Natural killer cells in HIV-1 infection: Role of NK cell-mediated non-cytolytic mechanisms in pathogenesis of HIV-1 infection

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Natural killer (NK) cells exhibit both cytolytic and non-cytolytic effector functions against HIV-infected targets. Their precise role in immunopathogenesis of HIV-1 infection is yet to be fully understood. This review addresses the non-cytolytic functions exhibited by NK cells, their potential role in pathogenesis of HIV-1 infection and the effect of HIV-1 viremia on NK cell functions. Activated NK cells are capable of secreting CC-chemokines and suppressing HIV-1 replication in a non-cytolytic fashion. However, HIV-1 viremia suppresses the ability of NK cells to secrete CC-chemokines. Suppression of HIV-1 viremia by highly active antiretroviral therapy (HAART) restores the ability of NK cells to secrete CC-chemokines and suppress endogenous HIV-1 replication by non-cytolytic mechanisms. Better understanding of the mechanisms involved in HIV-1-NK cell interactions would be helpful in delineating novel therapeutic strategies against HIV-1.

Keywords: CC-chemokines, HIV-1, NK cells

Natural killer cells are one of the major lymphocyte subsets which play a critical role in host immune response to a variety of intracellular pathogens. Unlike T and B-lymphocytes, NK cells do not productively rearrange T-cell receptor or immunoglobulin genes and also do not express highly variable, antigen specific receptors. However, NK cells can discriminate between normal cells and neoplastic or virus-infected cells. NK cells can also contribute to the elimination of infected and/or neoplastic cells during effector phase of the adaptive immune response. NK cells also secrete cytokines, such as, interferon-γ (IFN-γ), which promote differentiation of activated CD4+ T cells into TH1 effector cells. Thus, NK cells participate in innate and adaptive immune responses to intracellular pathogens and malignant tumors.

Cytolytic activity of NK cells is clearly distinguishable from that of cytotoxic CD8+ T-lymphocytes (CTL). NK cell cytotoxicity is spontaneous in the absence of prior sensitization and does not require expression of syngeneic major histocompatibility complex (MHC) antigens on the surface of the target cells. Unlike CTLs, the triggering of NK cell cytotoxicity reflects a balance between activating and inhibitory signals mediated by cell surface receptors. Inhibitory MHC class I binding receptors have a central role in current paradigms of target cell recognitions by NK cells. Ligation of one of these receptors by specific MHC class I allotypes delivers a dominant negative signal to NK cells that prevent target cell lysis (Fig. 1). Down-regulation of MHC class I molecules on the target cell surface that commonly occurs by neoplastic or viral transformation, releases NK cells from such inhibitory signals and allows lysis of the target cells. Positive signals for cytolyis are provided by ligation of several receptors including CD2, CD16, NKR-P1, 2B4, NKp30, NKp44, NKp46 and NKp80, which are expressed on NK cells. The functional regulation of these receptors are quite complex and are beyond the scope of this review. Readers are encouraged to refer to Moretta et al. 2001 and Lanier et al. 1997, as this aspect of NK cell function will not be further discussed in this review.

Among HIV-infected individuals CD8+ T cells have been shown to play an integral role in controlling HIV viremia by MHC-restricted cytolytic or non-MHC-restricted non-cytolytic responses. We have shown previously that CD8+ T cells provide potent antiviral activity against endogenous HIV replication via autologous cell-to-cell contact among long-term non-progressors (LTNPs) and those who are treated with HAART. Profound antiviral activity mediated by either cell-to-cell contact or soluble factor(s) has been demonstrated among HIV-infected individuals in whom HAART was initiated early in HIV infection. However, many of these patients...
A. Cytolytic

1. NK Cell

\[ \text{NCR} \rightarrow \text{INKR} \rightarrow \text{Target Cell} \]

2. NK Cell

\[ \text{NCR} \rightarrow + \rightarrow \text{Infected Target Cell} \rightarrow \text{Viral Replication} \]

