Evolution of Innate and Adaptive Effector Cell Functions during Acute HIV-1 Infection

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Early events during acute human immunodeficiency virus type 1 (HIV-1) infection are critical in determining the course of disease progression. Cells of the innate and adaptive immune responses are involved in this acute response to infection; however, little is known about the coevolution of innate and adaptive effector cell populations during the initial phase of HIV-1 infection. Here, we have characterized the development of innate natural killer (NK) cell and adaptive HIV-1–specific CD8+ T cell function during acute HIV-1 infection. Although NK cell populations were significantly expanded during acute infection before HIV-1 seroconversion, HIV-1–specific CD8+ T cell responses were absent or weak and were inversely correlated with the level of NK cell activity. NK cell activity was directly correlated with the level of viral replication during acute HIV-1 infection and declined rapidly in subjects who initiated highly active antiretroviral therapy, whereas NK cell activity remained elevated in subjects who did not initiate therapy. Yet, reexposure to HIV-1 antigen during treatment discontinuation in chronic infection resulted in a synchronous increase in NK and CD8+ T cell activity. Overall, these data demonstrate that expansion of the NK cell population precedes the development of adaptive HIV-1–specific CD8+ T cells during acute infection but that both effector cell subsets respond with similar kinetics during chronic HIV-1 infection.

Cytolytic effector cells are critical in the control of viral infections because they eliminate infected target cells. The immune system is endowed with 2 main subsets of cytotoxic effector cells: the NK cells of the innate immune system and the CD8+ T cells of the adaptive immune system. Both NK cells and CD8+ T cells have been shown to play a critical role in the immune response to viral infections in humans. Despite certain redundancies in their effector functions, deficiencies in either of these subsets are associated with recurrent infection and death in humans, suggesting that both cell types are independently required and are complementary in their antiviral activity [1, 2]. However, very little is known about the dynamics of these cytotoxic effector cell subsets during acute HIV-1 infection.

Acute HIV-1 infection represents the first interaction between the host and the incoming virus [3, 4]. Early during this time, HIV-1 replicates at high levels, reaching viral loads of $>1 \times 10^4$ viral copies/mL [5, 6]; viral replication subsequently declines to a viral set point of $\sim3 \times 10^4$ viral copies/mL. Previous experiments have demonstrated that simian immunodeficiency virus (SIV)–infected macaques depleted of CD8+ cells—including both T cells and NK cells that express CD8 in macaques—fail to control viral replication [7], suggesting a critical role for CD8+ effector cells in the containment of SIV-1 replication. Further evidence for a role for both CD8+ T cells and NK cells in the control of HIV-1 disease comes from epidemiological studies demonstrating significantly slower disease progression in both individuals positive for specific HLA class I molecules that present viral epitopes to CD8+ T cells [8] and individuals with specific killer immunoglobulin-like receptor (KIR) genotypes [9–11]. In particular, a recent study demonstrating a significant association
between the expression of KIR3DS1 (and of its putative ligand, HLA-Bw4 801) and slower HIV-1 disease progression suggested that cells that express KIR (NK cells and CD8+ T cells) are critical in the control of HIV-1 disease [9].

Several lines of evidence have demonstrated that the initial phase of HIV-1 infection is critical in determining the subsequent disease course [6, 12]. Here, we have characterized the dynamics of NK cells and HIV-1-specific CD8+ T cells starting very early during acute HIV-1 infection and have shown that NK cell activity is dramatically elevated during acute HIV-1 infection, when virus-specific CD8+ T cell responses are still absent or weak, and then declines after the emergence of HIV-1-specific CD8+ T cell responses. In contrast, we found that both NK and CD8+ T cell populations are significantly expanded during rebound of viral replication during treatment interruption in chronic HIV-1 infection. Taken together, these data suggest that, although NK cells represent the dominant cytolytic effector population during the acute phase of HIV-1 infection (before the development of adaptive immune responses), both NK cells and virus-specific CD8+ T cells may cooperate during the chronic phase of infection to contain the level of viral replication.

