Chemokines and other cytokines in human immunodeficiency virus type 1 (HIV-1) infection

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ABSTRACT
Cytokines, chemokines and their receptors are important factors that influence the pathogenesis of HIV-1 in vivo. HIV-1 infection stimulates the production of cytokines and chemokines from a great variety of cell types, which may either induce or inhibit viral replication. Furthermore, chemokine receptors act as viral co-receptors for viral entry into cells. Co-receptor expression and co-receptor availability are important determinants of the susceptibility to infection. Cytokines and chemokines modulate co-receptor expression and thus influence HIV pathogenesis.

In this review, we describe the importance of the distribution of chemokines and their receptors in the primary infection and in the later evolution of the disease, as well as the effect of relevant cytokines in viral replication and in the regulation of immune cell homeostasis. Finally, we also discuss the use of cytokines and chemokines as therapeutic agents.

KEY WORDS: Cytokine / Chemokine / HIV-1.

PALABRAS CLAVE: Citocina / Quimiocina / VIH-1.
INTRODUCTION

Cytokines are a group of low molecular weight proteins that mediate communication between immune system cells. They contribute to chemical signalling pathways that regulate development, tissue repair, haemopoiesis, inflammation and immune responses. Cytokines have pleiotropic activities and functional redundancy. They act in a complex network where one cytokine can influence the production of, and response to, many other cytokines. Chemokines are a large family of cytokines. They have chemoattractive functions and are involved in the normal traffic of leukocytes, not only to lymphoid organs but also to non-lymphoid organs. They also participate in the recruitment of leukocytes to injury and infection sites, in metastasis and angiogenesis\(^{[3]}\). Apart from the chemoattractive functions, they have inflammatory functions, such as activation, costimulation and differentiation of T cells and monocytes\(^{[4,6]}\).

The classification of chemokines is based on the number and spacing of the first cysteines in their amino acid sequence. Accordingly, there are four subfamilies named C, CC, CXC and CX(C)\(^{2}\). The CXC family (also named \(\alpha\) family) has a single amino acid between the initial two cysteines. In the CC family the two cysteines are adjacent to each other (also named \(\beta\) family). The CXC family has three amino acids between the cysteines. The C subfamily has only one of the two first cysteines in its sequence. Chemokines act through chemokine receptors, which are a subfamily of the 7-transmembrane G-protein-coupled receptors. Chemokine signalling through its receptors is characterized by redundancy: many chemokines bind to more than one chemokine receptor, and vice versa. This promiscuity is not general, since some chemokine receptors signal for just one ligand\(^{[9]}\). The classification of the receptors is based on the chemokine superfamily classification.

Cytokines and chemokines play an important role in HIV-1 infection, starting with the viral entry into the cell. Apart from their well established effects on viral entry, chemokines and cytokines have additional roles in HIV-1 pathogenesis. HIV-1 infection stimulates the production of several cytokines and chemokines from a variety of cell types. These events contribute to viral replication in many ways: some cytokines may limit viral spread whereas others may contribute to its propagation. Here, we will review the regulation of the chemokine/cytokine network along with HIV-1 infection and its contribution to viral pathogenesis and evolution.

CHEMOKINE RECEPTORS AND HIV ENTRY

CD4 antigen is the major receptor for HIV-1 entry into cells\(^{[11-14]}\). It is expressed mainly in T lymphocytes, but can be also expressed in other cellular types as macrophages, microglial and dendritic cells\(^{[11-14]}\). The sole presence of CD4 antigen in cells is not sufficient to permit viral infection. The need for a second receptor (i.e. co-receptor) is compulsory for viral infection to occur\(^{[15]}\). A subset of chemokine receptors act as viral entry co-receptors and the natural ligands of these receptors can inhibit viral infection in vitro\(^{[16-19]}\). The two main HIV-1 co-receptors that determine viral tropism are CCR5 and CXCR4. The CC-chemokines Regulated on Activation Normal T-cell Expressed and Secreted (RANTES or CCL-5), Macrophage Inflammatory Protein-1\(\alpha\) (MIP-1\(\alpha\) or CCL-3) and 1\(\beta\) (MIP-1\(\beta\) or CCL-4), were first identified as natural ligands for CCR5 receptor and as inhibitors of the R5 strains (strains that use CCR5 as co-receptor for viral entry) in vitro\(^{[16-19]}\). Later, Stromal Cell Derived Factor 1 (SDF-1 or CXCL12) was identified as the natural ligand of CXCR4 and a potent inhibitor of X4 variants (strains that use CXCR4 as co-receptor) in vitro\(^{[16-19]}\). Generally, inhibition of HIV-1 entry by chemokines depends on two possible mechanisms: a steric effect that consists in the competitive blockade of viral entry by direct union of the ligand to its receptor, or through the internalisation of the receptor after chemokine binding\(^{[20,21]}\). Alternatively, chemokine receptor dimerization may account for inhibition of HIV entry and virus replication\(^{[22-25]}\).

