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Innate immune defenses in HIV–1 infection: prospects for a novel immune therapy

Carlos J Montoya, Maria T Rugeles and Alan L Landay†

HIV-1 infection leads to a severe decrease of CD4+ T lymphocytes, dysregulation of several leukocyte subpopulations and generalized immune activation, with the subsequent development of opportunistic infections and malignancies. Administration of highly active antiretroviral therapy (HAART) has been successful in reducing HIV-1 plasma viremia; however, the ability of HAART to restore immunocompetence appears incomplete, particularly in patients with chronic and advanced disease. Several components of the innate immune system have direct anti-HIV-1 effects, and studies to analyze the benefits of enhancing the function of the innate response during HIV-1 infection are increasing. Development of any complementary therapeutic approaches to HIV-1 infection, particularly those able to compensate for the limitations of HAART, and enhance the anti-HIV-1 innate immune activity would be of interest. The stimulation of innate immune responses using Toll-like receptor agonists, such as monophosphoryl lipid A and oligodeoxynucleotides with CpG motifs, are currently being investigated and their benefit in HIV-1-infected patients are under evaluation.

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CONTENTS

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HIV-1 infection & AIDS

HIV-1, the etiological agent of AIDS, causes a progressive loss of $CD4^+$ T cells associated with other quantitative and qualitative dysregulations of the immune response [1,2]. Currently, HIV-1 infection is among the most important public health problems worldwide; at the end of 2005, there were 40.3 million people infected and, in that year, 4.9 million new cases were diagnosed and 3.1 million HIV-1-infected individuals died as a consequence of HIV-1-associated clinical complications [3].

The immunopathogenesis of HIV-1 infection involves multiple interactions between the virus and the host's immune system. Although there is activation of both innate and adaptive immune responses, the infection is fought with limited success. The dysregulation of the immune response appears early during HIV-1 infection characterized by the loss of T-lymphocyte proliferative responses to recall antigens, alloantigens and mitogens before the severe reduction in $CD4^+$ T-cell count occurs [2]. In addition to the direct elimination of $CD4^+$ T cells, HIV-1 replication may directly impair immune function through the immunosuppressive effect of viral proteins. Furthermore, chronic HIV-1 infection is characterized by a state of uncontrolled immune activation that leads to immunosuppression and accelerated $CD4^+$ T-cell death, resulting in severe immunodeficiency [4,5].

Despite nearly 20 years of research aiming to induce adaptive immune responses to HIV-1, no successful immunological therapy or vaccine has been developed. The current approved therapy for HIV-1 infection is highly active antiretroviral therapy (HAART), which decreases plasma HIV-1 RNA levels and increases the $CD4^+$ T-cell count. However, after HAART is discontinued, there is a rebound in viral load and a decrease in $CD4^+$ T-cell counts to levels similar to that before HAART administration. Furthermore, potent antiretroviral therapy administered for several months is unable to eliminate HIV-1 tissue reservoirs effectively and does not lead to a full recovery of immune

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responses [6]. Therefore, the cost and complexity of HAART regimens, the growing list of long-term side effects and the eventual development of resistance have underscored the immediate need for additional therapeutic approaches.

Potential pathogens are encountered on a daily basis, and most of them are detected and destroyed rapidly by innate immune responses. Reports from investigations in the last 10 years suggest that innate immunity plays more than a passive role during the evolution of HIV-1 infection. The importance of innate immune mechanisms in controlling HIV-1 infection is currently an area of intense interest, as several components of the innate immune system have direct anti-HIV-1 activity [7]. Considering the role of innate immunity in inducing long-lasting adaptive responses, and the fact that innate responses should not be affected by viral escape mutants, novel approaches should be developed to elicit this innate immune activity during HIV-1 infection.

Innate immune system

The innate immune system is an evolutionarily ancient and universal form of host defense found in all multicellular organisms. Innate immunity is not a function of a single defined cellular or soluble physiological element; rather, this response is very heterogeneous and consists of a wide variety of cells and soluble proteins that accomplish both recognition and effector functions [8]. This system is the first line of defense against invading pathogens and is particularly important in controlling bacterial and viral infections: innate immune cells patrol peripheral tissues and their rapid recruitment and activation at epithelial and mucosal surfaces is a hallmark of acute inflammatory responses.

The innate immune system uses at least two distinct strategies of immune recognition: recognition of 'microbial nonself' and recognition of 'missing self' [8]. In the first strategy, rather than recognizing small specific antigenic sequences, cells of the innate immune system have evolved germ line-encoded receptors (pattern recognition receptors [PRRs]) capable of broadly recognizing macromolecular patterns characteristic of pathogenic microbes (pathogen associated molecular patterns [PAMPs]). PAMPs recognition is the simplest way that a limited number of cellular PRRs can distinguish a large subset of potential pathogens. Innate immune PRRs not only recognize conserved microbial structures directly, as signatures of the class of pathogen, but also trigger an effector response that is tailored to the pathogen. The second strategy is based on the recognition of molecular structures expressed only on normal uninfected cells of the host. These structures function as molecular markers of normal-self as microorganisms do not produce them, and their expression is lost on infected and transformed cells.

Toll-like receptors (TLRs) are the most well-known PRRs expressed on cells of the innate immune system, which mediate nuclear factor (NF)-κB activation by a variety of bacterial, mycobacterial, fungal, spirochetal and viral PAMPs [9]. Currently, in humans, ten TLRs have been cloned and described: TLR-4 is the primary signaling receptor for enteric Gram-negative bacterial lipopolysaccharide (LPS) and chlamydial heat-shock protein 60, whereas TLR-2 is the signaling receptor for Gram-positive bacterial cell-wall components, bacterial, mycobacterial and spirochetal lipoproteins and yeast molecules (zymosan). TLR-3 transduces the response to double-stranded RNA and TLR-5 is the receptor for bacterial flagellin. TLR-7 and -8 have recently been shown to mediate response to single-stranded RNA and imidazoquinoline compounds, which have antiviral and antitumor activities. TLR-9 is the receptor for bacterial and viral DNA and synthetic oligodeoxynucleotides containing short unmethylated CpG dinucleotides. By the means of TLRs, the innate immune system is activated only by danger signals originating from pathogens or by injured cells, which induce both expression of costimulatory molecules and release of cytokines and chemokines [9].