B. Non-cytolytic

\[ \text{RANTES} \rightarrow \text{NK Cell} \rightarrow \text{MIP-1} \alpha \rightarrow \text{MIP-1} \beta \rightarrow \text{IFN-\gamma} \rightarrow \text{CD4}^+ \text{T Cell} \rightarrow \text{HIV} \rightarrow \text{Viral Replication} \]

Figure 1: Mechanisms by which NK cells affect viral replication—(A) Cytolytic: NK cells recognize self MHC-class I molecules on the surface of target cells using NK inhibitory (INKR) receptors. In the absence of MHC class I, as seen with viral or malignant transformation of target cells, NK cells are activated and lyse target cells using natural cytotoxicity receptors (NCRs). (B) Non-cytolytic: NK cells upon stimulation secrete cytokines, like interferon-\(\gamma\) and chemokines like RANTES, MIP-1\(\alpha\), MIP-1\(\beta\), and suppress viral replication. In the case of HIV-1 infection, NK cells secrete CC-chemokines and block viral entry into newer target cells.

Demonstrated potent antiviral activity mediated by soluble factor(s) independent of CC-chemokines. In this regard, the precise nature of NK cell-contact and soluble factor-mediated suppression of endogenous HIV replication among HIV-infected individuals has not yet been fully delineated. This review addresses the role of NK cells in suppression of HIV-1 replication mediated by secretion of soluble factors.

NK cells secrete CC-chemokines and are capable of suppressing HIV replication in vitro—Cell-mediated immune responses play an important role in host defense against viral infections. NK cells from HIV-1-infected individuals have been shown to induce MIP-1\(\alpha\) in response to IL-12 and IL-15. Furthermore, studies from our laboratory and others have shown that NK cells from HIV-infected individuals are also capable of secreting CC-chemokines and suppressing HIV replication in vitro. Fehniger et al. have compared the level of CC-chemokines secreted by NK cells isolated from both HIV-seronegative and HIV-seropositive individuals in response to IL-12 and IL-15. They have found that both HIV-seronegative and seropositive individuals produced significant amounts of CC-chemokines upon activation. They also demonstrated that despite the fact that HIV-infected donor NK cells produce lesser amounts of CC-chemokines, there is no statistically significant difference in CC-chemokine production by NK cells from HIV-seronegative and seropositive donors. This study has further examined
the ability of culture supernatants of monokine-activated NK cells from both HIV-seronegative and seropositive donors to suppress HIV replication in vitro. NK cell supernatants inhibit both R5-using HIV-1_SMN.SX and X4-using HIV-1_R4.3 in vitro. Collectively, these data indicate that NK cells from HIV-1 seronegative and seropositive individuals produce CC-chemokines and potentially other similar soluble factors that can suppress HIV-1 replication in vitro. The number of HIV-1 seropositive patients (n = 7) that participated in this study was too small to make generalized conclusions toward relationship between HIV-1 disease status and the ability of NK cells to produce CC-chemokines.

Previous studies from our laboratory have addressed the capacity of NK cells isolated from HIV-1 infected individuals to produce CC-chemokines and to suppress HIV-1 replication in autologous, endogenously infected cells. This study has utilized co-culture of enriched NK cells along with NK/CD8 depleted PBLS to address the role of NK cells in suppressing HIV-1 replication. The above study has found that NK cells from HIV-1 infected individuals produced higher number of copies of mRNA transcripts for CC-chemokines (RANTES, MIP-1α, MIP-1β) and also significant amount of RANTES, MIP-1α and MIP-1β, in response to IL-2 stimulation. In this study, CC-chemokines have shown that NK cells stimulated by cross linking of CD16 are able to suppress HIV-1 replication in autologous stimulated NK/CD8 depleted T cells in a co-culture system (range 46-97% suppression). In about half of these patients, the presence of neutralizing antibodies to CC-chemokines were able to overcome NK cell mediated suppression of HIV-1 replication, suggesting that CC-chemokines released by NK cells play a key role in suppression of HIV-1 replication. Overall, this study is unique in establishing that NK cells from HIV-1 infected individuals are capable of secreting sufficient amounts of CC-chemokines and inhibit endogenous HIV-1 replication.