CCR5 and CXCR4 are key receptors for HIV-1 pathogenesis; in fact, CCR5 is critical for the infection to occur. There is a 32 base pair deletion in the gene that codifies for the CCR5 receptor (CCR5\(\Delta32\)), generating a non-functional protein that is not expressed in the cell surface. The presence of CCR5\(\Delta32\) in homozygosis is associated to HIV-1 infection resistance\(^{[26,29]}\), although some cases of homozygous individuals have been infected with X4 strains\(^{[30]}\); in heterozygosis, the mutation has been associated to a delay in the progression of the disease\(^{[31]}\). Other mutations in the promoter region of the CCR5 gene could also affect the transmission or the progression of the disease but to a lesser extent than the CCR5\(\Delta32\) mutation\(^{[32,33]}\). Mutations in the cytoplasmic tail domain of the gene encoding CXCR4 have been associated to aberrant chemokine receptor function causing human disease\(^{[34]}\). However, no mutation has been described in the CXCR4 gene that may influence HIV-1 infection, probably because CXCR4 and SDF-1 are essential for embryonic development\(^{[35]}\), so that mutations in the gene codifying for CXCR4 could be lethal.

CCR5 and CXCR4 expression has been described in a great variety of cell types and tissues. Chemokine receptor expression in a specific cellular type may be constitutive or inducible. Macrophages express high levels of CCR5, whereas only 5% of circulating monocytes express the receptor\(^{[36]}\). Dendritic cells, Langerhans cells, endothelial cells and cells...
of the microglia also express CCR5(37,38). In CD4+ T lymphocytes, CCR5 is restricted to memory cells, whereas CXCR4 is expressed in both memory and naive cells, being much greater in naive T cells(39, 40). B lymphocytes, haematopoietic progenitor cells, endothelial cells and neurons also express CXCR4.

CHEMOKINES AND THEIR RECEPTORS DURING HIV-1 INFECTION

HIV-1 infection, in vivo, can be divided in three phases. In the primary infection the dissemination of the virus occurs in lymphoid and mucosal tissue(41) and other tissues including the central nervous system. In this phase, a peak of viral load and the development of specific immune responses is observed(42-44). Recent developments suggest that CCR5+ memory T cells are primed for infection, and massive infection, viral replication and virus-induced cell death occur in gut mucosal tissue during this early phase of HIV infection(45, 46).

Next, the chronic phase is characterized by a clinical, but not virological latency. In the last phase, named acquired immunodeficiency syndrome (AIDS), an augmentation of viral replication activity and a marked depletion of CD4 T cell numbers in peripheral blood occur.

The primary infection and the asymptomatic period of the disease are characterized by the presence of CCR5-using (R5) HIV-1 strains, whereas CXCR4-using (X4) strains appear in 50% of the cases in the last phase of the infection(47). X4 strains seem to be more pathogenic in cell culture experiments and their in vivo emergence is associated to a rapid drop in CD4 T cell numbers(48, 49), an accelerated disease progression and the appearance of AIDS(50, 51). The factors that determine the restriction of R5 strains to the transmission of the disease are not fully understood. Several authors have suggested that the R5 strains are preferentially transmitted due to the expression pattern of the receptors and their ligands in the sites of infection of the virus(52). The genital epithelium and rectal tissue are broadly populated with dendritic cells, macrophages and CD4 and CCR5 expressing cells(53). Dendritic, immature cells express 10 times more CCR5 than CXCR4(54), and in those cells R5 strains replicate selectively. It has also been suggested that the CCR5 expression levels in Langerhans cells, that belong to the dendritic cell family, are a determinant factor in the preferential transmission of R5 strains(55). On the other hand, CCR5 expression is also elevated in the intestinal epithelium, which could be important in the rectal transmission of R5 strains(56). Besides, the elevated levels of CXL12 in the intestine could be a suppressor factor for the X4 strains(57). In accordance to this model, the major abundance of local CCR5 expression in the sites of infection, in comparison with CXCR4, could explain the predominance of R5 HIV-1 transmission.