The cellular innate immune response is very heterogeneous and utilizes complex mechanisms to eradicate invading pathogens. Macrophages and natural killer (NK) cells are innate effectors that rapidly lyse infected cells, while plasmacytoid dendritic cells (pDCs) produce large amounts of α-interferon (IFN)-α to induce an antiviral state. The innate immune response provides time for the subsequent development of adaptive immune responses; myeloid dendritic cells (mDCs) and CD1d-restricted TCR-invariant NK T (iNKT) cells can bridge these two major immune systems and contribute to both the very rapid (innate) and the adaptive immune responses.

Dendritic cells

Dendritic cells (DCs) have a fundamental role in the activation and function of both innate and adaptive immune responses. In peripheral blood, DCs are heterogeneous and mostly immature, and comprise at least two major populations, mDCs and pDCs, which differ widely in many respects: they express different patterns of pathogen recognition receptors and respond to different microbial antigens. Furthermore, they express different chemokine receptors and secrete different cytokines [10,11].

The mDCs ($CD33^+$, $CD11c^+$, $HLA\text{-}DR^+$, but negative for the lineage markers CD3, CD14, CD16, CD19, CD20 and CD56) are sentinels patrolling peripheral tissues where they can efficiently intercept and capture invading pathogens either by phagocytosis, macropinocytosis or endocytosis [10]. Antigen capture triggers mDC maturation and migration to the secondary lymphoid tissue, where they present pathogen-derived antigens to specific naive T cells to initiate the adaptive immune response.

The pDCs ($CD123^+$, $HLA\text{-}DR^+$ and negative for CD11c, CD33 and lineage markers) migrate directly from blood to secondary lymphoid tissues and are potent producers of type-I IFNs; each pDC produces up to 1000-times more IFN-α than any other cell type in the body [12]. Therefore, they play an important role in controlling viral infections.

Natural killer cells

NK cells are a subpopulation of bone marrow-derived large granular lymphocytes; they are present as a mature population in blood and spleen, and at low frequencies in lymph nodes

where they accumulate following local stimulation. NK cells spontaneously kill tumor cells without prior sensitization; also, their activities are important in controlling viral infections, especially during early phases [13]. Furthermore, NK cells exhibit cytolytic activity towards antibody-coated cells (antibody-dependent cellular cytotoxicity [ADCC]) and allogeneic cells. NK cells also mediate noncytolytic suppression of viral replication through the secretion of several chemokines, such as CCL3 (also known as MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES), and cytokines, such as IFN-γ, tumor necrosis factor (TNF)-α and granulocyte–macrophage-colony stimulating factor (GM-CSF) [13].

NK cells are defined phenotypically by the expression of CD16, CD56, CD57 and NKR-P1 (CD161) molecules on their surface. However, these molecules are not specific for NK cells since a fraction of other cell types, such as $CD8^+$ cytotoxic T lymphocytes and iNKT cells, may also express them. Based on the relative expression of the cell surface markers CD16 and CD56, two distinct subsets of NK cells have been described. The $CD16^{low/-}CD56^{hi}$ subset corresponds to less than 10% of peripheral blood NK cells and its main function is the regulation of other cell types through elaboration of chemokines and cytokines. The $CD16^{hi}CD56^{low}$ subset constitutes 90% of circulating NK cells and is mainly responsible for natural cytotoxicity and ADCC [14].

A complex balance of inhibitory and stimulatory receptors expressed on the surface of NK cells regulates their activation. In recent years, many major histocompatibility complex (MHC) class I-binding receptors have been discovered on NK cells. These NK receptors are clonally expressed on overlapping subsets of NK cells and regulate NK cell function by binding to ligands expressed on the surface of target cells. NK receptors with MHC restriction are not homogeneous as these receptors can be classified into two main groups: killer cell immunoglobulin-like receptor and C-type lectin-like receptors of the NKG2/CD94 family. Recently, three novel surface molecules specific for NK cells were identified: NKp46, NKp30 and NKp44. They represent the first members of a novel emerging group of receptors collectively termed natural cytotoxicity receptors. The available data suggest an important role for these receptors in recognizing tumor cells [15].

iNKT cells

Natural killer T (NKT) cells, a rare subset of $CD3^+$ T lymphocytes, are considered part of the innate immune system and constitute a lymphocyte lineage sharing characteristics of both T and NK cells. A subgroup of NKT cells recognizes glycolipid antigens in the context of the monomorphic antigen-presenting molecule CD1d. Most of the CD1d-restricted NKT cells display an invariant TCR repertoire, in humans consisting of an invariant Vα24 chain preferentially paired with a semi-invariant Vβ11 chain (human iNKT cells) [16].

The first natural ligands recognized by iNKT cells in murine systems have been recently defined and consist of glycosylceramides from the cell wall of *Sphingomonas* [17]. Alternatively, Gram-negative, LPS-positive *Salmonella typhimurium* activate iNKT cells through the recognition of an endogenous lysosomal glycosphingolipid, iGb3, presented by LPS-activated DCs [17]. The synthetic molecule α-galactosylceramide (α-GalCer), which is a glycosphingolipid originally derived from a marine sponge, also activates both human and mouse iNKT cells in the context of CD1d-expressing antigen-presenting cells [18].

A potential role of iNKT cells in the regulation of innate and adaptive immunity has been hypothesized based on their apparent flexibility to rapidly release large amounts of T helper cell (Th)1 or Th2 cytokines (mainly IFN-γ and interleukin (IL)-4, respectively) upon activation [19]. iNKT cells play crucial roles in various types of immune-mediated responses, including antitumor, antimicrobial, tolerance and autoimmune reactions. There are few reports describing the frequency and phenotype of human iNKT cells and their subsets ($CD4^+$, $CD8^+$, $CD4^-/CD8^-$) in peripheral blood and other lymphoid tissues [20].

Innate immunity during HIV-1 infection

HIV-1 infection is associated with a significant activation of the immune system, but this does not lead to an effective adaptive immune response. It is well known that HIV-1 has a remarkable ability to generate mutations in almost all the specific antigenic sequences recognized by the adaptive immune system, therefore creating escape mutants [2]. Interestingly, the innate immune system recognizes pathogens by the pattern of their microbial surface components rather than by specific antigenic sequences. As the first line of defense, owing primarily to its tissue distribution and rapid activation, the innate immune system could potentially prevent HIV-1 transmission, and provide the time and conditions for adaptive immune responses to develop [7]. As several components of the innate immune system have direct anti-HIV-1 activity, the roles of innate immune mechanisms in controlling HIV-1 infection are currently an area of intense investigation.

