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HIV-1 Does Not Provoke Alteration of Cytokine Gene Expression in Lymphoid Tissue after Acute Infection Ex Vivo

Annette Audigé,* Erika Schlaepfer,* Athos Bonanomi,* Helene Joller,† Marlyse C. Knuchel,** Markus Weber,‡ David Nadal,§ and Roberto F. Speck2*

The cytokine response to invading microorganisms is critical for priming the adaptive immune response. During acute HIV infection, the response is disrupted, but the mechanism is poorly understood. We examined the cytokine response in human lymphoid tissue, acutely infected ex vivo with HIV. Lymphoid tissue was cultured either as blocks or as human lymphocyte aggregate cultures (HLAC) of tonsils and lymph nodes. This approach allowed us to examine the effects of HIV on cytokines using distinct culture techniques. In contrast to HLAC, mock-infected tissue blocks displayed a 50- to 100-fold up-regulation of mRNAs for IL-1β, -6, and -8 in the first 6 days of culture. Parallel increases were also noted at the protein level in the supernatants. Although IL-1β, -6, and -8 are known to synergistically enhance HIV replication, peak HIV replication (measured as p24Ag) was similar in tissue blocks and HLAC. Surprisingly, vigorous HIV replication of CXCR4- and CCR5-tropic HIV strains did not result in characteristic mRNA profiles for IL-1β, -2, -4, -6, -8, -10, -12, -15, IFN-γ, TNF-α, TGF-β, and β-chemokines in tissue blocks or HLAC. The increased expression of IL-1β, -6, and -8 in tissue blocks may approximate clinical situations with heightened immune activation; neutralization of these cytokines resulted in inhibition of HIV replication, suggesting that these cytokines may contribute to HIV replication in certain clinical settings. These results also indicate that different molecular mechanisms govern HIV replication in tissue blocks and HLAC. Prevention of effective cytokine responses may be an important mechanism that HIV uses during acute infection. The Journal of Immunology, 2004, 172: 2687–2696.

Human immunodeficiency virus severely compromises the cellular immune system; immunomodulation offers one promising therapeutic strategy (1–4). Currently, several drugs are in clinical trials based on their potential cytotoxic or antiviral actions in HIV infection. These include IL-2 (5–8), IFN-α (9–11), and GM-CSF (12).

A detailed understanding of how HIV succeeds in dysregulating the cytokine network would be highly beneficial to the development of immune-based therapies. Some studies have linked disease progression to a shift from a Th1 to a Th2 cytokine response (13–15); however, these findings are still controversial (16, 17). Others proposed a Th1 to Th0 switch or alteration of both Th1 and Th2 cytokine production without a polarized Th1/Th2 state (18, 19).

Long-term immune control of viral infections depends largely on the cytokine milieu generated by the innate immune system in the first 3–5 days (20). A number of in vitro studies have tried to recapitulate the early effects of HIV infection and viral gene products on cytokines (21–23). Results depended strongly on the experimental design and were contradictory even for the same HIV gene product. For example, Nef induced IL-2 in T cells stimulated with CD3 and CD28 (22) but down-regulated IL-2 in T cells treated with PHA or PMA (21). Another study reported no differences in the levels or kinetics of cytokine secretion between infected and acutely HIV-infected IL-2-stimulated PBMC (24).

Many reported in vitro findings are difficult to extrapolate to the in vivo situation. They were obtained in PBMC activated with Abs or polyclonal mitogens, monocyte-derived macrophages, or cell lines exposed to a high virus input or recombinant HIV proteins. However, the cytokine milieu generated in vivo after infection results from the interaction of many different cells. Furthermore, HIV is primarily a disease of lymphoid tissue, and thus, results obtained in PBMC may not adequately mirror the critical immunopathogenetic events in HIV infection.

Cultures of human lymphoid tissue as blocks, also called lymphoid histocultures, offer an excellent alternative model for the study of HIV pathogenesis (25). Histocultures preserve the rich repertoire of cellular subpopulations and tissue architecture of lymphoid tissue, and they support vigorous HIV replication without the prior activation needed by PBMC.

In the current work, we aimed to define the cytokine response in lymphoid tissue after ex vivo infection with HIV. We focused on the Th1 cytokines (IFN-γ, IL-2, and IL-12), the Th2 cytokines (IL-4 and IL-10), the proinflammatory cytokines (IL-1β, IL-6, and IL-8), and TNF-α. We also included IL-15, which shares bioologic activities with IL-2; TGF-β, which has complex immune regulatory functions; and the β-chemokines RANTES and macrophage-inflammatory protein (MIP)β-1α.

