The importance of the innate immune system in controlling HIV infection and disease

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The innate immune system is the first line of defense against invading pathogens and is particularly important in warding off bacterial and viral infections presenting at the mucosal cell surface. From this primitive immune response, the more sophisticated adaptive immune system was derived. Despite nearly two decades of research directed at inducing adaptive immune responses to HIV, no successful immunological therapy or vaccine has been developed. On the basis of recent observations, it is suggested that instead emphasis should now be placed on the alternative arm of the immune system, the innate immune response. Novel approaches should be developed to elicit this rapidly responding immune activity in HIV infection.

The innate immune system recognizes pathogens by the pattern of their microbial surface components rather than by a specific antigenic sequence (as used by the adaptive immune system). Pattern recognition is the simplest way that a limited number of cellular receptors can distinguish a large subset of potential pathogens. Both soluble and cellular components contribute to the innate immune response (Box 1). Major differences from the adaptive immune system include the speed of the innate response, the absence of memory and the generality of its recognition system. The innate immune response provides time, if needed, for the subsequent development of adaptive immune responses (Fig. 1). Dendritic cells (DCs), macrophages and some T cells can bridge these two major immune systems and contribute to both the very rapid (innate) and the delayed (adaptive) immune responses (Box 1). This article describes certain participants in the innate immune system that have activity against HIV, and comments on the potential of this immune response for the development of therapies and vaccines.

Anti-HIV soluble components of innate immunity

Among the soluble components of the innate immune system with anti-HIV activity are mannose-binding lectins (MBLs) and complement (Box 1). These soluble products bind to HIV and either lyse the virus directly or help viral phagocytosis by macrophages. In several studies, individuals with low levels of circulating MBLs have an increased risk of HIV infection and enhanced progression to disease. Complement can also rapidly inactivate HIV and serve as an opsonin for phagocytosis of the virus. Some studies suggest that complement lysed HIV in the presence of specific antiviral antibodies. Thus, the complement system participates in both innate and adaptive immunity.

Additional soluble components, including chemokines and other cytokines, are released following the interaction of pathogens with different cells of the innate immune system (Box 1). Innate immune cytokines, such as interleukin-12 (IL-12), IL-4 and IL-6, can determine whether a Th1 helper 1 (Th1) or Th2-type adaptive immune activity predominates. Some, such as tumor necrosis factor α (TNF-α) and the interferons (IFNs), can affect the extent of HIV replication. Chemokines can recruit natural killer (NK) cells, T cells and macrophages to the site of HIV infection and increase the cytotoxic function of these cells. The production of chemokines, and particularly the presence or absence of their receptors, might also influence the ability of HIV to infect cells. Importantly, the production of cytokines by the innate immune system induces both innate and adaptive cellular responses to HIV infection.

Anti-HIV cellular components of innate immunity

Two innate cell-mediated anti-HIV activities merit special consideration before discussing other innate immune cells involved in HIV infection.

Type I IFN-producing cells

IFN-producing cells (IPC), first described in the 1970s as T-associated plasmacytoid cells, initially had no known function. Subsequent studies demonstrated that this cell type is the major producer of type 1 IFNs (Ref. 10). IPCs produce up to 1000 times more IFN than other cell types in the body. Other reports indicate that these cells are immature DCs (pDC-2) that can mature into DCs (DC-2) and enhance Th2-type responses. IPCs secrete type 1 IFNs (Fig. 1) in response to exposure to herpetic simplex virus (HSV) or other pathogens. These cells express CD4 and the IL-3 receptor (IL-3R) but are negative for CD11c and lineage markers. They are found primarily in lymphoid tissues but also make up 0.2–0.9% of peripheral blood mononuclear cells (PBMCs). IPCs can be derived from CD34+ stem cells by granulocyte colony-stimulating factor (G-CSF) and Flt3 ligand stimulation and can be cultured for a few days in the presence of IL-3 (Ref. 13). Conditions for maintenance of their function are not yet known.

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Clinical studies strongly suggest the importance of IPCs in HIV infection. Recently, a loss of IPCs and IFN-α production has been associated with high HIV RNA levels and AIDS (Ref. 12). In some HIV-infected Kaposi’s sarcoma (KS) patients who are no longer developing new lesions, the number of IPCs remains within the normal range. By contrast, KS patients who continue to develop lesions have low IPC numbers12. Moreover, normal IPC numbers and IFN-α production have been found in individuals who remain healthy, despite being infected for >10 years and having very low CD4+ T-cell counts (<100 cells µl-1); these patients were not receiving any treatment for HIV or other pathogens12. Such studies suggest that IPCs protect HIV-infected individuals from cancer and opportunistic infections, through IFN production and the induction of innate and adaptive immune responses. Furthermore, the studies indicate the flexibility that is inherent in the immune system; an innate immune response can protect these individuals from disease after the loss of an adaptive immune cell function (i.e. CD4+ T cells).