Mechanism by which NK cells restrict HIV-1 replication in vitro is still not clearly delineated. Whether the state of HIV-1 infection influences the ability of NK cells to secrete CC-chemokines and suppress HIV-1 replication, has also not yet been determined. Since NK cells are capable of suppressing HIV-1 replication by means of cell-to-cell contact and by secretion of soluble factors, it would be important to distinguish between these two different mechanisms involved in the inhibition of HIV-1 replication. Finally, it is not clear whether NK cells are capable of producing soluble factors other than CC-chemokines, similar to CD8+ T cells, that may suppress HIV-1 replication.

**Suppression of viral replication by autologous NK cells in viremic patients**—To determine the degree of suppression of viral replication in endogenously infected, stimulated CD4+ T cells by autologous NK cells from viremic patients who were not receiving HAART, we set up the following culture conditions—CD4+ T cells alone, CD4+ T and NK cell co-culture. CD4+ T cells with supernatant from NK cells, and CD4+ T cells with supernatant from NK cells in the presence or absence of anti-CC-chemokine antibodies. Upon optimal stimulation of purified CD4+ T cells, the peak p24 values from day 12 cultures were observed (Fig. 2A). When CD4+ T cells were co-cultured with NK cells at 1:1 ratios, mean 2.25 log reduction in HIV replication was observed for NK cells when compared with viral replication in CD4+ T cells alone. However, when supernatants from cultures of NK cells were added to CD4+ T cells, relatively low degree of viral suppression mediated by NK cells was observed (mean 1.42 log); (Fig. 2A). The presence of anti-CC-chemokine antibodies in culture in which supernatant from NK cells was added to stimulated CD4+ T cells, had minimal effect on the levels of viral suppression (Fig. 2A). These data indicated that NK cells from HIV viremic individuals exhibited only modest antiviral activity, which was mediated by cell to cell contact. In this context, soluble factors including CC-chemokines played only a minor role in suppressing viral replication in CD4+ T cells from chronically infected individuals who were not receiving antiretroviral therapy.

**Suppression of viral replication by autologous NK cells in infected aviremic patients**—It has been previously demonstrated that CD8+ T cell-mediated suppressive activity against HIV declines with disease progression. However, studies addressing the precise role of NK cell-mediated suppressive factors in the inhibition of endogenous HIV replication in aviremic patients have been lacking. In order to further examine the role of NK cell-mediated suppression of endogenous HIV replication, cells from patients receiving HAART, in whom aviremia was achieved, were subjected to the same culture conditions as described above. Profound suppression of NK cell-mediated endogenous HIV replication (mean of 3.25 log) was noted (Fig. 2B). Furthermore, there was substantial suppression of
HIV replication in CD4+ T cells when supernatants from NK cells (mean 2.90 log) were added (Fig. 2B)4). Of note, in the presence of excess anti-CC-chemokine antibodies, the suppressive effect mediated by NK cell released soluble factors was almost completely abrogated (mean 0.41 log) relative to replication of HIV in CD4+ T cells (Fig. 2B)4), indicating that CC-chemokines played an important role in NK-cell mediated suppression of endogenous replication of HIV in this ex vivo system. These findings suggested that in infected patients who initiate HAART and in whom aviremia was achieved, cell-contact and soluble factor-mediated suppression of HIV by NK cells was preserved. Furthermore, these data suggested that suppression exhibited by NK cells in this system was virtually exclusively mediated by CC-chemokines while that observed with CD8+ T cells was mediated predominantly by non-CC-chemokine factors8,9.