**SUBJECTS, MATERIALS, AND METHODS**

**Study subjects.** Thirty-five HIV-1–infected subjects and 7 HIV-1–negative control subjects were included in this study. Fifteen subjects were recruited during acute HIV-1 infection before the development of any detectable antibodies by a p24 ELISA (hereafter, “acute seronegative”); 14 subjects were recruited during acute HIV-1 infection at a time when they had detectable antibody responses (positive by the p24 ELISA) but <3 bands in an HIV-1 Western blot (hereafter, “acute seropositive”); and 6 chronically infected subjects receiving highly active antiretroviral therapy (HAART) were recruited from a supervised treatment interruption (STI) trial. The median viral load for the cohort of 29 subjects with acute HIV-1 infection was $1.9 \times 10^8$ RNA copies/mL (range, $1.24 \times 10^5$–$13 \times 10^6$ RNA copies/mL), and the median CD4+ T cell count was 471 cells/μL (range, 289–1038 cells/μL). The acute seronegative subjects had significantly higher viral loads (median, $2.7 \times 10^7$ RNA copies/mL; range, $0.2 \times 10^4$–$13 \times 10^6$ RNA copies/mL) than did the subjects identified after seroconversion (acute seropositive), who had a median viral load of $3.8 \times 10^5$ RNA copies/mL (range, $1.24 \times 10^4$–$6.8 \times 10^5$ RNA copies/mL) ($P = .003$), but the 2 groups did not differ significantly with respect to CD4+ T cell counts. After 1 year of infection, the average viral loads for the acute seronegative and acute seropositive subjects who did not initiate HAART were $3.3 \times 10^5$ and $5.1 \times 10^5$ RNA copies/mL ($P > .5$), respectively, whereas all study subjects who received HAART had viral loads below the limit of detection (<50 RNA copies/mL). The chronically infected subjects all had undetectable viral loads (<50 RNA copies/mL) and a median CD4+ T cell count of 728 cells/μL (range, 516–1012 cells/μL) while receiving HAART before STI. The Massachusetts General Hospital Institutional Review Board approved the present study, and each subject provided written, informed consent for participation in the study.

**Flow cytometric analysis of cell numbers, intracellular cytokine production, and CD107a expression.** NK cell populations were defined as lymphocytes that were CD3 negative and were further defined by their expression of CD56 and/or CD16 (CD3–CD56+CD16−, CD3+CD56−CD16+, and CD3+CD56+CD16+), as described elsewhere [13]. CD8+ T cells were defined as CD3+ lymphocytes that expressed CD8 but not CD4. Intracellular cytokine staining was performed as described elsewhere [25, 26]. NK cell activation was quantified [13] after stimulation of peripheral-blood mononuclear cells (PBMCs) with major histocompatibility complex (MHC)–devoid K562 cells (ATCC) at an effector-to-target cell ratio of 10:1; medium alone served as the negative control, and PMA/ionomycin (2.5 and 0.5 μg/mL, respectively) served as the positive control. In contrast, HIV-1–specific CD8+ T cell activity was assessed after stimulation of PBMCs with peptide pools derived from Gag, Pol, Nef accessory (Vif, Vpu, Vpr, Tat, and Rev), and Env (final concentration for each peptide, 2 μmol/L); 1 μg/mL each of the monoclonal antibodies anti-CD28 and anti-CD49d (BD Biosciences); and 20 μg/mL CD107a-PeCy5 antibody (BD Biosciences) added directly. Brefeldin A (Sigma) and monensin (Golgi block; BD Biosciences), at final concentrations of 5 and 0.3 μg/mL, respectively, were added immediately to NK cell tubes but were added after 1 h for the CD8+ T cell assays, and the total stimulation lasted for 6 h at 37°C in 5% CO2. PBMCs were then stained for NK cell surface markers with CD56-PeCy7, CD16-APC-Cy7 (BD Biosciences), and CD3–PECy5 (BD Biosciences) added directly. Flow cytometric analysis of cell numbers, intracellular cytokine production, and CD107a expression was performed as described elsewhere [13]. IFN-γ enzyme-linked immunospot (ELISpot) assay to detect HIV-1–specific CD8+ T cell responses. HIV-1–specific CD8+ T cell responses were quantified by IFN-γ ELISpot assay, using a panel of peptides corresponding to optimal clade B cytotoxic T lymphocyte epitopes that have been described elsewhere [14]. PBMCs were plated at $1 \times 10^5$ cells/well with peptides at a final

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concentration of $1 \times 10^{-3}$ molar, as described elsewhere [15]. PBMCs were incubated with medium alone (negative control) or phytohemagglutinin (positive control). The numbers of specific IFN-γ–secreting T cells were counted using an automated ELISpot reader (AID); the average negative-control value was subtracted, and the results were expressed as spot-forming cells per $1 \times 10^{6}$ input cells. Negative controls were always $\leq 30$ sfc/1 $\times 10^{6}$ input cells. A response was considered to be positive if it was $\geq 50$ sfc/1 $\times 10^{6}$ input cells and was at least 3 times greater than the mean background activity.

Statistical analysis. To test for differences in means between several populations, we used 1-way analysis of variance; for all comparisons for which $P < .05$, a Tukey correction was made. $P < .05$ was considered to be significant.