This oversimplified model does not explain the unique presence of R5 strains in vertical (mother to child) infections and in intravenous infections. In these types of transmissions, the R5 strains should not be selected since in these cases there is no epithelium to act as a barrier, and so the X4 strains should be satisfactorily transmitted. A hypothesis has been proposed in which dendritic cells could bind HIV-1 viral particles by CCR5-dependent and CXCR4-independent mechanisms. Dendritic cells internalize the virus in endocytic intracellular vacuoles, without being productively infected. After virus internalisation, HIV-1 viral particles remain infectious for 5 days. Hereinafter, cells transport viral particles to the lymphoid node where viral particles will be presented to B and T cells and where the adaptive immune response will start. In T-cell zones of the lymphoid nodes, R5 strains could be highly favoured due to the high level expression of CCR5 in CD4 activated T cells present in these zones. So, the selective amplification of R5 strains in the lymphoid tissue could be independent of the mode of transmission(55, 56).

During the asymptomatic phase, viral replication takes place in lymphoid organs(57). Five to ten years after the initial infection, 50% of the patients progress to more advanced stages of the disease. Although chemokines are known to be important in the progression of HIV infection, the exact role they play is not well established. Intuitively, it can be envisaged that chemokines may be modelling their receptor expression in vivo. Besides, immunological or genetic alterations that affect chemokine levels may alter the susceptibility to HIV-1 infection or the rate of progression once the infection is established. CXCL12 levels are highly variable among healthy donors, however, it seems that these levels keep stable in time, independent of the absolute value. CXCL12 is not an inducible chemokine. Rather, it is constitutively expressed by stromal cells in many tissues, where it plays important homeostatic functions, so it is not strange that its plasma levels are stable. In HIV-1 infection there is no consensus about CXCL12 levels. Derdeyn et al. found a positive correlation between CXCL12 plasma levels and CD4 T cell numbers in a cross-sectional study of HIV-1 positive individuals(60). This suggested a relationship between low CXCL12 levels and HIV-1 progression. Conversely, Soriano et al. suggested that HIV infection increases CXCL12 levels. Moreover, Ikekawa et al. described that HIV-1 infection induces CXCL12 production, particularly in the last phase of the disease(62), suggesting that an elevated CXCL12 level may be a prognostic indicator of an advanced
state of the disease. Similarly to healthy donors, the levels of the chemokine are very variable among HIV-1 patients. The factors that influence and/or determine CXCL12 production are unknown. It has been proposed that a polymorphism in the gene that codifies for CXCL12 may explain the disparity of the chemokine levels. A genetic variant consisting in a transition of SDF-1 (CXCL12) 3’ untranslated region at the base 801, G-A (SDF-1 3’ A), has been associated to a delay in AIDS development when it appears in homozygosis\(^{(63)}\). The protection mechanism of this mutation is not clear; some authors have proposed that this alteration may generate an augmentation in stability or a better mRNA processing, so that the mutation would generate an over-expression of CXCL12, which would delay the appearance of X4 strains. In other studies, the presence of the mutation in homozygosis has been associated with an accelerated disease progression or without effect on progression\(^{(64, 65)}\). We have found that patients infected with HIV-1 X4 strains have significantly lower plasma levels of CXCL12 than patients infected with HIV-1 R5 strains. However, CXCL12 plasma levels were independent of plasma viral load, of CD4 T cell numbers or the presence or absence of the SDF-1 polymorphic variant\(^{(66)}\). Our own studies suggest that the levels of CXCL12 remain stable or may vary in a manner that is not dependent on HIV viral load or antiretroviral treatment after longitudinal follow up of HIV positive individuals (unpublished observation).

As with CXCL12, there is also controversy about the role of CC-chemokines in the progression of HIV-1 infection. It is well established that CCL5 levels are significantly higher in HIV-1 individuals than in healthy donors\(^{(67, 68)}\). In fact, \textit{ex vivo} studies have shown that CD4 T cells, but not CD8 T cells of asymptomatic HIV-1 infected individuals produce elevated amounts of CC-chemokines, capable of inhibiting HIV-1 replication. Other studies have shown that CD8 T cells of asymptomatic patients produce higher levels of CCL3 and CCL4, but not CCL5, than CD8 T cells from healthy donors or patients with rapid disease progression\(^{(69, 70)}\). However, several authors have not found any correlation between CCL5 levels and disease progression\(^{(71, 72)}\). It is also important to take into account that T cells are not the only producers of CC-chemokines. For example, activated NK cells produce CCL5, CCL3 and CCL4\(^{(73)}\). Another important source of CCL5 are platelets, which store it in granules and release it in great amounts when they are activated\(^{(74)}\). The presence of opportunistic infections, apart from HIV-1, may also induce CC-chemokine production. In HIV-1 infected individuals, the co-infection with herpervirus-6 induces the production of CCL5, but not that of CCL3 or CCL4 in lymphoid tissue. Furthermore, the production of CCL5 is associated with the suppression of HIV-1 R5 strains\(^{(75)}\). In another study, the co-infection with \textit{Mycobacterium tuberculosis} was associated with CC-chemokine production and an accelerated disease progression\(^{(76)}\). Conversely, coinfection of HIV-1 with GB virus type C appears not to alter circulating levels of chemokine expression\(^{(77)}\).