4 Abbreviations used in this paper: MIP, macrophage-inflammatory protein; R5, CCR5 tropic; X4, CXCR4 tropic; HLAC, human lymphocyte aggregate culture; DC, dendritic cell; HBMS, hydroxymethylbilane synthase; NFIB-2, NF I; MNE, mean normalized gene expression; SEB, staphylococcal enterotoxin B; p.i., postinfection; PDC, plasmacytoid DC; MDC, myeloid DC.

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We hypothesized that the cytokine milieu differs between infections with CCR5-tropic (R5) or CXCR4-tropic (X4) HIV strains. Notably, R5 strains use CD4 and CCR5 to enter cells. Cells such as memory T cells and macrophages express CCR5 and are therefore permissive to R5 strains (26). Typically, R5 strains are involved in the early infection and predominate until late-stage AIDS. X4 strains use CD4 and CXCR4, receptors found predominantly on T cells. Emergence of X4 strains usually occurs in late-stage HIV disease and is associated with an acceleration of the immunodeficiency. The elucidation of the cytokine response to R5 and X4 strains may help to resolve their temporal appearance in HIV (27, 28).

Although most studies of HIV pathogenesis in primary lymphoid tissue were performed with tissue blocks, human lymphocyte aggregate cultures (HLAC) have been reported to be an equivalent value as an experimental system but much easier to handle (29). However, different culture modes may affect the initial HIV-host interactions and subsequent cytokine responses. Therefore, we performed our analyses in parallel in cultures of lymphoid tissue blocks and HLAC.

Materials and Methods

Lymphoid tissue

The acquisition and processing of lymphoid tissue were approved by the local ethical committee. Human tonsils from otherwise healthy patients (18–70 years of age) were obtained within 1–5 h after tonsillectomy from the Department of the Ear, Nose, and Throat Surgery at University Hospital of Zurich. The median age of the patients was 26 years. Lymph nodes of intestinal or inguinal origin were obtained from organ donors from the Department of Surgery, University Hospital of Zurich.

Lymphoid tissue was divided into tissue blocks of 2–3 mm, and three or two blocks from tonsils or lymph nodes, respectively, were placed at the air–interface of a collagen sponge gel (Pharmacia and Upjohn, Kalamazoo, MI) covered by a membrane (pore size, 4 or 70 μm; BD Biosciences, San Jose, CA) and grinding the tissue through the sieve with a syringe plunger. Erythrocytes were lysed with ACK cell lysing buffer (Cambrex, Walkersville, MD). Lymphoid cells were washed and transferred to a 96-well plate at a concentration of 2 × 10^6 cells/well.

Culture blocks and HLAC were from the same donor. Tissue blocks and HLAC were cultured in RPMI 1640 containing 15% fetal bovine serum, 1% penicillin, 1% streptomycin, 2 μg/ml fungizone, 2 μM L-glutamine, 1 mM sodium pyruvate, and 1% nonessential amino acids.

One experiment comprised data from one donor; the numbers of experiments and replicates are given in the legends. In each single experiment, blocks and HLAC were harvested for RNA extraction, samples were collected 1, 3, and 6 h for blocks; 50 μl for HLAC, and 1% oxygen. Samples were harvested for RNA extraction after 6, 16, 26, and 48 h. For determining the effects of mitogens on cytokine mRNA expression, tissue blocks and HLAC were immersed in culture medium containing either PMA (0.5 μg/ml) and ionomycin (0.05 μg/ml), staphylococcal enterotoxin B (SEB; 1 μg/ml), or PHA (5 μg/ml) (all reagents from Sigma-Aldrich, St. Louis, MO) for 30 min at room temperature before being cultured as described above. Mitogens were maintained during culturing. Blocks and HLAC were harvested for RNA extraction after 6, 24, and 72 h of stimulation.

HIV infection of human lymphoid tissue ex vivo

Each tissue block was infected by inoculation with 5–15 μl of viral stock containing 1 ng of p24, which is consistent with described protocols (33). HLAC were incubated overnight in 100 μl containing 5 ng of p24, washed three times with PBS the next morning, and resuspended in 200 μl of fresh medium. Culture medium (500 μl for blocks; 50–100 μl for HLAC) was replaced every 3–4 days. HIV replication was followed by measuring the concentration of p24 in the culture medium with an in-house p24 ELISA. For RNA extraction, samples were collected 1, 3, and 6 days postinfection (p.i.).