**Relationship between suppression of viral replication by NK cells and virologic and immunologic parameters**—In order to address the relationship between the capacity of NK cells to suppress viral replication in CD4+ T cells and various virologic and immunologic parameters of HIV-infected patients, we obtained HIV RNA levels and CD4+ and CD8+ T cell counts at the time of study for all patients. When statistical tests of associations were performed between the degree of suppression of HIV replication by NK cells either by cell-to-cell contact or by soluble factors and levels of plasma viremia at the time of study. The ability of NK cells to suppress endogenous HIV replication either by cell-to-cell contact (Fig. 3A) or by soluble factors (Fig. 3B) correlated inversely with plasma viremia at the time of study4). These data suggested that the higher the plasma viral load, the lower the ability of NK cells to suppress endogenous HIV replication in CD4+ T cells.

In addition, statistical comparisons were made between the log suppression of viral replication in both culture conditions (cell-contact and supernatant) and CD4+ and CD8+ T cell counts at the time of study. There was no statistically significant correlation between NK cell-contact or soluble factor-mediated suppression of HIV replication and CD4+ T cell counts (p > 0.05), or CD8+ T cell counts (p = 0.07 with cell-contact-mediated and p = 0.16 with supernatant-mediated suppression)4).

**Relationship between levels of CC-chemokines secreted by NK cells and plasma viremia**—Since the ability of NK cells to suppress endogenous HIV replication in CD4+ T cells is predominantly mediated by secretion of CC-chemokines and inversely correlates with plasma viremia (see above), next we investigated whether plasma viremia had any effect on the levels of CC-chemokines secreted by NK cells. Supernatants from purified NK cells were tested for levels of RANTES, MIP-1α, and MIP-1β as measured by ELISA. The levels of CC-chemokines secreted by NK cells on day 3 were higher among patients who were aviremic and receiving HAART compared to those patients who were viremic at the time of study. The levels of both RANTES and MIP-1α secreted by NK cells inversely correlated with the levels of
plasma viremia (Fig. 4)\textsuperscript{11}. These data indicated that higher degree of HIV viremia, lowered the ability of NK cells to secrete CC-chemokines. It was likely that HIV-induced suppression of CC-chemokines was accountable for the inability of NK cells to suppress endogenous HIV replication in autologous CD4\textsuperscript{+} T cells.

Effect of HIV-1 viremia on NK cell effector functions and its implications to pathogenesis of HIV-1 infection—NK cells can mediate suppression of endogenous HIV replication in autologous CD4\textsuperscript{+} T cells of HIV-infected individuals to a level comparable to that of CD8\textsuperscript{+} T cells. Of note is the fact that the degree of suppression by NK cells is more profound in aviremic patients compared to viremic patients in both systems (cell to cell contact and supernatant) and there is an inverse correlation between the level of plasma viremia and the ability to suppress HIV replication. Furthermore, suppression of HIV replication by soluble factors is virtually exclusively mediated by CC-chemokines in NK cells, whereas non-chemokine factors are predominantly involved in CD8\textsuperscript{+} T cells\textsuperscript{6,11}. In this regard, anti-CC-chemokine antibodies completely abrogate the suppression of HIV replication mediated by NK-derived soluble factors. Although it has been shown in previous studies that CD8\textsuperscript{+} T cells are capable of suppressing endogenous HIV replication by both cell-to-cell contact and by secreting soluble factors\textsuperscript{9,10}, the suppressive activity of autologous NK cells against HIV replication in CD4\textsuperscript{+} T cells provides novel insights into immune defenses against HIV-1.

Several studies have correlated high frequency of NK cells or NK cell activity with reduced

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\caption{Relationship between the ability of NK cells to suppress endogenous HIV replication and plasma HIV viremia at the time of study. The relationship between the level of plasma viremia and the suppression of HIV replication in culture conditions of (A) cell-to-cell contact; and (B) soluble factors. There is a strong inverse correlation between the ability of NK cells to suppress HIV replication, either by cell-cell contact or by soluble factors, and the level of plasma viremia.}
\end{figure}

