between tissue explants, pooled media from 27 tonsillar or four rectosigmoid tissue blocks from each donor were used for each experimental condition, and each experiment was repeated with tissues from a number of donors, denoted below as n. The absolute and relative replication of these viral variants differed between the two types of tissues (Fig. 1a,b and Fig. 2a,b).

Replication of R5SF162 in rectosigmoid tissue was greater than in tonsillar tissue. When recalculated on a per block basis, an average block of R5SF162 HIV-1-infected tonsillar tissue produced between days 3 and

**Fig. 1. Replication of X4 and R5 HIV-1 in tonsillar tissues ex vivo.** Blocks of tonsillar tissues were infected ex vivo with (a) R5SF162 or (b) X4LAI.04. Culture medium bathing the 27 tissue blocks was changed every 3 days and analysed for HIV RNA using real-time polymerase chain reaction and for p24 with enzyme-linked immunosorbent assay. The graphs represent the replication of X4LAI.04 in five donors and R5SF162 in four donors. The donors are identified by a number on top of each graph. The measurements of viral replication by p24 and by viral RNA were well correlated, as shown by the linear regression analyses of the concentrations of p24 and viral RNA released by tissues infected ex vivo with X4LAI.04 (c) and R5SF162 (d). Cumulative amount of HIV-1 released between days 3 and 12 postinoculation by tonsil explants infected with X4LAI.04 and R5SF162 measured by p24 (e) and viral RNA (f) (mean ± SEM).
12 postinfection 0.86 ± 0.12 ng of p24, whereas over the same period an average block of rectosigmoid tissue infected with R5SF162 produced 4 ± 0.98 ng of p24. Replication of X4LAI.04 was greater in tonsil blocks than in rectal biopsies with an average production of 26.7 ± 0.8 and 8 ± 1.53 ng of p24 per block of tonsillar and rectosigmoid tissue, respectively.

We compared R5 and X4 replication in matched blocks of tissues; in tonsils, R5SF162 replication was less than X4LAI.04 replication. The total accumulation of p24 in supernatants from R5-infected tonsillar tissues reached 12.4 ± 7.6% of that from X4-infected tissues (Fig. 1c) and the total production of viral RNA from R5-infected tissues constituted 15 ± 9% of that in X4-infected tissues (Fig. 1f). In contrast, in rectosigmoid tissue, R5 and X4 replications were similar, with the former reaching 54 ± 17% (P = 0.3) of the latter (n = 5; Fig. 2e,f). This difference between the HIV-1 replication of R5 and X4 in the two types of tissues was not associated with large differences in replication kinetics [18,23].

There were correlations between the accumulation of p24 and RNA within each tissue type. For tonsillar tissues from each donor, the release of p24 strongly correlated with the release of viral RNA: r varied between 0.92 and 0.99 for X4LAI.04 and between 0.95 and 0.999 for R5SF162. The correlation between these two parameters remained strong when data points from five different donors were pooled, r = 0.90, P < 0.001 and r = 0.94, P < 0.001 for X4LAI.04 and R5 isolates, respectively (Fig. 1c,d). For rectosigmoid tissue, the correlation between the production of p24 and viral RNA was not as tight as in tonsils (Fig. 2a,b). This may reflect the larger number of tonsil (27) versus rectosigmoid (four) explants. In individual rectosigmoid experiments, r varied between 0.75 and 0.99 for X4LAI.04-infected tissue and between 0.78 and 0.99 for R5SF162-infected tissue. For pooled experiments r = 0.79, P < 0.001 for X4LAI.04 and r = 0.84, P < 0.001 for R5SF162, respectively (Fig. 2e,d).

Analysis of the infection of rectosigmoid and tonsillar tissues thus revealed similarities in the kinetics of R5 and X4 HIV-1 replication and a strong correlation between p24 and viral RNA release for both viral variants in both types of tissues. Relative R5 replication was much more efficient in rectosigmoid than in tonsillar tissue.

**Lymphocyte subsets in rectosigmoid and tonsillar tissue**

To compare HIV-1 infection of rectosigmoid and tonsillar tissues at the cellular level, we phenotyped lymphocytes isolated from tissue blocks [19,24]. The resultant cell suspensions were stained for CD45, CD3, CD4, CD8, CCR5, CXCR4, CD19, CD16, CD56, and p24 and subjected to flow cytometry.