The exact role of chemokines in HIV-1 pathogenesis remains obscure, probably due to the fact that multiple chemokines or cytokines may have different effects on viral replication and pathogenesis, or their effects may be compromised by viral factors. The inhibitory effect of HIV-1 replication by CXCR4 or CCR5 agonists may depend on the signalling through G proteins. Therefore chemokines may be exerting a more complex function apart from the inhibitory effect on viral entry. In fact, chemokine signalling may induce chemotaxis of CD4 T cells to sites of active virus replication, but may also induce death or survival signals. The predominant activation of one pathway or the other may depend on the concentration of each chemokine\(^{(78, 79)}\). CCL5, CCL3 and CCL4 can increase viral replication in monocytes, macrophages and lymphocytes that have already been infected with HIV but this effect depends on protein G signalling because it is sensitive to pertussis toxin\(^{(80)}\). Dolei et al. suggested that CC-chemokines may induce viral entry of X4 strains in peripheral blood mononuclear cells (PBMC) as a consequence of the accumulation of CXCR4 transcript. Kinter et al published that CC-chemokines have no direct effect on CXCR4 expression, however, they induce the co-localization of CD4 and CXCR4, facilitating X4 strains entry into cells\(^{(81, 82)}\). Other authors have suggested that the signalling of chemokines through G proteins activates pathways involved in the regulation of viral replication, such as integration or reverse transcription. CXCL12 may also have opposite effects on viral replication because it has been described that it induces viral replication by stimulating the transcription of the provirus through Tat (an accessory gene of HIV-1)\(^{(76)}\).

Other chemokines have been described to affect HIV-1 replication, although they are not involved in the entry process. For example, interferon-γ-inducible protein 10 (IP-10/CXCL10) and monocyte chemotactic protein-1 (MCP-1 or CCL2) have been detected in the cerebrospinal fluid of HIV individuals. IP-10 has been described to induce viral replication in monocytes\(^{(83)}\), whereas MCP-1 is one of the major chemoattractants for monocytes\(^{(84)}\). The central nervous system is often affected in the course of HIV infection, with HIV-associated dementia occurring in approximately 15% of individuals with AIDS. The monocyctic lineage is the major cell type infected with HIV in the brain. Besides, IP-10 and MCP-1 are produced by astrocytes treated with HIV Tat\(^{(85)}\).
It has been suggested that once the virus has reached the brain, viral proteins may stimulate the production of MCP-1 and IP-10, which would recruit monocytes to the brain and could induce viral replication. Another chemokine that may also affect HIV-1 replication is IL-8 (CXCL8). It is a potent chemotactic factor for neutrophil granulocytes and T lymphocytes. Increased levels of circulating IL-8 have been detected in HIV-infected individuals. It has been suggested that during HIV infection, IL-8 may play an important role in the recruitment of CD4 T cells to the lymph nodes, where continuous viral replication takes place.

CYTOKINES AND THE INFLAMMATORY RESPONSE TO HIV INFECTION

The production of pro-inflammatory cytokines during viral infection is essential for the development of innate immunity. Early inflammatory responses are also important for the development of adaptive immunity, since they are involved in the process of generating antigen-specific effector cells. HIV infection elicits the production of a broad range of cytokines and chemokines as part of the inflammatory response (Table I).

The acute host response to HIV-1 infection is characterized by a cytokine profile that includes proinflammatory cytokines (IL-1, IL-6, TNF-α and INFα/β/γ) and anti-inflammatory cytokines (IL-4, IL-10 and IL-13) with a peak in INFγ expression. At later stages of the infection the pattern of cytokine production shifts, showing a peak in TNF-α, IL-10 and IL-6 expression. Apart from the chronic activation status of the immune system, HIV may promote cytokine production by the direct action of viral proteins: HIV-1 glycoprotein gp120, which interacts with CD4, CXCR4 and CCR5, induces the secretion of IL-1, IL-6, TNF-α, INFα/β/γ, IL-4, IL-13 and IL-10. HIV-1 Tat, a regulatory protein essential for viral replication, stimulates the production of many cytokines, such as IL-2, IL-6, IL-8, TGF-β1, TNF-α and MCP-1.