Viruses

Virus stocks were obtained by calcium phosphate-mediated transfection (Promega) of 293T cells with pNL4-3, pHIV-2 (National Institutes of...
Table I. Expression of cell surface markers on tonsillar lymphocytes at baseline

<table>
<thead>
<tr>
<th>CD4+</th>
<th>CD8+</th>
<th>CD25+</th>
<th>CD45RA</th>
<th>CCR5</th>
<th>CXCR4</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 (35–56)</td>
<td>8 (3–12)</td>
<td>36 (14–61)</td>
<td>42 (13–91)</td>
<td>16 (9–32)</td>
<td>56 (29–79)</td>
</tr>
<tr>
<td>CD25+</td>
<td>CD45RA</td>
<td>CCR5</td>
<td>CXCR4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (1–22)</td>
<td>66 (32–95)</td>
<td>48 (14–82)</td>
<td>33 (14–72)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as median (minimum to maximum) (n = 12).

† Percentage of positive cells among the lymphocyte population.

‡ Percentage of positive cells of CD4+ T cells.

§ Percentage of positive cells of CD8+ T cells.

In tissue blocks, mRNAs for the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 were strongly up-regulated over time; this increase was already apparent after 1 day of culturing (Table II). In contrast, expression of IL-1β, -6, and -8 mRNAs was not substantially increased in HLAC (range of relative mRNA expression, 1.2–to 3.1-fold). IL-4 (Th2) was the only cytokine that was down-regulated over time, particularly in HLAC. Transcriptional up-regulation of other cytokines (i.e., IL-2, -10, -12, -15, IFN-γ, TNF-α, and TGFB) was less pronounced over time (relative expression, <1.0-fold), and no substantial differences were detected between tissue blocks and HLAC. A similar pattern was observed in tissue blocks from lymph nodes of intestinal or inguinal origin (Table III).
Cytokine mRNA expression in tonsillar tissue blocks over time was paralleled by increases in the respective protein concentrations in the culture fluids (Table IV).

**Cytokine mRNA expression in HLAC exposed to hypoxic conditions**

We thought that stress from hypoxia might explain the dramatic increases in proinflammatory cytokine mRNA levels in the tissue blocks. To determine the effects of hypoxia, we measured cytokine mRNA levels in HLAC exposed to hypoxic conditions for 2 days. In general, levels of transcripts of proinflammatory cytokines were higher in hypoxia-stimulated than in unstimulated HLAC (Fig. 1). The increase in replication for both X4 and R5 viruses was generally slower in tissue blocks than in HLAC. Cultured blocks of lymph nodes of intestinal and inguinal origin also supported productive infection by NL4-3 or YU-2 (data not shown). In a minority of experiments, tissue blocks did not support HIV replication exposed to either R5 or X4 strains.

**Cytokine mRNA expression in tonsillar tissue blocks and HLAC upon stimulation with polyclonal mitogens**

To test whether the tissue is able to react to exogenous stimuli, tissue blocks and HLAC were exposed to polyclonal mitogens (Fig. 2). PMA/ionomycin stimulation resulted in strong mRNA up-regulations of IL-2 and -4 and a severalfold increase of IFN-γ mRNA. SEB generated a very similar pattern. PHA markedly induced IL-4 and -2 in HLAC and increased IL-4 severalfold in blocks. Thus, tonsillar tissue blocks and HLAC react to exogenous stimuli by up-regulating Th1 and Th2 cytokines.

**HIV replication in lymphoid tissue blocks or HLAC**

To examine the effects of different coreceptors, we tested well-characterized HIV strains that use the coreceptors CXCR4 (NL4-3 and 134), CCR5 (49.5 and YU-2), or both (89.6). In most experiments, tonsillar blocks and HLAC supported replication of both X4 and R5 viruses (Fig. 3). Peak levels of HIV replication were typically higher after infection with X4 than with R5 strains. The increase in replication for both X4 and R5 viruses was generally slower in tissue blocks than in HLAC. Cultured blocks of lymph nodes of intestinal and inguinal origin also supported productive infection by NL4-3 or YU-2 (data not shown). In a minority of experiments, tissue blocks did not support HIV replication exposed to either R5 or X4 strains.