It has also been demonstrated, however, that several innate immune cells are susceptible to HIV-1 infection as they express CD4, CCR5 and CXCR4 coreceptors for HIV-1 entry [21–23]. Different functional and quantitative alterations in cells of the innate immune system have been characterized in HIV-1-infected individuals (BOX 1) [24–26]. Patients with HIV-1 infection at both early and late stages of disease exhibit immunoregulatory defects, such as autoimmunity, tumors and opportunistic infections that might precede $CD4^+$ T-cell depletion. Abnormal frequency or function of innate immune cells during HIV-1 infection may contribute to the functional impairment of HIV-1-specific $CD4^+$ T cells and might be responsible for the reduced cytokine responsiveness [27–29]. However, in HIV-1-infected individuals the correlation of the loss or dysfunction of innate immune cells and disease progression on response to antiretroviral therapy is not well established.

Box 1. Innate immune response alterations observed in HIV–1–infected patients.

- Reduced number of pDCs, mDCs, NK and iNKT cells
- Decreased expression of costimulatory molecules: CD80 and CD86
- Defects in antigen presentation by mDCs and macrophages
- Reduced production of interferon-α by pDC
- Abnormal activity of NK cells: low expression of natural cytotoxicity receptors and increased expression of several inhibitory receptors (KIR)
- Low production of perforin in cytotoxic-cell granules from lymphoid tissues
- Functional alterations in neutrophils including low chemotactic response and abnormal respiratory burst

iNKT: CD1d-restricted T cell receptor-invariant natural killer T cells; KIR: Killer immunoglobulin-superfamily receptor; mDC: Myeloid dendritic cells; NCR: Natural cytotoxicity receptor; NK: Natural killer; pDC: Plasmacytoid dendritic cell.

Dendritic cells & HIV-1 infection

Owing to their tissue distribution and critical role in inducing immune responses, DCs have been postulated as immune elements with potential anti-HIV-1 activity. Through the production of IFN-α, pDCs mediate a variety of important rapid antiviral and antitumor activities. IFN-α blocks HIV-1 replication, and activates the function of other innate immune cellular components, such as NK and iNKT cells [30,31]. Furthermore, IFN-α increases the recognition of HIV-1 by the adaptive immune system by enhancing MHC class I and B7 expression on antigen-presenting cells [32].

Some clinical observations support the anti-HIV-1 activity of pDCs and IFN-α. In HIV-1-infected patients who had limited lesions of Kaposi's sarcoma, the numbers of pDCs remain within the normal range, while in HIV-1-infected patients who continue developing new lesions the pDC number was persistently low. Moreover, normal pDC frequency and IFN-α production have been found in individuals who remained healthy, despite being infected for more than 10 years and having very low $CD4^+$ T-cell counts; these patients were not receiving any treatment for HIV-1 nor infected by other pathogens [24].

On the other hand, immature DCs express the molecules required for viral entry [33]. Furthermore, the HIV-1 envelope glycoprotein (gp)120 can interact with several C-type lectin molecules expressed on the surface of DCs, such as DC-SIGN (CD209) and mannose receptor [34,35]. Viral infection of DCs isolated both from peripheral blood and lymphoid tissues has been demonstrated in HIV-1-infected individuals. DCs can be productively infected by HIV-1, although with a lower efficiency than $CD4^+$ T cells and macrophages [36,37].

Quantitative and qualitative abnormalities in DCs induced by HIV-1 have been reported in the last few years. Both mDC and pDC numbers may be reduced in peripheral blood, and most significantly in those patients with active viral replication [21,38]. DCs from HIV-1-infected patients show a reduced capacity to stimulate autologous T-cell proliferation and IL-2 production [39]. This functional impairment may be dependent on the reduced expression of costimulatory molecules, such as CD80 and CD86 [40]. Also, a decrease in IFN-α production by pDCs was associated with high viral load and the development of AIDS [24].

NK cells in HIV-1 infection

NK cells can contribute significantly to host defense against HIV-1 through the production of soluble factors and cytolytic mechanisms. β-chemokines produced by NK cells block entry of CRR5-tropic viruses to target cells by competitive inhibition of receptor binding [41]. NK cells can kill HIV-1-infected targets directly or through ADCC owing to the presence of gp120 antibodies in serum or mucosal sites. In addition, the role of NK cells in resistance to HIV-1 infection *in vivo* has been recently postulated. The NK cells from a group of Vietnamese intravenous drug users who remained HIV-1 seronegative despite exposure to HIV, produced considerably more β-chemokines, IFN-γ and TNF-α than did NK cells from HIV-1-positive individuals and HIV-1-negative volunteers [42]. A higher production of IFN-γ by NK cells from exposed seronegative individuals has also been suggested as a mechanism of natural resistance to HIV-1 infection [43].

By contrast, there are reports indicating that NK cell-mediated immunity is compromized in HIV-1-infected individuals. HIV-1 might exert its effects on NK cells in three ways: direct infection of NK cells, direct binding of HIV-1 to chemokine receptors at the surface of NK cells, and indirect effects as a result of generalized HIV-1-induced immune activation. Decreases in the frequency of peripheral blood NK cells have been observed in some studies [44], although functional impairment is more common during HIV-1 infection [45,46]. The reduction in the number of NK cells might be the result of direct infection, as a small subpopulation of NK cells expresses the molecules required for viral entry [22]. Alterations in NK-cell function appear early in the course of infection and persist at all stages of disease progression [47]. The secretion of β-chemokines by NK cells is deficient in HIV-1-infected individuals and correlates inversely with the level of viremia [48].