HIV-1 Nef can also induce the production of IL-1β, IL-6, IL-10, IL-15, TNF-β and INF-γ.

The biphasic response in cytokine expression may indicate the involvement of different cell types along the course of the infection. Although cytokines are produced by different cell types, the main producers are T cells and macrophages. CD4 T cells can be divided into two groups (Th1 and Th2) according to the type of cytokines they produce. Th1 cells secrete IL-2 and INF-γ, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13. Cytokines produced by Th1 cells are involved in cellular immune responses, such as proliferation of cytotoxic T cells (CTLs), whereas Th2 responses are characterized by reduced or undetectable cellular response accompanied by an increase in B cell activity. In 1993, Clerici and Shearer presented the Th1-Th2 hypothesis of HIV-1 infection. According to this hypothesis, in HIV-1 infected individuals a reduction of Th1 cell activity and an augmentation of Th2 cell activity would be taking place along with the progression of the disease. This shift would generate a change in cytokine balance that would be critical for the development of AIDS. One possible explanation for this effect would be the differential effects of Th1 (INF-γ and Th2 (IL-4) cytokines on CXCR4 and CCR5 expression and accessibility by HIV. In T cells, IL-4 up-regulates the expression of CXCR4 and CC-chemokines (i.e. CCL5) and down-regulates CCR5, whereas INF-γ is able to induce CC-chemokine secretion. In fact, Th1 cells express 4 times more CCR5 than Th2, whereas CXCR4 is expressed 4 times more in Th2 than Th1 cells. IL-18 promotes Th1 and Th2 polarization, depending on the profile of other circulating cytokines. It has been hypothesized that during infection, when accompanied by INF-γ and IL-12, IL-18 is capable of inducing Th1 responses and enhancing T and NK-cell cytotoxicity. During the late state of the disease, when INF-γ and IL-12 are diminished, chronic elevation of IL-18 may promote Th2 immune responses and persistent viral replication.

However, whether progression of HIV-1 disease is associated with a shift from Th1 to Th2-type response remains controversial.

INTERLEUKIN-7 (IL-7) AND CD4 T CELL HOMEOSTASIS

Apart from the inflammatory process, cytokines are also involved in important homeostatic functions. Possibly, IL-7 is the main cytokine in the maintenance of CD4 T cell homeostasis in HIV-1 infected individuals. The loss of peripheral blood CD4 T cells that hallmark HIV infection is biphasic, with a weak drop that characterizes the chronic phase, followed by a rapid and massive loss of T cells in the last phase of the infection. During the chronic phase, the CD4 T cell pool size appears to be constant probably because of the immune system’s capacity to repopulate after cellular depletion. Two alternative mechanisms are possible: 1) a thymus-dependent de novo generation of naive T cells and 2) the peripheral proliferation of mature CD4 T cells in a thymus-independent manner. IL-7 has been shown to induce the generation of CD4 T cells by these two mechanisms. There are evidences supporting both hypothesis; on one hand, 50% of HIV-1 infected individuals have abundant thymic tissue. An increase in thymic tissue and in the T cell rearrangement excision circles (TRECs, a surrogate for thymic tissue) may be a sign of thymic hypoplasia or thymic regeneration after effective antiretroviral therapy.
### TABLE I. Summary of cytokines and their relation to HIV infection*