**Cytokine mRNA expression in lymphoid tissue infected ex vivo with HIV**

We focused on early time points (i.e., 1, 3, and 6 days p.i.) with the objective to elucidate the cytokine responses following immediate exposure to virus, when virus spreading starts, and finally when virus production is readily detectable but before virus-triggered damage. Overall, cytokine mRNA expression in tonsillar tissue blocks or HLAC with the prototype viruses NL4-3 or YU-2 was not different from that in mock-infected samples at any time point (Fig. 4). Similar results were obtained for the β-chemokine RANTES and MIP-1α (Fig. 5). In lymph nodes, cytokine mRNA levels also generally showed only minor differences between NL4-3-, YU-2-, and mock-infected blocks on days 1 and 3 p.i. (Table IV).
In some cases, cytokine mRNA expression in lymphoid cultures was >2-fold or <0.5-fold, indicating up- or down-regulation, respectively, of certain cytokines in cultures from some donor tissues.

Consistent with the mRNA data, protein concentrations of the cytokines IFN-γ, TNF-α, IL-1β, -6, and -8, and of the β-chemokines RANTES and MIP-1α in HIV-infected cultures from tonsillar tissue blocks were not significantly different from those in mock-infected cultures (Fig. 6).

HIV replication in tonsillar tissue blocks treated with anti-cytokine Abs

To assess the significance of proinflammatory cytokines, the mRNAs of which were up-regulated in mock-infected blocks but not HLAC, for HIV replication, we monitored NL4-3 replication in tonsillar tissue blocks in the presence of Abs against either IL-1β, -6, or -8. This treatment resulted in substantial inhibition of NL4-3 replication; as expected, treatment of HLAC with Abs had only a minor effect on virus replication (Fig. 7). In both tissue blocks and HLAC, culturing with anti-cytokine Abs had no influence on cell viability compared with addition of isotype Abs.

Discussion

Throughout the course of infection, HIV subverts the immune system. However, the innate immune response, which largely determines the efficiency of the adaptive immune response to HIV, has been poorly characterized. The cytokine response is a major component of the innate immune system. We investigated the cytokine response to HIV in lymphoid tissue infected ex vivo as a model of acute HIV infection. Our main observations were as follows: 1) endogenous cytokine mRNA expression was substantially different between lymphoid tissue blocks and HLAC; 2) tissue blocks and HLAC supported replication of HIV strains equally well regardless of coreceptor usage; 3) infection of lymphoid cultures ex vivo with either X4 or R5 HIV strain did not result in a specific cytokine profile; and 4) treatment of tonsillar tissue blocks but not HLAC with neutralizing Abs against either IL-1β, -6, or -8 markedly diminished HIV replication. Thus, the cytokine milieu after HIV infection within an HIV-naive lymphoid microenvironment does not show a Th1 or Th2 profile, which may reflect a hiding strategy of the virus rather than the inability of the tissue to mount an efficient immune response. Furthermore, different mechanisms seem to govern HIV replication in tissue blocks and HLAC.

We first characterized the cell repertoire and the endogenous cytokine milieu over time in mock-infected tonsillar tissue cultured ex vivo either as blocks or HLAC. Flow-cytometric analysis demonstrated that the preparation of HLAC did not alter the percentages of CD4+ and CD8+ T cells, PDC, MDC, as well as NK cells. Therefore, cultures of lymphoid tissue with their preserved rich cell repertoire are very promising for the study of the innate immune response to HIV.

To a great extent, cytokines are transcriptionally regulated and expressed only transiently in response to stimuli (36–38). HIV itself is known to affect gene transcription of host cells (e.g., de
novo methylation of the IFN-γ promoter and subsequent down-regulation of IFN-γ production) (39). Therefore, the innate immune response to HIV and the effects of the virus on cytokines are directly reflected by the transcriptional level of cytokines. Consequently, we examined the cytokine milieu primarily by quantitative real-time PCR. Changes in mRNA expression were also verified at the protein level. We found that the culture mode significantly influenced the constitutive cytokine expression. In mock-infected lymphoid tissue blocks of tonsillar, inguinal, or intestinal origin, IL-1β, -6, and -8 mRNAs were strongly up-regulated over time. In contrast, IL-1β, -6, and -8 mRNAs showed no mRNA up-regulations in HLAC. The other cytokines analyzed showed less pronounced mRNA changes whether tissue was cultured as tissue blocks or HLAC. Cytokines at the protein level paralleled the changes in mRNA with significant increased concentrations of IL-1β, -6, and -8 similar to reported findings (40). No increase was observed for the other cytokines analyzed.