Regarding the expression of NK receptors in patients infected by HIV-1, normal or increased expression of killer cell immunoglobulin-like receptor molecules and a down-modulation of some activating natural cytotoxicity receptors have been demonstrated [49]. An aberrant expression of these receptors might impair the cytolytic and secretory functions of NK cells, and help in explaining alterations in cytotoxic activity observed in HIV-1-positive patients.

iNKT cells in HIV-1 infection

Currently, little is known about the potential role of iNKT cells in human infectious diseases. Although it has been reported that iNKT cells exert antiviral effects through IFN-γ, a cytokine that is also capable of inhibiting HIV-1 replication [50], little data is available on the role of iNKT cells in controlling HIV-1 infection. Indeed, the effects of HIV-1 infection on iNKT-cell frequency and function and the role of these cells in the immune response to HIV-1 and associated opportunistic infections have barely been explored [26,51,52].

iNKT cells are susceptible to HIV-1 infection as they express the molecules required for viral entry: CD4, CCR5 and CXCR4 [23,51]. Quantitative alterations of iNKT cells in HIV-1-infected patients have been reported [26,51,52]. Apparently, the $CD4^+$ iNKT cells constitute the main subpopulation affected by HIV-1 infection with a decreased frequency that correlates with viral load [51]. It can be postulated that the loss of iNKT cells in HIV-1-infected individuals could lead to autoimmunity or autoimmune-like disorders. Also, diminished iNKT cell-mediated antitumor responses could contribute to an increased incidence of tumors such as Kaposi's sarcoma and non-Hodgkin's lymphoma in AIDS patients.

Immune reconstitution with HAART

The current drug treatment for HIV-1 infection is HAART, which produces dramatic decreases in plasma HIV-1 RNA levels and increases in $CD4^+$ T-lymphocyte count leading to marked decreases in the incidence of opportunistic infections and mortality [53]. Beside the virological effects, HAART has improved immunological function in advanced, moderate and early HIV-1 disease, as demonstrated by improved *in vitro* responses to recall antigens and polyclonal mitogenic stimuli [54–57]. However, responses to immunization are heterogeneous among HAART-treated patients, even when $CD4^+$ T-cell counts are comparable.

Despite these beneficial effects of HAART, discordant results have been reported regarding the improvement of immunological function in HIV-1-infected individuals receiving this regimen [57,58]. HAART does not have the ability to normalize all immune system parameters, such as $CD4^+$ and $CD8^+$ T-cell hyperactivation. It is not clear whether HAART leads to recovery of the $CD4^+$ and $CD8^+$ T-cell repertoire, and it is likely that this is a delayed and incomplete process [59,60]. Furthermore, it is not known whether the persistent abnormalities will resolve with continued suppression of HIV-1 replication.

Potential explanations for incomplete immune restoration include residual viral replication, bone marrow and thymic insufficiency, persistent trapping of cells in lymphoid organs and irreversible damage of lymphopoietic organs after prolonged uncontrolled HIV-1 replication [6].

With the aim of determining the level of immune reconstitution with HAART, one study in HIV-1-infected individuals evaluated the restoration of responses to recall antigens after immunization, or the development of responses to neoantigens. Most patients with moderately advanced HIV-1 infection treated with HAART developed antibody delayed-type hypersentivity responses, and lymphocyte proliferative response (LPR) following immunization to both recall and neoantigens. However, LPR to tetanus toxoid tended to increase only modestly and transiently. The response was related to the number of naive and memory $CD4^+$ T cells and to the expression of the costimulatory molecule CD28 on $CD4^+$ cells. Also, the response was inversely related to the degree of immune activation and to plasma HIV-1 RNA levels [61].

Window of opportunity: immune restoration in early HIV-1 disease

It is predicted that HIV-1-infected individuals in early HIV-1 disease are the most likely group to achieve immune reconstitution following HAART. Results from recent investigations demonstrate that in those patients there was a significant increase in total, naive and memory $CD4^+$ T cells, and reduction of total and activated $CD8^+$ T cells and activated $CD4^+$ T cells. The LPR to anti-$CD3^+$ CD28 antibodies, antigens and allogeneic stimulation was restored. Interestingly and more importantly, the HIV-1-specific Th1 response was preserved [56]. Unfortunately, this strategy reduces the level of the HIV-1-specific $CD8^+$ cytotoxic T-cell response. These findings suggest that early intervention with potent HAART may reverse most of the immune defects induced by HIV-1 infection and the delay in HAART initiation might result in sustained functional immune impairment, even in persons with optimal $CD4^+$ T-cell increases and sustained viral suppression. The issue that is currently under evaluation is one of life-long therapy with HAART and the resulting problem with drug toxicity and the development of resistance.

Immune recovery in advanced disease

HIV-1-infected individuals with advanced disease respond both virologically and immunologically to therapy with a potent antiretroviral regimen, as was demonstrated by increase in total, naive and memory $CD4^+$ T lymphocytes and decrease in the proportion of activated $CD8^+$ DR^+ $CD38^+$ T cells; however, individuals older than 40 years of age demonstrated reduced immunological recovery [62]. Even if patients normalize the $CD4^+$ T-cell counts, the immunological function may remain impaired if patients delay initiation of HAART.

Little reconstitution of HIV-1-specific $CD4^+$ T-cell responses is found in patients with moderate or advanced disease, in contrast to patients with early disease, who show an improved response to specific HIV-1 proteins, such as p24 after the administration of HAART [56,63,64]. Also, HIV-1-specific $CD8^+$ cytotoxic T-lymphocyte levels declined after chronic suppression of viral antigens by HAART [55,65]. Therefore, immune-based therapy strategies aimed at restoring immunity in advanced HIV-1 disease could be a rational option in conjunction with HAART-mediated potent HIV-1 suppression.

Restoration of innate immunity with HAART

For antiretroviral therapies, a decrease in plasma HIV-1 RNA level is the accepted marker of virological control, whereas the $CD4^+$ T-cell count and the response to immunization with different antigens are the main markers of immune restoration. Usually, quantitative and functional parameters of innate immunity are not included in studies that evaluate the beneficial effect of HAART on immune reconstitution.

As observed for adaptive immune responses, several reports indicate that innate cell number and function, particularly DCs and NK cells, partially improve after HAART [46,66–68]. Regarding IFN-α, the production of this cytokine was reconstituted during HIV-suppressive therapy; reconstitution of IFN-α generation to levels commensurate with protection against opportunistic infection occurs prior to similar restoration of CD4 counts [69]. In the outcomes analyses, such immune reconstitution was associated with protection from recurrent or new opportunistic infection [69].

Is there a need for immune-based therapy?

Despite the fact that patients treated with HAART experience a significant decrease in the levels of HIV-1 RNA (<50 copies/ml), HIV-1 eradication has not been achieved. Furthermore, as stated previously, HAART alone does not lead to a full recovery of immune competence. It is clear that the goals of immune reconstitution should not only be to increase $CD4^+$ T-cell numbers and reduce T-cell activation but also lead to the production of functional innate and adaptive cells. In addition, the cost of HAART regimens, their frequent intolerance and adverse events, and the development of resistance have underscored the immediate need for additional therapeutic approaches [70–72]. Considerable efforts are focused on therapies that improve immune reconstitution and the overall clinical outcome (BOX 2).