Through the production of IFNs, IPCs mediate a variety of important rapid antiviral and antitumor activities, besides modulating the immune system. The IFNs can have direct anti-HIV effects and can block HIV replication14, as well as activate the function of other innate immune cellular components (e.g. NK cells)15. Type I IFNs can increase the recognition of HIV by the adaptive immune system by enhancing MHC class I and B7 expression on antigen-presenting cells16. The type I IFNs also increase IFN-γ production by CD4+ T cells17 and prolong T-cell survival; they have been found to promote Th1-type immune responses18. Moreover, besides involvement in innate immunity through IFN production, the maturation of IPCs into DCs (DC-2) can result in the production of cytokines that help Th2-dependent adaptive immune activity11.

**CD8+ noncytotoxic anti-HIV T cells**

Our studies suggest that the CD8+ T cell is another example of an immune component that can play a role in both innate and adaptive anti-HIV immune responses. CD8+ T cells can control HIV replication in infected cells both through classic cytotoxic T-lymphocyte (CTL) activity19 and a CD8+ T-cell noncytotoxic antiviral response (CNAR)20 (Box 2).
Opinion

Box 2. Features of the CD8+ T-cell noncytotoxic antiviral response (CNAR)

- Observed only with CD8+ T cells, predominantly those expressing HLA-DR and CD28.
- Blocks HIV replication in acutely infected CD4+ T cells and macrophages.
- Does not involve cell killing.
- Does not require HLA compatibility.
- Suppression of HIV replication is dose-dependent (correlates directly with the number of CD8+ T cells added to infected CD4+ T cells in culture) and can occur at low CD8+:CD4+ T-cell ratios (e.g. 1:4).
- Shows clinical relevance; it has strong activity in healthy individuals and decreased activity with progression to disease.
- Active against all HIV-1, HIV-2 and SIV strains tested: both syncytium-inducing and nonsyncytium-inducing viruses are affected.
- Inhibits HIV replication at the level of transcription and does not affect any process before virus integration.
- Mediated (at least in part) by a soluble protein, termed CD8+ T-cell antiviral factor (CAF).

CTL function is an adaptive immune response, whereas CNAR resembles an innate immune response (Box 3). This latter function of CD8+ T cells was noted several years ago, when studies of healthy HIV-infected individuals showed that virus could be recovered from isolated PBMCs only when CD8+ T cells were removed. Subsequent re-addition of CD8+ T cells to the virus-producing CD4+ T cells suppressed virus replication in a dose-dependent manner. Yet, no elimination of virus-infected cells took place (Box 3).

This noncytotoxic antiviral function of CD8+ T cells is clinically relevant because CNAR is associated with a long-term asymptomatic state and the response decreases concomitant with the development of disease. CNAR can be demonstrated in vitro by mixing CD8+ T cells from HIV-infected individuals with infected CD4+ T cells; very few CD8+ T cells (CD8+ :CD4+ T-cell ratio of 1:4) are needed to block HIV replication. A similar type of CNAR has been described with lentivirus infections in nonhuman primates and cats, and has also been reported in human T-cell leukemia virus (HTLV) infection.

CNAR has many characteristics of an innate immune response (Box 3). The activity is not HIV-specific (Box 2) nor species-specific, is not restricted by HLA class I or class II molecules and occurs rapidly after acute HIV infection. It does not appear to be induced by particular HIV proteins, or to show specificity for any antigen. Polyclonal CD8+ T cells and CD8+ T-cell clones from some uninfected individuals can show this antiviral activity. Finally, CNAR can be found in HIV-exposed uninfected individuals and does not appear to involve a memory response.

As with other innate immune activities, CNAR is mediated, at least in part, by a secreted cytokine called the CD8+ T-cell antiviral factor (CAF). CAF appears to be novel; it is unlike other known cellular proteins with antiviral activity, including the IFNs and the chemokines. In cell culture, chemokines have been shown to block HIV replication by competing with the chemokine coreceptor for HIV infection. However, their presence does not correlate with CAF activity. Moreover, CAF blocks infection by β-chemokine-insensitive viruses, acting at the level of transcription and not virus entry. A similar type of antiviral factor has been described, produced by an Epstein–Barr virus (EBV)-responsive CD8+ T-cell clone, CD8+ T cells from feline immunodeficiency virus (FIV)-infected cats and some CD8+ T-cell clones from uninfected individuals.

Other innate immune cells involved in HIV infection

Among the other innate immune cells that are important in warding off microbial infections (Box 1), neutrophils, DCs, NK cells and γδ T cells deserve special consideration in HIV infection. Neutrophils are the most abundant innate immune cells responding early to infections. In addition to their phagocytic activity, they release many proteins and inflammatory cytokines that help to control microbial infection. Neutrophil function can be decreased in HIV infection, DCs express chemokine coreceptors used for HIV entry into the cell. Chemokine receptors attract DCs to areas of inflammation and DCs elicit protection from HIV infection through their production of chemokines and type I IFNs. DCs are also important mediators of antigen recognition and activate both innate and adaptive immune responses through the secretion of cytokines (e.g. IL-7, IL-1, TNF-α and IL-12). NK cells eliminate HIV-infected cells directly or through antibody–directed cellular cytotoxicity (ADCC). ADCC can be clinically relevant in HIV infection; indeed, strong NK cell function is associated with a healthy clinical state. NK cells are also a source of several cytokines (e.g. IFN-γ, TNF-α and granulocyte–macrophage colony-stimulating factor (GM-CSF)) as well as β-chemokines, and have been shown to inhibit HIV replication in vitro. γδ T cells, found commonly at mucosal surfaces, generally do not recognize peptide antigens presented by MHC molecules, but instead interact directly with nonpeptide antigens or with cellular stress proteins (e.g. heat shock proteins (HSPs)). These cells can lyse HIV-infected targets and in some in vitro studies