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cell source</th>
<th>Normal function</th>
<th>Relation to HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-1</strong></td>
<td>Monocytes, lymphocytes, endothelia, keratinocytes, epithelia, microglia</td>
<td>Induces IL-6 production, haematopoiesis, co-stimulates T cells, acute-inflammation response</td>
<td>Induces viral replication in vitro by activation of NFkB Elevated levels in HIV+ patients</td>
</tr>
<tr>
<td><strong>IL-2</strong></td>
<td>Activated T cells (Th1)</td>
<td>Stimulates proliferation and cytolytic activity of activated T cells, T cell differentiation, and thymocyte and B cell proliferation, induces secretion of INFγ, IL4 and TNF, enhances NK and monocyte activities</td>
<td>Impaired production in HIV+ patients</td>
</tr>
<tr>
<td><strong>IL-4</strong></td>
<td>T cells, mast cells, eosinophils, basophils</td>
<td>B cell proliferation and differentiation, T cell proliferation, monocyte activation and mast cell proliferation</td>
<td>Induces the production of CC-chemokines. Up-regulates CXCR4 and down-regulates CCR5</td>
</tr>
<tr>
<td><strong>IL-10</strong></td>
<td>Monocytes, B and T cells, epithelial cells, keratinocytes Th2</td>
<td>Inhibition of proinflammatory cytokine production Inhibition of IL2 production Inhibition of T cell activation and cytokine production B cell co-stimulator</td>
<td>High concentration: inhibits HIV replication Low concentration: induces HIV replication</td>
</tr>
<tr>
<td><strong>IL-12</strong></td>
<td>Monocytes/macrophages</td>
<td>Activates NK and T cells Induces INFγ production by NK and T cells Induces Th1 development</td>
<td>Inductive and inhibitory effects on HIV replication in vitro Impaired production in HIV+ patients</td>
</tr>
<tr>
<td><strong>IL-13</strong></td>
<td>Activated T cells Mast cells B cells Th2</td>
<td>B cell growth and differentiation factor No effect on T cells Anti-inflammatory cytokine: inhibits pro-inflammatory cytokine production by monocytes</td>
<td>Down-regulates CD4, CXCR4 and CCR5 Inhibits HIV replication in macrophages</td>
</tr>
<tr>
<td><strong>IL-15</strong></td>
<td>Monocytes/macrophages, PBMCs...</td>
<td>Stimulation of activated T cells, B cells, NK cells</td>
<td>Stimulates HIV-specific CTL Impaired production in HIV+ patients</td>
</tr>
<tr>
<td><strong>IL-16</strong></td>
<td>CD8T cells, eosinophils, epithelial cells</td>
<td>Chemotactracts CD4 T cells, Eosinophils adhesion</td>
<td>Inhibits HIV replication</td>
</tr>
<tr>
<td><strong>TNF</strong></td>
<td>Monocytes/macrophages T cells</td>
<td>Cytotoxic for tumour cells Induces necrotic or apoptotic cell death Pro-inflammatory activity: induces IL1, IL6 and IL8</td>
<td>Induces viral replication in vitro by activation of NFkB Elevated levels in HIV+ patients</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>Monocytes/macrophages, T cells, epithelial cells and keratinocytes and other cells types</td>
<td>Induces responses in B cells, T-h cells, CTL and NK cells. Anti-inflammatory functions</td>
<td>Induces viral replication in vitro of T cells and macrophages by activation of NFkB. Elevated levels in HIV+ patients</td>
</tr>
<tr>
<td><strong>IL-7</strong></td>
<td>Stromal cells (thymus, bone marrow) and epithelial cells (keratinocytes)</td>
<td>Promotes B and T lymphoid development Monocyte cytokine secretion</td>
<td>High levels in HIV patients Induces HIV replication in vitro</td>
</tr>
<tr>
<td><strong>IL-18</strong></td>
<td>Monocytes/macrophages</td>
<td>Activates macrophages, NK and T cells Enhances IL1, TNF-α and IFN-γ production by activated T cells Regulates Th1 and Th2 responses</td>
<td>Induces viral replication in vitro Elevated levels in HIV+ individuals</td>
</tr>
</tbody>
</table>

*Data from references 108, 171-177.*
marker of thymus activity) has been observed in infected children after retroviral therapy (114, 115). An increase of thymocyte proliferation and in TREC levels after exogenous addition of IL-7 in human thymic tissue in culture has also been described (116). Conversely, an increase of peripheral T cell proliferation in response to IL-7 (measured as Ki-67 expression) was observed in humans and a macaque model (117).

Furthermore, in thymectomized and T cell depleted mice, IL-7 treatment is able to reconstitute the immune system by peripheral proliferation of the injected T cell inoculum (118). IL-7 is produced by stromal cells from the thymus and from the bone marrow, but other tissue cells may also produce IL-7, including the intestinal epithelium, keratinocytes, or liver and dendritic cells (119-122). IL-7 plays an important role in the development of immature B and T cells. Mutations that prevent CD127 (IL-7 receptor, IL7r) expression can induce a severe immunodeficiency in mice, and exogenous IL-7 increases TCR rearrangement, inducing the novo generation of naïve T cells (123-126). IL-7 can also modulate mature T cell function: it acts as a co-stimulator of T cells, it induces CXCR4 expression in CD4 T cells and it inhibits programmed cell death (apoptosis) through the production of proteins from the Bcl-2-molecular family (116, 127-131).