RESULTS

HIV-1–specific CD8+ T cell responses preceded by increased NK cell numbers and activity during acute HIV-1 infection. Acute viral infections are marked by rapid cellular proliferation and cytokine secretion [16–19]. Although it is well established that the CD8+ T cell population expands early during HIV-1 infection, the kinetics of the expansion of the NK cell population during acute HIV-1 infection, as well as the dynamics between the expansions of the NK and CD8+ T cell populations, are not well characterized. Thus, we measured total CD8+ T and NK cell numbers in the peripheral blood of subjects identified very early during acute HIV-1 infection, when antibodies directed against HIV-1 were not yet detectable (the acute seronegative subjects), and compared them with the numbers in subjects with acute HIV-1 infection who were p24 antibody positive but had an indeterminate HIV-1 Western blot (< 3 bands; the acute seropositive subjects). We observed significantly ($P = .02$) higher proportions of NK cells in the subjects who were seronegative than in either the subjects who were seropositive but still Western blot indeterminate or the HIV-1–uninfected control subjects (figure 1A). In line with this, the absolute number of NK cells also was significantly increased in the subjects with acute HIV-1 infection, being a median of 375 NK cells/μL (range, 251–616 NK cells/μL) in the uninfected control subjects, compared with 573 NK cells/μL (range, 221–1067 NK cells/μL) in the acute seronegative subjects and 416 NK cells/μL (range, 175–676 NK cells/μL) in the acute seropositive subjects ($P = .01$ and not significant, respectively), whereas total CD3+ T cell numbers were stable ($P > .5$, for all comparisons). Furthermore, in line with the findings of previous studies [13], we observed an early increase in the proportion of CD3+ CD56dim NK cells during acute infection, with a concomitant reduction in CD3+ CD56bright NK cells (data not shown). However, these changes were not accompanied by any accumulation of CD3+ CD56− CD16+ NK cells at this early stage of HIV-1 infection, as described previously [13]. In contrast to this early expansion of the NK cell population, the expansion of the population of total CD8+ T cells was delayed, and there was a trend toward higher CD8+ T cell numbers in the subjects with acute HIV-1 infection who were seropositive ($P = .06$) (figure 1D). Thus, overall, the population of NK cells are the earliest cytolytic effector cells to expand during acute HIV-1 infection, followed later by an expansion of the CD8+ T cell population.

Given these changes in NK and CD8+ T cell numbers, we were interested in characterizing the dynamics of NK and HIV-1–specific CD8+ T cell activity during the acute phase of HIV-1 infection. The level of NK cell activity was quantified on the basis of the production of IFN-γ and the induction of CD107a expression on NK cells after a 6-h stimulation with MHC-devoid target cells, whereas the level of HIV-specific CD8+ T cell responses was measured using an IFN-γ ELISpot assay. In parallel with the increase in NK cell numbers, NK cell degranulation and IFN-γ secretion in response to MHC-devoid target cells was significantly stronger in the acute seronegative subjects than in the acute seropositive subjects ($P = .01$ and $P = .04$, respectively) (figure 1B and 1C). In contrast, as expected from the numeric changes in CD8+ T cells, there was a trend toward stronger HIV-1–specific CD8+ T cell responses ($P = .07$) that targeted a significantly broader number of CD8+ T cell epitopes ($P = .03$) in the subjects who were p24 seropositive than in those who were still p24 seronegative (figure 1E and 1F). Furthermore, the level of CD107a expression on NK cells was inversely correlated with the magnitude of HIV-1–specific CD8+ T cell responses (figure 1G), further demonstrating the distinct dynamics of the innate and adaptive effector cell functions during the earliest phase of HIV-1 infection.

Distinct and opposite trajectories for NK cell activity and HIV-1–specific CD8+ T cell responses during acute HIV-1 infection. To further characterize the evolution of both CD8+ T cell and NK cell responses over the course of acute HIV-1 infection, CD8+ T and NK cell activity was monitored longitudinally over 6 months in 2 subjects with symptomatic acute HIV-1 infection who were seronegative at their first visit and who initiated HAART during acute infection. Although NK cell activity was analyzed after stimulation of PBMCs with MHC-devoid target cells, HIV-1–specific CD8+ T cell cytokine secretion and degranulation was measured after stimulation of PBMCs with pools of peptides derived from Gag, Pol, Nef, Tat, Rev, Vif, Vpu, and Vpr. As described elsewhere [15, 20], HIV-1–specific CD8+ T cell activity, as measured by degranulation and IFN-γ secretion by use of flow cytometry, increased throughout the first few months of infection as the level of viral replication decreased. In contrast, the ability of NK cells to secrete antiviral cytokines and to degranulate was most pronounced at the first time point during seronegative acute infection (figure 2A–2D). NK cell activity subsequently declined...
Figure 1. NK and CD8+ T cell dynamics during acute HIV-1 infection. For the different study populations, dot plots represent the proportion of NK cells (A); the proportion of degranulating CD107+ NK cells after stimulation with major histocompatibility complex (MHC)–devoid target cells for 6 h (B); the proportion of interferon (IFN)–γ+ NK cells after stimulation with MHC-devoid target cells for 6 h (C); the proportion of CD8+ T cells (D); the magnitude of the HIV-specific CD8+ T cell response, as determined by IFN-γ enzyme-linked immunospot assay, after stimulation with HLA-restricted peptides (E); and the breadth of the HIV-specific CD8+ T cell response, represented as the number of epitopes recognized (F). Panel G shows the correlation between the percentage of CD107+ NK cells after stimulation and the magnitude of the HIV-specific CD8+ T cell response within the same individual.