\begin{figure}[h]
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\caption{Relationship between RANTES and MIP-1\alpha secretion by NK cells and plasma viremia. The relationship between plasma viremia of all the participating patients and the levels of — (A) RANTES; and (B) MIP-1\alpha secretion has been shown. There was a strong inverse correlation between RANTES and plasma viremia. The levels of RANTES and MIP-1\alpha measured by ELISA on day 3 have been shown.}
\end{figure}
suspensibility in certain individuals to HIV infection and with the control of initial plasma viremia in simian immunodeficiency virus model. Loss of NK cell activity has been correlated with HIV disease progression, particularly in individuals with opportunistic infection. Since there is no correlation between NK-mediated cytotoxicity and plasma viremia, it appears that autologous NK cells exert cell-contact-mediated antiviral activity against CD4+ T cells independent of cytolytic activity. It is also possible that NK cells could have cell membrane-bound molecules that may inhibit HIV replication in CD4+ T cells.

Current data clearly demonstrate that the ability of NK cell supernatant-mediated suppression of endogenous HIV replication is related to their ability to secrete CC-chemokines. Ability of NK cells to secrete CC-chemokines is significantly depressed among viremic patients in comparison with aviremic patients. This indicates that HIV-induced inhibition of NK cell function may involve mechanisms leading to depressed secretion of CC-chemokines.

These studies have potentially important implications for understanding the full scope of host defense mechanism against HIV infection including the innate immune system. NK cells likely play an important role in protection against both infection and progression of HIV disease. In addition, these data provide yet another example of the multifaceted deleterious effects that active virus replication has on immune function.

**Future directions**— These studies have clearly demonstrated the inhibitory effect of HIV-1 on NK cell function. However, it is still unclear how HIV-1 specifically interacts with NK cells in order to render them incapable of secreting CC-chemokines. This aspect of HIV-1-NK cell interaction needs to be further studied at the molecular level.

One approach involves establishment of an in vitro system in which one can study the effect of HIV-1 proteins (Env Gag and Nef) on NK cell function. Such studies would enable us to further elucidate underlying mechanisms involved in HIV-1 induced suppression of NK cell function. Of note, exposure of HIV-1 envelope proteins to NK cells have been shown to inhibit NK cell proliferation, cytotoxicity and cytokine production. Newer technologies have provided us with unique opportunity to study the profile of gene expression of NK cells from patients. Studies of microarray analyses using purified NK cells from HIV-1 viremic, viremic and HIV-1 seronegative patients would allow us to explore the overall effect of HIV-1 on NK cell gene expression. Such studies would allow us focus on research of specific gene products, thereby allowing us to dissect out HIV-NK interaction at the molecular level. Finally, two phenotypically and functionally distinct subtypes of NK cells have been described. These include CD56dim CD16+ cells, which express CXCR1, CXCR2, CXCR3 and CXCR4 and CX3CR1, but no detectable levels of CC-chemokine receptors on cell surface and CD56bright CD16- NK cells expressing CCR5 and CCR7. Several studies have determined that fresh NK cells of CD56dim CD16+ subset are more naturally cytotoxic than their counterparts. There is also evidence that in HIV-infected individuals, there is a selective loss of CD56dim CD16+ subset of NK cells with disease progression. It is appealing to study the nature of these subsets in HIV-1 infected individuals in relation to their ability to suppress HIV replication. If there is in fact a selective depletion of a subset of NK cells that secretes CC-chemokines, that would prove to be yet another mechanism by which HIV-1 evades immune defense system. Understanding the nature and mechanisms involved in selective depletion of a subset of NK cells would enable us identify NK cell-specific immune-modulatory effects of HIV-1 infection.

**Conclusion**

NK cells play a critical role in the immunopathogenesis of HIV-1 infection. NK cells affect HIV-1 replication in vivo by both cytolytic and non-cytolytic mechanisms. HIV-1 viremia has a depressive effect on NK cell non-cytolytic mechanisms. Delineating the exact role of these mechanisms could have long standing therapeutic implications for HIV-1 infection. Further studies on the precise mechanism of the effects of NK cell and of innate immune mechanisms on HIV replication will be important not only in further our understanding of HIV pathogenesis, but in widening the potential for immune-based intervention in HIV disease.

**References**