In vivo, IL-7 levels augment as a part of the homeostatic response to lymphopenia. In fact, a strong negative correlation between the CD4 T cell number and plasma IL-7 levels in HIV-1 infected patients has been described (132, 133). However, it is still unclear whether IL-7 levels augment because of an increase in free IL-7 in plasma, as a consequence of the reduction in the number of T cells that express IL7R (the

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**TABLE II. Summary of chemokines and their relation to HIV infection**

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Cell source</th>
<th>Normal function</th>
<th>Relation to HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL5 (RANTES)</td>
<td>NK and T cells, Monocytes, Basophils, Platelets, Epithelial cells</td>
<td>Chemoattractant of monocytes, lymphocytes, NK, eosinophils and basophils.</td>
<td>Suppresses HIV replication in vitro by blocking R5 strains entry into cells, Stimulates viral replication, Elevated levels in HIV+ individuals</td>
</tr>
<tr>
<td>CCL3 (MIP-1α)</td>
<td>Haematopoietic cells, Macrophages, Eosinophils, Mast cells, Platelets, Langerhans cells</td>
<td>Pro-inflammatory properties: chemoattractant for T and B cells, Its production is suppressed by IL10, IL4, INF-γ.</td>
<td>Suppresses HIV replication in vitro by blocking R5 strains entry into cells, Stimulates viral replication</td>
</tr>
<tr>
<td>CCL4 (MIP-1β)</td>
<td>Macrophages, T cells, Platelets, Langerhans cells</td>
<td>Chemoattractant of monocytes, T cells, NK.</td>
<td>Suppresses HIV replication in vitro by blocking R5 strains entry into cells, Stimulates viral replication</td>
</tr>
<tr>
<td>CCL2 (MCP-1)</td>
<td>Monocytes, Fibroblasts, Epithelial cells, Keratinocytes</td>
<td>Chemoattractant for monocytes, T cells and NK cells, Its production is induced by IL-1, INF-γ, TNF-α, PHA...</td>
<td>Stimulates HIV replication; Induction in AIDS dementia</td>
</tr>
<tr>
<td>CXCL10 (IP-10)</td>
<td>Endothelial cells, Monocytes, Fibroblasts, Keratinocytes</td>
<td>Chemoattractant for Tcells, monocytes and NK cells, Modulation of adhesion molecule expression, Development of Th1 cells</td>
<td>Stimulates HIV replication; Induction in AIDS dementia</td>
</tr>
<tr>
<td>CXCL12 (SDF-1)</td>
<td>Thymus and bone marrow stromal cells, Dendritic cells, Mucosal epithelia</td>
<td>Chemoattractant for monocytes, B and T cells, haematopoietic progenitors</td>
<td>Suppresses HIV replication in vitro by blocking X4 strains entry into cells, Stimulates viral replication</td>
</tr>
<tr>
<td>CXCL8 (IL8)</td>
<td>Monocyte and Lymphocytes, Endothelial and epithelial cells, Keratinocytes</td>
<td>Neutrophils chemotaxis and activation, T cell chemotaxis, keratinocytes chemotaxis and mitogenesis...</td>
<td>Elevated levels in HIV+ individuals, Recruitment of lymphocytes to sites of infection</td>
</tr>
</tbody>
</table>

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CYTOKINES AND CHEMOKINES IN ANTI-HIV THERAPY

The use of cytokines and chemokines as possible therapeutic agents is a controversial point. Highly active antiretroviral therapy (HAART), that is, combination of 2, 3 or more antiretrovirals belonging to at least two different classes of drugs, has substantially modified the well being and life expectancy of HIV positive individuals and constitutes, nowadays, the paradigm of anti-HIV treatment. However, immune reconstitution, restoration of an effective immune response and the elimination of a latent viral pool in cell reservoirs or tissue compartments has not been achieved in the vast majority of HIV positive individuals that advance to AIDS. Alternative or complementary treatment options are needed.

Interleukin-2 (IL-2) is a key regulator of T cell function. It stimulates proliferation of activated T cells, causes the proliferation of resting T cells and also stimulates the production of other cytokines such as INF-γ, IL-4 and TNF. IL-2 is produced by CD4 and CD8 T cells. A defect in IL-2 production has been associated to HIV-1 infection and immunodeficiency. IL-2 treatment was expected to improve immune responses. It was also hypothesized that it could be useful in eradicating the latent viral pool by the activation of infected T cells in the presence of HAART. IL-2 has been evaluated in a large number of clinical studies, and its use has been associated with consistent improvements in CD4 T cell numbers\(^{137}\). However, increases in plasma HIV-1 RNA levels and worsening in clinical status have been observed in some patients. Thus, despite a large amount of data on HIV-infected patients, no clear-cut consensus has been reached on the beneficial effects of IL-2 treatment.