Exogenous addition of IL-1β, -6, and -8 is known to enhance HIV replication in in vitro models (41–43). Therefore, we expected a more vigorous HIV replication in tissue blocks than in HLAC. A direct comparison of HIV replication between these two culture modes is problematic, because cell number and viability in tissue blocks cannot be controlled over time. Furthermore, the infection protocols are different. In any case, both culture systems showed vigorous HIV replication after infection. Along with distinct cytokine profiles, the similar HIV replication efficiencies suggest that different molecular mechanisms likely govern HIV replication in HLAC and tissue blocks. Notably, R5 and X4 strains replicated equally well in both culture systems, albeit they are known to target distinct cell subsets.

We hypothesized that infection of lymphoid tissue blocks and HLAC ex vivo with HIV would result in characteristic cytokine responses, especially that cytokine responses would differ between HIV strains with selective coreceptor usage. However, we observed no consistent changes in mRNA levels for proinflammatory, Th1, or Th2

**FIGURE 4.** HIV does not provoke alteration of cytokine gene expression in lymphoid tissue after acute infection ex vivo. Cytokine mRNA amounts in NL4-3-infected (■) and YU-2-infected (□) tonsillar cultures are given as x-fold relative to mock-infected samples. For each sample, cytokine mRNA expression was normalized to mRNA expression of the housekeeping gene HMBS. Data are given as median (maximum-minimum) from five experiments (from four for day 3 p.i.).

**FIGURE 5.** HIV does not provoke alteration of chemokine gene expression in lymphoid tissue after acute infection ex vivo. Cytokine mRNA amounts in NL4-3-infected (■, ■, A, ■, ▲) and YU-2-infected (A, ■, B, ■, ▲, ◆) tonsillar cultures are given as x-fold relative to mock-infected samples. For each sample, cytokine mRNA expression was normalized to mRNA expression of the housekeeping gene HMBS. In A, data are given as median (maximum-minimum) from five experiments (from four for day 3 p.i.).
cytokines or β-chemokines. Parallel changes at the protein level excluded a major effect of HIV on posttranscriptional or translational modulation of gene expression. This was particularly surprising, because we had selected the cytokines based on their reported changes during HIV infection or their immunopathogenic relevance for the innate immune response. No consistent and marked alterations were found either, when we expanded our analyses to IFN type I (data not shown), which is induced in various in vitro acute HIV infection models (44–46). Furthermore, mRNA levels of cytokines at very early time points after infection (i.e., 6 and 16 h after HIV infection) were also similar for HIV-infected and mock-infected samples (data not shown).

Because we analyzed mRNA from whole-tissue lysates, distinct cytokine responses within subsets of cells may have been missed. However, it seems rather unlikely that selective expression of cytokines within subsets of cells will be balanced so that no difference or pattern was observed. Furthermore, some of the analyzed cytokines are specifically produced by cell subsets, such as IL-2 by T cells (47, 48), IFN-γ by T cells, NK cells, or macrophages (49, 50).

Importantly, the similar cytokine patterns after challenge with R5 and X4 HIV strains argue against a specific role for the examined cytokines in the distinct temporal appearance of viral strains. This is all the more surprising, because R5 and X4 viruses target distinct cell types with definite abilities to produce cytokines.

In findings similar to ours, Kinter et al. (24) did not observe significant differences in the levels or kinetics of cytokine secretion between uninfected and HIV-infected samples with IL-2-stimulated PBMC. Margolis et al. (51), who also examined the chemokine response to HIV in lymphoid tissue, found no differences of MIP-1α, -β, and RANTES at earlier time points after infection with a X4 strain but found a substantial increase after 8 days; no change at all was noted after infection with a R5 strain. These and our studies are distinguished from most other studies in that the

**Table V. Cytokine mRNA expression in NL4-3- and YU-2-infected relative to mock-infected tissue blocks from lymph nodes**