Box 2. Immune-based therapies for HIV-1-infected patients. Strategies of immunotherapy.

- Blocking immune activation and apoptosis:
  - Cyclosporin A
  - Corticosteroids: prednisone
  - IL-10
  - TNF inhibitors: mAbs, thalidomide
  - Mycophenolate
  - Hydroxyurea
  - Fas/Fas-ligand inhibition
- Enhance production, expansion or functional activity:
  - IL-2
  - IL-7
  - IL-12
  - IL-15
  - IL-16
  - GM-CSF
  - Growth hormone
  - Adoptive immunotherapy
- Induction of specific anti-HIV-1 immunity:
  - Therapeutic vaccines
  - Structured therapeutic interruptions
  - Adoptive immunotherapy
- Other immunomodulators:
  - CpG ODNs
  - MPL
  - TLR-7 and -8 agonists
  - α-GalCer
  - IFN-α

α-GalCer: α-galactosylceramide; CpG ODNs: Oligodeoxynucleotides with CpG motifs; GM-CSF: Granulocyte-macrophage colony stimulating factor; IFN: Interferon; IL: Interleukin; mAb: Monoclonal antibody; MPL: Monophosphoryl lipid A; TLR: Toll-like receptor; TNF: Tumor necrosis factor.

Stimulation of innate immunity: prospects in HIV-1 infection

Considering the fundamental role of innate immunity in controlling viral infections and instructing adaptive immunity, additional therapeutic strategies aiming to enhance the innate immune response appear promising (FIGURE 1). An additional advantage of potentiating the innate immune response during HIV-1 infection is that this response will not be affected when the viral escape mutants arise. An interesting strategy is the use of activating signals mediated by receptors belonging to the TLR family, differentially expressed by innate immune cells. Synthetic and purified components of microbial extracts that exhibit strong stimulatory effects on innate immune cells could be used to accomplish this objective [73,74]. TLR agonists include a variety of lipids and glycolipids (LPS, monophosphoryl lipid A [MPL], mycolic acid, lipoarabinomannan, lipoteichoic acid), polynucleotides (poli-I:C, bacterial DNA, CpG oligodeoxynucleotides (ODNs) and double-stranded RNA), antiviral drugs (imidazoquinolines) and lipoproteins. Some of these TLR agonists have been evaluated as potential adjuvants in the generation of new immune responses utilizing protective vaccines; however, their use as tools to reconstitute immune function is still speculative given the paucity of data on their efficacy.

TLR-4 agonists: monophosphoryl lipid A

The attenuation of LPS toxicity by chemical procedures has allowed the production of a molecule referred to as MPL, which retains the immunostimulating activities of the parent LPS molecule but has no toxicity [75]. Recently, other analogs of MPL have been evaluated for adjuvant activity [76]. These molecules activate TLR-4 and increase the function of different

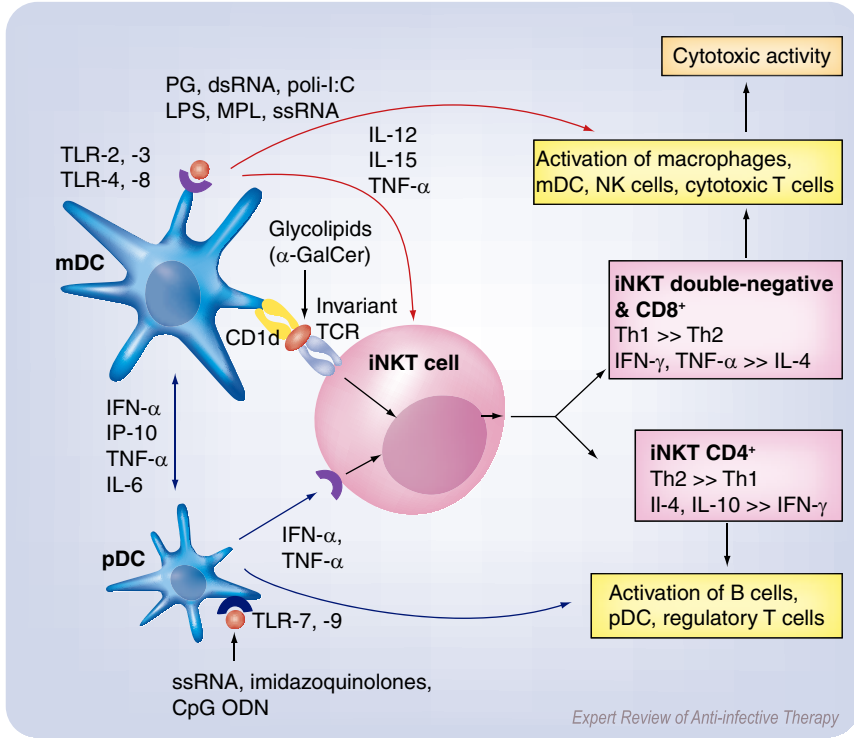

Figure 1. Crosstalk of innate immune cells activated with CD1d–restricted glycolipids and TLR agonists: potential role on immune reconstitution in HIV–1–infected individuals. Innate immune cells can be activated using synthetic molecules mimicking natural innate ligands. TLR-7 and -9 agonists (e.g., ssRNA, imidazoquinolines and CpG ODNs) enhance innate and adaptive immune responses through the stimulation of pDCs to secrete proinflammatory cytokines such as IFN-α and TNF-α. mDCs express TLR-2, -3, -4, -6 and -8 and are activated by agonists of these receptors (e.g., PG, dsRNA, poli-I:C, LPS, MPL, ssRNA and imidazoquinolines) to secrete the immunoregulatory cytokines IL-12, IL-15 and TNF-α. On the other hand, glycolipids (such as α-GalCer) function as antigens presented by CD1d to iNKT cells, delivering strong activation signals to secrete Th1 cytokines (IFN-γ, IL-2, TNF-α) by $CD8^+$ and double-negative iNKT cells, or Th2 cytokines (IL-4 and -10) by $CD4^+$ iNKT cells. All these cytokines, and probably cell-to-cell interactions, are important to enhance the functional activity of innate effector cells, such as macrophages and NK cells, to develop the maturation program in mDCs and pDCs and their antigen presentation activity, and to stimulate the establishment of adaptive responses mediated by $CD4^+$, $CD8^+$ and B lymphocytes. Altogether, these responses induced by innate immunity agonists might be important in the modulation and restoration of immune system responses of HIV-1-infected individuals.
α-GalCer: α-galactosylceramide; CpG ODNs: Oligodeoxynucleotides with CpG motifs; dsRNA: Double-stranded RNA; IFN: Interferon; IL: Interleukin; iNKT: CD1d-restricted TCR-invariant natural killer T cells; IP: Interferon-induced protein; LPS: Lipopolysaccharide; mDC: Myeloid dendritic cells; MPL: Monophosphoryl lipid A; NK: Natural killer; pDC: Plasmacytoid dendritic cells; PG: Peptidoglycan; ssRNA: Single-stranded RNA; Th: T helper cell; TLR: Toll-like receptor; TNF: Tumor necrosis factor.