More recently, IL-7 has been also proposed as an adjuvant in antiretroviral treatment. It has an effect on thymopoiesis and it is able to stimulate the latent viral reservoirs by thymus-independent proliferation. These effects have been thought as relevant in the context of an immune-based treatment of HIV infection. Some authors support the use of IL-7 in therapy because it induces activation of quiescent cells. IL-7 would allow viral replication but the virus would be restrained by treatment with HAART. This strategy would eliminate the virus from its latent reservoir\(^{138-140}\). IL-7 may induce proviral reactivation from resting CD4 T cells from HIV-infected patients on HAART regimen\(^{139}\). However, studies made in PBMCs in vitro and in thymic tissue ex vivo have shown that IL-7 induces HIV-1 replication\(^{141, 142}\). Our own studies do not support the use of IL-7 as an immuno-
therapy agent for two reasons. First, the association between the X4 viral phenotype and elevated plasma IL-7 levels, and second, its possible effect on CXCR4 up-regulation in vivo. It may be possible that in patients with R5 strains, IL-7 treatment may favour the emergence of the more pathogenic X4 strains, accelerating disease progression.

Chemokines CCL3, CCL4, CCL5 and CXCL12 have also been proposed as possible alternatives to antiretroviral therapy because of their capacity to inhibit viral replication in cell culture. Whilst this approach has not been successful, it has paved the way to the development of chemokine analogues and chemokine receptor antagonists as potent anti-HIV agents in cell culture and in humans.

The identification of chemokine receptors as cofactors for HIV entry has suggested new targets for inhibition of HIV entry. Natural chemokines block HIV entry and infection mediated by the corresponding chemokine receptor, either by direct blockadage or by downregulation of the receptor. These include the ligands for CCR5 and CXCR4, or minor co-receptors: CCR3, CCR4 and CCR8. Alternatively, chemokine-based synthetic peptides such as Aminoosypentane (AOP)-RANTES and N-nonanoyl (NNY)-RANTES and N-(nonanoyl)-des-Ser (PSC)-RANTES, which induce CCR5 internalisation, have been also developed. Similarly, small molecule inhibitors of chemokine receptors have already been identified. Agents that block CXCR4 include small peptides (Allelix-40-4C, T22 and its analogues), SCH-C and SCH-D, from Shering-Plough, and Pfizer’s Maraviroc (UK-427,857) as well as a CCR5 antagonist from GlaxoSmithKline function and mostly reside in effector lymphoid tissue in the gut.

The role of chemokines in HIV-1 pathogenesis must be considered in the context of the cytokine regulatory network. The complexity of the interplay between cytokines, chemokines and HIV-1 makes it difficult to assess the role of a single cytokine/chemokine in the regulation of HIV-1 replication in vivo. Apart from the host factors that we have stated here, other host factors, viral factors and the presence of other opportunistic pathogens may be altering CXCR4 and CCR5 expression and the production of cytokines and chemokines, which will finally influence viral replication and pathogenesis.

A most relevant caveat in our understanding of the complex role of cytokines and chemokines in HIV infection is the fact that most experimental findings and their conclusions stem from work done in peripheral blood cells and secondary lymphoid organs. A new paradigm could be established by recent findings suggesting that HIV pathogenesis is derived from a massive destruction of immune cells in the gut during the early phase of acute infection. The slow decay of the immune system may only be a reflection of what has already happened but went unnoticed. That is, prior to the establishment of chronic HIV infection, direct and overwhelming cell killing has taken place in the gut but driven by strong chemokine signalling of peripheral and other lymphoid tissue cells to effector lymphoid tissue where they are targeted for destruction. The fact that memory, CCR5+ CD4 T cells are the ones permissive for HIV infection and mostly reside in effector lymphoid tissue in the gut suggests that new CCR5-blocking agents in development may play a prominent role in the treatment of HIV infection.

CONCLUDING REMARKS. THE CONJUROR HAS ALREADY DONE THE TRICK

The role of chemokines in HIV-1 pathogenesis must be considered in the context of the cytokine regulatory network. The complexity of the interplay between cytokines, chemokines and HIV-1 makes it difficult to assess the role of a single cytokine/chemokine in the regulation of HIV-1 replication in vivo. Apart from the host factors that we have stated here, other host factors, viral factors and the presence of other opportunistic pathogens may be altering CXCR4 and CCR5 expression and the production of cytokines and chemokines, which will finally influence viral replication and pathogenesis.

ACKNOWLEDGEMENTS

Work in our laboratory has been supported by grants from the Ministerio de Educación y Ciencia (MEC) project BFI-2003-00405, FIPSE 36293/02B, Fondo de Investigación Sanitaria (FIS), Fundació Marató de TV3 (project 020930) and the European TRIOH Consortium (LSHB-CT-2003-503980).

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