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NL4-3</td>
<td>YU-2</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.7 (1.0–2.9)</td>
<td>1.3 (1.0–2.0)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.8 (0.9–8.7)</td>
<td>1.6 (1.2–6.7)</td>
</tr>
<tr>
<td>IL-2</td>
<td>1.6 (0.8–3.6)</td>
<td>1.1 (0.9–1.8)</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.8 (0.6–7.2)</td>
<td>1.6 (0.8–3.5)</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.4 (0.7–12.1)</td>
<td>1.6 (1.0–2.6)</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.7 (1.0–3.4)</td>
<td>1.9 (1.1–3.3)</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.2 (1.2–5.4)</td>
<td>1.8 (1.3–3.8)</td>
</tr>
<tr>
<td>IL-12</td>
<td>1.5 (1.2–2.3)</td>
<td>1.2 (1.1–1.9)</td>
</tr>
<tr>
<td>IL-15</td>
<td>1.8 (1.6–3.3)</td>
<td>1.8 (1.0–4.3)</td>
</tr>
<tr>
<td>TGF-β</td>
<td>1.3 (0.9–1.9)</td>
<td>0.9 (0.7–1.1)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.3 (0.6–1.6)</td>
<td>1.1 (1.0–1.7)</td>
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*Data are shown as median (minimum to maximum).
cytokine profiles were examined in the context of spreading HIV infection of PBMC or lymphoid tissue. In most other studies, specific cytokine profiles were obtained using recombinant HIV proteins (52), an extremely high multiplicity of infection (53), or monocyte-derived macrophages (54). For example, expression of Nef in monocyte-derived macrophages results in the release of MIP-1α and MIP-1β that has been interpreted as critical for attracting CD4+ T lymphocytes for subsequent HIV dissemination (23). The work by Margolis et al. and our laboratory do not corroborate these findings. Indeed, macrophages in lymphoid tissue cultures are selectively infected by R5 strains (55). Therefore, we would expect that infection of lymphoid tissue with R5 strains should recapitulate the reported increase of β-chemokine release. However, exactly the opposite was true with increased β-chemokine release after infection with X4 strains (51), which notably do not productively infect macrophages.

In vivo studies of patients with primary HIV infection reported some increases, mainly for proinflammatory cytokines, but without a marked and characteristic profile similar to the results we observed (16, 56, 57). Strikingly, patients with acute mononucleosis (EBV) showed higher levels of cytokines in the serum than patients with primary HIV infection, suggesting that an absent or weak cytokine response is specific to HIV infection (57). Only a very small number of patients with acute HIV infection have been studied for their cytokine profiles, because most patients are not aware of acute HIV transmission and do not experience symptomatic infection. Thus, the cytokine responses in patients with asymptomatic primary HIV infection are unknown. The data we present are consistent with moderate and not acute cytokine responses described in patients with primary HIV infection.

The critical role of the experimental system in investigating the cytokine response to HIV is illustrated by the following facts. In the CD4+ lymphoblastoid T cell line CEM 72 h after infection, HIV infection resulted in a ≥500-fold up-regulation of mRNA expression of the NFIB-2 (31). In tonsillar tissue blocks, HIV infection did not result in up-regulation of NFIB-2 mRNA (data not shown). These opposing findings emphasize the importance of primary cultures for HIV research.

The lack of a distinct cytokine response to HIV may be explained by the lymphoid tissue cultured ex vivo being anergic. This is certainly not the case, because selective cytokine responses were seen after exposing the tissue to polyclonal mitogens. This suggests that the dominance of IL-1β, -6, or -8 in tissue blocks will not override specific cytokine responses and that the lack of cytokine responses we observed is real.

Hypoxic stress or surgery may partially trigger the increased generation of IL-1β, -6, and -8 in tissue blocks. Although these cytokine increases may represent an in vitro artifact, they may approximate clinical situations of heightened immune activation. We made use of this phenomenon for dissecting the roles of IL-1β, -6, and -8 for HIV replication. In tissue blocks, neutralization of individual cytokines resulted in substantial inhibition of HIV replication, whereas in HLAC, neutralization showed only minor effects. Similar to our findings, neutralization of TNF-α, IL-1β, or IL-6 resulted in decreased HIV replication in IL-2-stimulated PBMC (24). These findings indicate the requirement for synergistic activity of multiple cytokines for efficient HIV replication in certain situations. Consistent with our observations, the combined administration of TNF-α, IL-2, IL-6, and TNF-α is required for induction of HIV in latently infected CD4+ T cells (58).

In HLAC, an activated cellular phenotype of CD4+ T cells, as illustrated by higher expression of the IL-2Rα (CD25), may compensate for the lack of increased IL-1β, -6, and -8 in the observed vigorous HIV replication. HLAC also reveal an increased proportion of T cells expressing the activation marker CD95.5

In summary, although both blocks and HLAC of lymphoid tissue cultured ex vivo support HIV replication, they differ in their endogenous cytokine profiles. Irrespective of the mode of tissue culturing, we found no selective changes in cytokines after HIV infection, suggesting that the initial infection process does not provoke a cytokine response. This, in turn, may justify the proposal that, during early infection of an HIV-naïve host, the virus uses prevention of effective cytokine responses as a mechanism to escape host defenses and rapidly establish progeny. The data we present are consistent with moderate and not acute cytokine responses described in patients with primary HIV infection.

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