In our analysis of tonsillar cell subsets, we pooled 27 or 54 tissue blocks each containing on average 159 380.3 ± 24 469 T cells, which constituted 56 ± 6% of all tissue lymphocytes. CD4 and CD8 T cells accounted for almost the entire CD3 cell subset, with an average ratio of 4.5 ± 0.4 (n = 5). A majority, 66 ± 5%, of CD4 T cells expressed CXCR4 but not CCR5, whereas only 6 ± 2% expressed CCR5 without any apparent expression of CXCR4 and 9 ± 2% expressed both CXCR4 and CCR5 (n = 15; Fig. 3a). On day 12 of
culture, there were no significant changes, with T cells constituting 55.2 ± 4.3% (n = 5) of lymphocytes with a CD4:CD8 ratio of 5.7 ± 1.07 (n = 5), and B cells accounting for 29.5 ± 5.7% of lymphocytes (n = 5). Further analysis showed that 70 ± 4% of CD4 T cells expressed CXCR4, whereas only 5.4 ± 1% of CD4 T cells exclusively expressed CCR5. Only 3.36 ± 0.6% of CD4 T cells were double positive, whereas the remaining cells were double negative (n = 19).

An average block of rectosigmoid tissue contained 367 820 ± 76 394 T cells, which constituted 60 ± 10% of lymphocytes, whereas B cells constituted 32 ± 10% of lymphocytes. CD4 and CD8 T cells accounted for almost the entire CD3 cell subset, with an average CD4:CD8 ratio of 2.3 ± 0.5 (n = 5). HIV-1 co-receptor expression on rectosigmoid CD4 T cells was in agreement with previous reports from our group [21,25,26]; 11 ± 3% of CD4 T cells expressed CXCR4 only, 31 ± 5% selectively expressed CCR5, and 40 ± 8% expressed both CXCR4 and CCR5 (Fig. 3b).

At days 7–10 of culture, T cells made up 86 ± 6% and B cells 11 ± 5% of lymphocytes (n = 4). The distribution of T-cell subsets was similar to that observed at day 0, the average CD4:CD8 ratio remained at the level of 2.54 ± 0.34 (n = 4). The expression of chemokine receptors changed slightly from that in freshly isolated tissues and reflected an increase in CXCR4 expression, with 36.3% of CD4 T cells exclusively expressing CXCR4, 47.8% expressing both CXCR4 and CCR5, whereas 12% of CD4 T cells exclusively expressed CCR5.
HIV-1 infection of tonsillar and rectal tissue

HIV-1 infection of both tissues resulted in CD4 T-cell depletion. In tonsils, X4LAI.04 infection depleted 80 ± 1% of CD4 T cells (n = 3, P = 0.026), whereas R5SF162 infection depleted 4.57 ± 4.54% (n = 3, P = 0.9) of CD4 T cells (Fig. 4a). In rectosigmoid tissue, X4LAI.04 infection depleted 79 ± 6% (P = 0.02, n = 3) of CD4 T cells, whereas R5SF162 depleted 34 ± 7% (P = 0.05, n = 3) of these cells (Fig. 4b).

HIV-1-infected CD4 T lymphocytes were identified by intracellular p24 staining. To include CD4 T cells that downregulated CD4 cells because of HIV-1 infection, we analysed CD3⁺CD8⁻ cells because almost all T cells in uninfected tissue express either CD4 or CD8. In tonsillar tissues on day 12 post-X4LAI.04 infection, 4.7 ± 1.7% (n = 5, P = 0.025) of CD3⁺CD8⁻ cells were p24-positive, whereas in tissues infected with R5SF162 the p24-positive fraction constituted 1.22 ± 0.3% (n = 5, P < 0.01) of these cells. In X4LAI.04-infected rectosigmoid tissue, 10 ± 5% (n = 5) of CD3⁺CD8⁻ lymphocytes were p24-positive, whereas in matched samples infected with R5SF162, 3.47 ± 1.57% (n = 5) of these cells were p24 positive.

The frequency of CCR5⁺CD4⁺ T cells in rectosigmoid tissue was approximately fivefold greater than in tonsillar tissue and, accordingly, the frequency of R5 HIV-1

Fig. 2 (Continued).

Fig. 3. Expression of HIV-1 co-receptors on CD4 T cells in tonsillar and rectosigmoid tissues. Lymphocytes isolated from tonsillar (a) and rectosigmoid (b) tissues at the time of acquisition were stained for the surface expression of CD3, CD4, CD8, CCR5 and CXCR4 and analysed using flow cytometry. Presented are average distributions of co-receptors on CD4 T cells in tissues from 15 (tonsil) and six (rectosigmoid) donors (mean ± SEM).
productively infected CD4 T cells was greater in rectosigmoid compared with tonsillar tissue.