antigen-presenting cells, enhancing both the cellular and humoral effector arms of immunity [75]. In the murine model, it was recently demonstrated that iNKT cells express TLR-4 and that LPS activates iNKT-cell and B-1-cell collaboration in an IL-4-dependent manner [77].

MPL has been administered safely to humans in various clinical vaccine trials [78]. It was observed that MPL combined with GM-CSF increased the immunogenicity of a vaccine with HIV envelope subunit [79]. Similarly, MPL was used as an adjuvant in an *in vivo* investigation to test the immunogenicity of the HIV-1 rgp120W61D; the induction of specific T-cell responses and secretion of β-chemokines were observed with this vaccine strategy [80].

MPL has not been directly evaluated as an immunotherapy for immune reconstitution in HIV-1-infected individuals; however, the authors preliminary evaluation of MPL stimulatory activity on innate immune cells from HIV-1-infected individuals showed that MPL increases the expression of CD80 and CD86 on mDCs and pDCs, and the activation marker CD69 on NK and iNKT cells. The costimulation with MPL and CpG ODNs synergistically upregulated the expression of CD69 on NK and iNKT cells [UNPUBLISHED RESULTS]. It would be important to continue exploring, *in vitro* and *in vivo,* the role of TLR-4-mediated costimulation on functional activity of innate immunity in HIV-1-positive individuals, and its effect on HIV-1 transcription and replication.

TLR-7 & TLR-8 agonists

Single-stranded RNA represents a physiological ligand for TLR-7 and -8 [81]; also, these receptors mediate signals after the interaction with antiviral drugs of the imidazoquinoline family (imiquimod and R-848). pDCs express TLR-7 and respond to imidazoquinolines enhancing the expression of costimulatory molecules and producing IFN-α and IL-12p70; mDCs express TLR-8 and respond to these molecules with phenotypic maturation and high secretion of IL-12p70 without producing detectable IFN-α [82]. These effects of TLR-7 and -8 agonists can be useful in enhancing the ability of DCs to activate virus-specific T cells and support the experimental and future clinical use of these agonists as adjuvants for vaccines or immunomodulating therapy. Alternatively, it was recently observed that distinct indirect pathways (IL-18, IL-12p70 and type-I IFN) control human NK-cell activation by TLR-7 and -8 agonists [83].

Studies with optimally TLR-7/-8 ligand-stimulated pDCs or mDCs exposed to cytomegalovirus (CMV) or HIV-1 antigens demonstrated that this treatment enhances autologous CMV- and HIV-1-specific memory T-cell responses as measured by effector cytokine production [82]. Also, nonhuman primates immunized with an HIV Gag protein and a TLR-7/-8 agonist had significantly increased Gag-specific Th1 and antibody responses [84]. These data point to the fact that TLR-7/-8 agonists may prove to be valuable agents to reconstitute the innate and adaptive responses in HIV-1-infected patients, or as adjuvant formulations in preventive or therapeutic vaccines [85].

TLR-9 agonists: CpG oligodeoxynucleotides

Unmethylated CpG motifs are prevalent in bacterial, but not in vertebrate genomic DNA; they are recognized by the TLR-9 and activate host defense mechanisms enhancing innate and acquired immune responses [86]. CpG ODNs are synthetic molecules of unmethylated DNA that mimic bacterial DNA and are also recognized by TLR-9 [87]. Certain CpG motifs (A-class CpG ODNs) are particularly potent at inducing IFN-α production by pDCs and activating NK cells, whereas other motifs (B-class CpG ODNs) are especially potent as B-cell activators [86,88].

CpG ODN-induced activation of innate immunity protects against lethal challenge with a wide variety of pathogens and has therapeutic activity in murine models of cancer and allergy [86]. CpG ODN administration enhances innate immune effector responses through stimulation of pDCs and production of cytokines, including IFN-α, IL-12, -15 and -18 [86,87]. IFN-α inhibits HIV-1 replication *in vitro* and *in vivo* [32]. CpG ODNs also enhance Th1 cytokine production by activated DCs, NK and iNKT cells, and stimulate monocytes and macrophages. The enhancement of the functional activity of DCs, NK and iNKT cells with CpG ODNs may result in immune restoration and may be a goal for immunotherapy to enhance viral control [86].

The effect of synthetic immunostimulatory CpG ODNs as an adjuvant for an HIV-1 immunogen has been evaluated recently [89–92]. In one study, the addition of CpG ODNs to HIV-1 antigens in incomplete Freund's adjuvant was the optimal combination for the induction of HIV-1-specific immune responses, as measured by the production of IFN-γ, RANTES and IgG antibodies [89]. These results suggest that the addition of CpG ODNs immunostimulatory sequences to HIV-1 antigens may enhance HIV-1-specific immune responses. Considering the role of CpG ODN in the activation of innate immunity, further studies exploring their potential use during HIV-1 infection are required.