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Opinion

Box 4. Important aspects of the innate immune system in HIV infection

- Rapid first line of immune defense (minutes to hours).
- Found at mucosal surfaces – a major entry point for HIV.
- Active against many pathogens, as well as HIV, depending on ‘pattern’ recognition.
- Could have protected several HIV-exposed individuals from infection (e.g. through CNAR).
- Could have prevented disease progression in some HIV-infected individuals (e.g. through IPCs and CNAR).
- Could be involved in limiting virus spread in acute infection (e.g. through MBL, complement and CNAR).
- Can protect against the development of cancer (e.g. through NK cells and IPCs).
- Activates the adaptive immune system.
- Chemokine production brings immune cells to the site of infection and other innate cytokines induce the function of cells from both the innate and adaptive immune systems.
- Can prevent HIV infection through modulation of immune responses and chemokine receptor expression.
- Several cytokines (e.g. CD8\(^+\) T-cell antiviral factor) and chemokines might block HIV infection and spread.

Conclusion

The value of the innate immune system has been well-documented in burn patients, in patients with cystic fibrosis (lacking lung surfactants) and in people with mutations in genes encoding cytokines or other innate immune cell factors\(^{45,46}\). Recent observations strongly suggest that the innate immune system is important in combating HIV infection and disease (Box 4). As the first line of defense, this immune system can prevent HIV transmission and ward off opportunistic infections and cancer. Potential pathogens are encountered on a daily basis; most of them are detected and destroyed rapidly by innate immune responses. If this protection is not complete, the rapid immune activity provides the time and conditions for adaptive immune responses to develop. Innate immune activity, mediated by MBLs, neutrophils and γδ T cells, could prevent multiple bacterial and fungal infections in individuals initially infected with HIV. MBL and complement could also be responsible for protection from HIV infection in some exposed uninfected (EU) people and for reducing viral load during acute infection. Innate immune components, such as CNAR, also appear to protect EU individuals from infection\(^{33}\). Perhaps the absence of disease in naturally infected primates\(^{47}\) and the protection from infection or disease in experimentally simian immunodeficiency virus (SIV)-infected animals\(^{48}\) results from innate immune responses. The importance of innate immunity in HIV infection is also reflected in healthy long-term survivors who show a persistent CNAR (Ref. 22) and high IPC numbers\(^ {32}\). IPCs might also play an important protective role in infected untreated individuals with very low CD4\(^+\) T-cell numbers but no evidence of opportunistic infection or cancer\(^ {12}\).

Future directions

The above observations indicate that it would be beneficial to take advantage of the innate immune activity in HIV infection. Unfortunately, induction of CNAR by an HIV vaccine has not yet been achieved and this response is reduced following long-term antiretroviral therapy\(^ {46}\). Nevertheless, several recent results suggest that eliciting and utilizing the innate immune response in HIV infection will provide therapeutic advantages. Cytokines, such as the IFNs (α and γ), TNF-α and IL-12, produced by innate immune cells help to activate NK cells and enhance their cytotoxic function\(^ {7}\). Indeed, immunization with an HSP linked to specific SIV antigens generates both adaptive and innate immune responses to SIV (Ref. 50). DNA vaccines carrying unmethylated CpG, which is recognized by Toll-like receptors on innate immune cells, can be helpful in enhancing a strong adaptive cellular immune response, by induction of IL-12 expression\(^ {51}\). The use of the CpG motif in HIV DNA vaccines to induce CNAR is under study in primate models (J. A. Levy, unpublished). G-CSF and Flt3 ligand have been shown to increase circulating IPC numbers\(^ {52}\) and other components of innate immunity with potentially important anti-HIV activities, including soluble substances in milk, tears, saliva and, recently, the placenta\(^ {33,54}\). Two areas worth pursuing are the identification of the ‘pattern’ that induces CNAR and the mechanism by which IPCs can be maintained or restored in HIV infection. For example, identifying the pattern that induces CNAR.

have suppressed HIV replication through chemokines and other soluble antiviral factors\(^ {43}\). Their production of Th1- and Th2-type cytokines can influence the adaptive immune response. Finally, B1 cells have a rapid antibody response (i.e. IgM) to polysaccharide antigens. Perhaps the anti-Tat IgM antibodies found in normal individuals\(^ {44}\) represent an innate anti-HIV immune response.

Opinion

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is currently under investigation by studying if normal CD8+ T cells exposed to virus-infected macrophages or DCs can be induced to show this antiviral activity (J. A. Levy, unpublished).

Approaches aimed at maximizing the role of innate immune activity in HIV infection need to be emphasized as they will prove valuable in treatment strategies and vaccine development.

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