**Cytokines of rectosigmoid and tonsillar tissue**

We analysed the modulation of cytokine secretion by HIV-1 infection in rectosigmoid tissues because such a modulation in human tonsils was previously documented [23].

As was shown earlier and confirmed here (Fig. 5), the infection of tonsillar tissue with X4 LAI.04 increased the secretion of four measured chemokines: from the basal level of 901 ± 120 pg/ml, MIP-1β increased 8.95 ± 1.14-fold \((P < 0.001)\), MIP-1α increased 4.30 ± 1.11-fold (basal level 479 ± 70 pg/ml, \(P < 0.001\)) and RANTES increased 2.5-fold (basal level 1256 ± 437 pg/ml, \(n = 5\)), although the increase in the latter chemokine was not statistically significant. SDF-1α increased 5.57 ± 0.5-fold (basal level 1295 ± 286 pg/ml, \(P < 0.001\)). Also, X4 LAI.04 infection increased the secretion of TNF-α 1.65 ± 0.24-fold (basal level 77 ± 4 pg/ml, \(P = 0.024\)) and IFN-γ 2.07 ± 0.24-fold (basal level 253 ± 33 pg/ml, \(P < 0.001\)). There was no increase in IL-1α, IL-1β, IP-10, IL-10 or MIG.
Notably, R5SF162 did not change the production of any of the tested chemokines/cytokines.

In rectosigmoid tissue, neither X4 LAI.04 nor R5SF162 modulated the production of chemokines; RANTES, MIP-1α and MIP-1β remained at the basal levels of 1700 ± 167 pg/ml, 7189 ± 900 pg/ml and 25 553 ± 5102 pg/ml, respectively. The production of several cytokines increased upon HIV-1 infection: IP-10 increased 10.21 ± 3.7-fold (P < 0.001) in X4LAI.04 and 12.22 ± 6.6-fold (P = 0.003) in R5SF162-infected tissues from a basal level of 5067 ± 2348 pg/ml; MIG increased 5.13 ± 1.5-fold (P < 0.001) in X4LAI.04-infected tissues and 25.4 ± 19-fold (P ≤ 0.001) in R5SF162-infected tissues from a basal level of 39.6 ± 27.6 ng/ml and IL-10 increased 17 ± 9-fold (P = 0.003) in X4LAI.04 and 20.39 ± 11-fold in R5SF162-infected tissues from a basal level of 1565 ± 884 pg/ml.

To test whether the lack of HIV-1-mediated chemokine induction in rectosigmoid tissue was caused by an inherent incapacity to secrete these chemokines, we activated *ex vivo* rectosigmoid cultures with phytohemagglutinin and measured the secretion of β chemokines. A 3-day phytohemagglutinin activation (10 μg/ml) increased chemokine secretion over the next 7 days: MIP-1B increased 23.1 ± 9.8-fold, MIP-1α increased 11.9 ± 4.4-fold and RANTES increased 16.25 ± 3.6-fold (n = 3). The α chemokine SDF-1α was increased 20.5 ± 9.7-fold. Rectosigmoid tissue *ex vivo* is thus capable of chemokine secretion upon stimulation, but in contrast to tonsillar tissue fails to do this in response to HIV-1 infection.

Therefore, in rectosigmoid tissue, neither X4 nor R5 upregulate co-receptor blocking chemokines in contrast to tonsillar tissue, in which X4 infection does. In both types of tissues, however, several immunomodulatory cytokines were upregulated in the course of HIV-1 infection.

**Discussion**

Whereas both X4 and R5 HIV-1 variants are commonly present in body fluids, R5 HIV-1 is thought to initiate infection selectively, predominantly in lymphoid tissue and dominates its early stages [27]. GALT was recently shown to be the main primary target for HIV-1 at the earliest stages of infection [10–12], not only in cases of anal intercourse but also in other routes of viral transmission. In secondary lymphoid organs, such as lymph nodes or tonsils, infection is established more slowly and viral replication may continue for years [28].