Other activators of innate immunity

Glycolipid-mediated activation of DCs & iNKT cells

In the last few years, there have been reports regarding the immunomodulatory properties of α-GalCer, a glycolipid originally extracted from marine sponges on the basis of its antitumor properties. Unlike common microbial adjuvants, which signal through TLRs, α-GalCer functions as an antigen presented by CD1d to iNKT cells. Like microbial adjuvants, α-GalCer activates DCs indirectly through cognate interaction with CD1d-restricted iNKT cells (FIGURE 1) [93,94].

iNKT cells are activated by α-GalCer, leading to the rapid production of several cytokines such as IFN-γ and IL-4 [95,96]. In mice, α-GalCer stimulation of iNKT cells regulates autoimmunity, as well as resistance to infection and tumors; preliminary evidence indicating a similar effect in human cells has been demonstrated [97,98]. Human iNKT cells can also be expanded in culture after stimulation with α-GalCer, in the presence of cytokines, such as IL-2, -7 and -15 [99]. Considering that the activation of iNKT cells *in vivo* leads to the subsequent activation of DCs, monocytes, NK and B cells (FIGURE 1), the potential benefit of an immunotherapy in HIV-1-infected individuals with α-GalCer should be explored in the next few years.

One of the most frequent coinfections observed during HIV-1 infection is opportunistic mycobacterial infections. iNKT cells have been shown to recognize and respond to mycobacterial infections [100]; glycolipid antigens from mycobacteria are presented by CD1d molecules and activate human iNKT cells to display effector functions, such as the secretion of cytokines and cytotoxicity against mycobacteria-infected cells [101]. Administration of α-GalCer in a CD1d-restricted manner prolongs the survival of mice infected with *Mycobacterium tuberculosis* [102]. Interestingly, iNKT cells were also found to have direct antimycobacterial activity through the release of an antimicrobial peptide, granulysin [103]. It remains to be determined whether activation of iNKT cells *in vivo* in *M. tuberculosis*-infected HIV-1-infected patients will be useful as an adjuvant therapy.

Acyclic nucleoside phosphonates

Molecules belonging to the acyclic nucleoside phosphonate (ANP) family enhanced the activity of NK cells and the secretion of IFN-γ in *in vitro* and *in vivo* studies [104–106]. The first molecule of this group, tenofovir, has been approved for therapy of HIV-1 infection based on its potent effect in controlling HIV-1 replication and decreasing viral load [107]. However, the beneficial effect of tenofovir on innate immunity, independent of its effect on virological suppression, is not known. A tenofovir-dependent activation of innate immunity may explain why some HIV-1-positive patients receiving this medication exhibit clinical improvement despite the absence of an effect on viral load. Exploring the role that tenofovir and other ANPs have on the activation of innate immune responses will open a new era of antiretroviral therapy with molecules that have dual function: direct inhibition of HIV-1 replication and stimulation of anti-HIV-1 immune response.

Immune-based therapies that enhance innate immunity

New therapeutic approaches for managing HIV-1 infection are focusing on cell-mediated immune responses to improve immunological control over HIV-1 replication. The complexity of HIV-1 immunopathogenesis has prompted multiple strategic approaches to re-establish normal cellular immune responses (BOX 2), including the blockage of immune activation, the enhancement of T-cell production and function, and the induction of specific anti-HIV-1 immunity. The key facts of other immunotherapeutic strategies currently evaluated that enhance the innate immune response and complement the benefits of HAART in HIV-1-infected patients will now be briefly reviewed.

Interleukin 2

HIV-1 infection significantly impairs IL-2 production [108,109], and it was thought that the exogenous administration of IL-2 could help to restore immune function in HIV-1-infected

individuals [110]. IL-2 is synthesized by activated $CD4^+$ T cells and has several immunomodulatory effects, including the differentiation and proliferation of $CD4^+$ and $CD8^+$ T lymphocytes.

Several studies have demonstrated that administration of IL-2 to HIV-1-infected patients induces a significant expansion of $CD4^+$ T lymphocytes with an increase in both naive and memory cells, enhances the expression of CD28 and helps to restore the *in vivo* proliferative response to mitogens and recall antigens [110–112]. A major concern with the use of IL-2 is the risk of stimulating HIV-1 replication. However, randomized studies in patients receiving HAART demonstrated no significant increases in HIV-1 RNA plasma levels in subjects who received IL-2 [110].

The effects of IL-2 therapy in combination with HAART in asymptomatic HIV-1-infected patients were reported recently [113]. Compared with patients receiving HAART alone, those who received IL-2 combined with HAART had a greater increase in $CD4^+$ T cells and a similar decrease in viral load. IL-2 induced a greater increase of naive and memory $CD4^+$ T lymphocytes and enhanced the expression of CD28 and CD25. In addition, patients treated with IL-2 combined with HAART experienced greater restoration and/or a preservation of functional immunity towards memory antigens and a higher *in vivo* antibody response to tetanus vaccination [114]. Recently, it was observed that administration of IL-2 combined with HAART in HIV-1-infected patients resulted in an increase in the number of NK cells [114] and a significant expansion of both $CD4^-$ and $CD4^+$ iNKT cells, while their expression of CCR5 was reduced [115]. This clinical observation suggested that IL-2 therapy in combination with HAART might be beneficial for the restoration of innate cell-mediated immunity. Clinical endpoint trials are currently underway to evaluate the role of IL-2 in early study of IL-2 in people with low $CD4^+$ T-cell counts on active anti-HIV therapy (SILCAAT) and advanced study of IL-2 in people with $CD4^+$ T-cell counts greater than 300/μl on active anti-HIV therapy (ESPRIT) HIV-1 disease.

Interleukin 7

There are several pathways for T-cell reconstitution in HIV-1-infected patients, including stimulating thymic differentiation, extrathymic differentiation and peripheral expansion. IL-7 appears to be a cytokine that can accomplish two of these tasks: enhance new T-cell synthesis by acting directly on the source of new T cells (the thymus) and expand the pre-existing pool of T cells [116].

Along with IL-15, IL-7 is crucial in the maintenance of memory $CD8^+$ T cells. It can also lead to the expansion of naive T cells without altering their naive phenotypic integrity. IL-7 also exerts an antiapoptotic effect through the increased expression of BCL-2, contributing to the positive effects of IL-7 on naive T-cell expansion prior to T-cell receptor rearrangement.

In HIV-1-infected children and adults, there was a strong inverse correlation between serum IL-7 levels and $CD4^+$ T-cell counts [117], indicating that changes in IL-7 levels are a naturally occurring homeostatic response. Alternatively, IL-7 may have negative effects during HIV-1 infection by enhancing infection of naive T cells; this effect has been seen *in vitro* and needs to be evaluated *in vivo* [118].

Although IL-7 is not necessary for maturation of NK cells, it was demonstrated that this cytokine augments NK-cell function, and it was suggested that IL-7 might be a possible immunotherapy for HIV-1 infection, alone or in combination with IL-15 [119].

Interleukin 15

IL-15 is a pleiotropic cytokine that has a diverse array of distinct biological effects in the body; it shares some activities with IL-2 and is produced by activated monocytes and macrophages. IL-15 is essential for the development and differentiation of NK cells, and for homeostatic expansion of $CD8^+$ memory T cells, NKT cells and B lymphocytes. IL-15 can also enhance *in vitro* production of IFN-γ and IL-12 during an immune response [120].

Several studies have demonstrated that IL-15 production is compromised in HIV-1-infected patients and exogenous IL-15 enhances immune cell reactivity in those patients [120,121]. IL-15 was found to enhance the *in vitro* proliferative capacity of peripheral mononuclear cells and purified $CD4^+$ T cells from HIV-1-infected patients, upon stimulation with mitogens, recall antigens, and HIV-1-specific antigens [122]. It was also reported that IL-15 enhances the *in vivo* cytotoxicity of $CD8^+$ T lymphocytes and NK cells from HIV-1-infected patients [123]. Recently, it was published that *in vitro* IL-15 priming induced a significant increase of IFN-γ production in both viremic and aviremic patients, and stimulated NK cells to produce β-chemokine quantities that are reported to be capable of inhibiting HIV infection and replication [124].

These data indicate that IL-15 may represent a good choice for innate and adaptive immune reconstitution in HIV-1-infected individuals [119]. However, it is not clear how viral load may be affected during *in vivo* administration of IL-15, given that it upregulates the expression of CCR5 and may increase the replication of M-tropic viruses.

Conclusion

Although HIV-1 infection has been classically defined as a chronic infection causing depletion of $CD4^+$ T cells, it is well known that this infection is also associated with depletion and functional alterations of different subsets of innate immune cells. Quantitative and qualitative abnormalities in these cells could contribute to HIV-1 pathogenesis because of:

- Their role in directing adaptive immune responses;
- Their role in controlling peripheral tolerance and autoimmunity;
- Their possible involvement in tumor rejection and defense against opportunistic pathogens.

The restoration of host immunity may be an important factor in controlling HIV-1 infection and slowing or preventing disease progression. HAART can partially, but not completely, achieve this goal. Studies need to be carried out to better define the effect of antiretroviral therapy, TLR agonists, CD1d-restricted glycolipids and immunotherapeutic strategies on the number and function of innate immune cells in HIV-1-infected patients. Future therapy for HIV-1 infection could include the stimulation of innate immune cells with TLR agonists, such as CpG ODNs and MPL, to reverse the innate and adaptive immune alterations by enhancing the production of cytokines such as IFN-α, -γ, IL-12, -15 and -18.

Expert commentary

The field of immune-based therapy for HIV-1 infection has advanced significantly over the past 15 years, but current approaches focused on enhancing adaptive immune response have not resulted in an effective therapy. Understanding the role of the innate immune response in HIV-1 disease is only in its infancy, and it needs to be carefully addressed by pathogenesis studies performed in well defined cohorts in order to provide clear insights into the functional deficits of innate immunity. In addition, studies focused on activating innate immune cells, such as pDCs and mDCs with agents, such as TLR ligands (in both *in vitro* and *in vivo* settings) needs to be pursued.

Innate immune modulators need to be evaluated as potential immune-based therapeutics on their own or as adjuvants in the setting of therapeutic HIV-1 vaccines. Demonstration of the importance of innate immunity in HIV-1 disease will require integrated bench science and clinical collaborations.

Five-year view

We have only just begun to understand the role of the innate immune system in HIV-1 infection. We now have data that demonstrates qualitative and quantitative changes in innate immune cells that include pDCs, mDCs, iNKT and NK cells. We also have the tools available that allow us to effectively quantitate these cell populations among the more prominent adaptive immune cells. We have also learned a significant amount of information regarding the major pathogen recognition receptors on innate immune cells, namely the TLRs, and have identified the ligands that lead to cellular activation. Over the next 5 years the major advances in this area will be in defining how one can harness the innate immune response for an effective HIV-1 immune therapy. There are already efforts in utilizing the TLR-9 agonists CPG ODNs for cancer immune therapy and treating HCV infection. Furthermore, α-GalCer is being tested *in vivo* in cancer subjects. The TLR-4 agonists have been included in human vaccines as highly effective adjuvants.

The appropriate agents are available to move forward in evaluating the potential of innate immune modulators for HIV-1 disease. However, one has to be certain in defining in which clinical situations this modulation will be most effective, as activating innate immune pathways does lead to very robust inflammatory responses that might actually enhance HIV-1 replication.

With this in mind, the focus of the first trials should be in subjects with well-controlled viral replication on HAART. In addition, we will clearly begin to test TLR agonists as adjuvants for both prophylactic and therapeutic HIV-1 vaccines. Finally, additional pathogenesis studies will identify potentially new molecules and receptors for activating the innate immune response.

Key issues

- As the first line of defense, the innate immune system has the potential to prevent HIV-1 transmission and provide the time and conditions for adaptive immune responses to develop.
- The innate immune system recognizes pathogens by the pattern of their microbial surface components rather than by a specific antigen sequence.
- The cellular component of the innate immune system has a dual role during HIV-1 pathogenesis: different cellular subsets are targets for viral infection and also have direct anti-HIV-1 effects.
- Different functional and quantitative alterations in cells of the innate immune system have been characterized in HIV-1-infected individuals, contributing to the immunosuppressive state characteristic of this infection.
- Despite the beneficial effects of highly active antiretroviral therapy on reducing viral load and increasing the number of $CD4^+$ T cells, its effect on restoration of immune function is limited.
- Based on the role of innate immunity in controlling viral infections and regulating adaptive immunity, the innate immune system is a perfect target for immune-based therapy for HIV-1 infection.
- The immunotherapeutic potential of Toll-like receptor ligands during HIV-1 infection has been suggested based on their safety, proven efficacy in vaccine clinical trials and their ability to potentiate the effector functions of innate immune cells.
- The immunomodulatory properties of the glycolipid α-galactosylceramide points to its potential use during HIV-1 infection.
- The effects of several cytokines, such as interleukin-2, -7, -15 and interferons on viral replication emphasizes the need for carrying out carefully designed clinical studies to define their potential use as therapeutic agents during HIV-1 infection.

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