To test whether GALT is particularly vulnerable to R5 HIV-1 infection, we compared infection by R5 and X4 in the tonsils, a secondary lymphoid organ, and in rectosigmoid tissue *ex vivo*. Both human tonsillar and rectosigmoid tissues *ex vivo* support productive infection by R5 and X4 HIV-1 without exogenous stimulation. Another human rectosigmoid explant system was developed earlier by the Dezzutti group for microbicide testing [29], but it requires phytohemagglutinin activation for efficient HIV-1 infection, probably because the semipolarization of explants in matrigel [29] reduces HIV-1 accessibility to lymphocytes.

Although the absolute levels of HIV-1 replication significantly varied from donor to donor, the relative replication levels of the two viruses in tissues from different donors were similar [30]. This allowed us to pool together data from experiments with different donors.

An average block of rectosigmoid tissue produced more R5 and less X4 HIV-1 than a tonsillar tissue block. On the basis of the CD4 T-cell number, p24 production in tonsils was four to seven times greater than in rectosigmoid tissue for both R5 and X4. The measurement of the relative production of X4 and R5 HIV-1 seems to be more adequate. The fraction of CCR5-expressing cells in rectosigmoid tissue was five times greater than in tonsillar tissue. The majority of these cells in rectosigmoid tissue also expressed CXCR4. The expression of CCR5 by CXCR4-positive CD4 T cells may reflect their constitutive activation in GALT [25]. Also, memory CCR5-positive CD4 T cells are prevalent in rectosigmoid tissue [26]. Therefore, in rectosigmoid tissue, R5 potential targets were much more abundant than in tonsillar tissue. Also, other factors, including a twofold difference in the fraction of CD8 cell anti-HIV factor (CAF)-producing CD8 T cells [31,32] may contribute to the difference in replication in tonsillar and rectosigmoid tissues.

To enumerate the actual R5 and X4 targets we identified them by intracellular p24 staining. The number of productively R5-infected lymphocytes was significantly greater in rectosigmoid than in tonsillar tissue, in agreement with the greater replication of R5 in this tissue. Unfortunately, these data do not provide a basis for the comparison of viral productivity of individual R5 and X4-infected cells in tonsillar and rectosigmoid tissue, because p24 measurement in culture medium represents a cumulative production of virus between media changes, whereas intracellular p24 staining is a snapshot of productive cells that have not yet died from infection.

The relative replication levels of R5 HIV-1 in both tissues correlated with the frequency of CCR5-positive T cells, which in rectosigmoid tissue constituted approximately 70% of the total CD4 T cells at the time of infection, whereas in tonsillar tissue, on average, these cells made up approximately 15% of CD4 T cells. This difference provides the simplest explanation for the difference in the efficiency of R5 HIV-1 infection in these tissues. The abundance of CCR5-positive CD4 T cells in
rectosigmoid tissue makes the cytopathic impact of R5 more pronounced than in tonsillar tissue. In the latter, R5 depletion is barely noticeable, whereas in rectosigmoid tissue more than 34% of CD4 T cells were depleted. These data may be relevant to the predominant mucosal transmission of R5 virus and the rapid depletion of GALT T lymphocytes at the early stages of HIV-1 infection.

Although R5 replicated more efficiently in rectosigmoid compared with tonsillar tissue, in both tissues X4 targets, the CXCR4-positive CD4 T lymphocytes, comprise the majority of the CD4 T cell population [21,33,34], and both tissues support X4 infection. With the improvement in staining techniques, it becomes apparent that virtually all the CD4 T cells are potential targets for X4.

In addition to the chemokine receptors, chemokines themselves may modulate HIV-1 infection as one of the first lines of antimicrobial defence. The upregulation of chemokines occurs in human tonsillar tissue ex vivo upon X4 but not R5 HIV-1 infection. It thus appears that at a local level, secondary lymphoid tissue is capable of mounting a defence, although not broadly effective, as X4 but not R5 HIV-1 infection. It thus appears that at a local level, secondary lymphoid tissue is capable of mounting a defence, although not broadly effective, as X4 but not R5 HIV-1 infection. In conclusion, by comparing tonsillar and rectosigmoid tissue susceptibility to R5 and X4 HIV-1, we showed that the latter is more susceptible to R5 infection than the former. This difference seems to be related to the much greater expression of CCR5 co-receptors in rectosigmoid than in tonsillar tissue. The majority of rectosigmoid CD4 lymphocytes still express CXCR4, however, either alone or in combination with CCR5, and therefore this tissue is also readily infected with X4 ex vivo. This strongly suggests that co-receptor expression alone is not sufficient to explain the R5 predominance seen in early infection. Undoubtedly, there are additional mechanisms that serve as partial ‘gatekeepers’ restricting X4 infection in vivo [27].

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