Longitudinal changes in NK cell activity after acute infection in the presence or absence of HAART. 

Studies characterizing the longitudinal changes in HIV-1–specific CD8+ T cell activity starting during acute HIV-1 infection have demonstrated an increase in virus-specific CD8+ T cell responses after acute HIV-1 infection in subjects who do not initiate HAART [15, 20, 21]. In contrast, HIV-1–specific CD8+ T cell responses remain stable or decline in subjects who initiate HAART during the acute phase of infection [21]. However, very little is known about changes in NK cell numbers and activity in subjects who do or do not initiate HAART during acute HIV-1 infection. Thus, we monitored the evolution of NK cell activity in 7 individuals who initiated HAART during acute HIV-1 infection and in 7 individuals who did not. NK cell activity, after stimulation of NK cells with MHC-devoid target cells, declined in all subjects over the course of the first year of infection (figure 3A and 3B). This decline in NK cell activity reached statistical significance in the subjects who initiated HAART. Furthermore, the level of NK cell degranulation was significantly associated with the level of viral replication (figure 3C). Overall, these data demonstrate that, after the resolution of acute HIV-1 infection, NK cell activity declines, in particular in subjects treated with HAART.
Synergistic expansion of the populations of NK cells and HIV-1–specific CD8+ T cells during treatment interruption in chronic infection. The above data demonstrate that the expansion of the NK cell population preceded the expansion of the HIV-1–specific CD8+ T cell population during the earliest phase of acute HIV-1 infection, when the host immune system and the virus first encounter. However, little is known about the kinetics of the expansion of NK cell responses during treatment interruption in chronic infection at a time when large numbers of virus-specific CD8+ T cells are present. Thus, changes in NK and CD8+ T cell numbers and function were evaluated in 6 treated subjects who discontinued HAART for an STI in chronic infection; these subjects were evaluated for both their NK cell responses to MHC-devoid target cells and their HIV-1–specific CD8+ T cell responses to pools of HIV-1 peptides at 3 time points: before the STI, at the peak HIV-1 rebound during the STI, and at a time when the viral load was again undetectable after the reinitiation of HAART.

Dramatic increase in NK cell numbers was accompanied by a preferential expansion of the KIR+ NK cell population (figure 4B) that demonstrated strong activity after stimulation with MHC-devoid target cells (figure 4C and 4D). Similarly, HIV-1–specific CD8+ T cell activity increased rapidly after the rebound of viral replication (figure 4E and 4F), with the level of HIV-1–specific CD8+ T cell IFN-γ secretion increasing 3-fold during the STI on average. After the suppression of HIV-1 viremia following the reinitiation of HAART, both NK and HIV-1–specific CD8+ T cell activity declined rapidly. Taken together, these data demonstrate that the kinetics of the expansion of NK and CD8+ T cell responses are similar during chronic HIV-1 infection, suggesting that, once primed, HIV-1–specific CD8+ T cells respond with similar sensitivity to changes in viral replication, as do the effector cells of the innate immune system.

DISCUSSION

Two cellular subsets of the innate and adaptive immune system are critical in the containment of viral infection: NK cells and virus-specific CD8+ T cells. New evidence demonstrating that NK cells can specifically lyse HIV-1–infected CD4+ T cells [22], as well as the significant association observed between the expression of KIR3DS1 (and of its putative ligand, HLA-Bw4 80I)
Figure 3. Decline in NK cell activity after acute HIV-1 infection. The dot plots represent changes in the level of NK cell activity at baseline and after 1 year in subjects who did not initiate therapy (untreated) and those who initiated highly active antiretroviral therapy (treated). The levels of interferon (IFN)-γ secretion (A) and degranulation (B) were quantified in NK cells after stimulation of peripheral-blood mononuclear cells with major histocompatibility complex (MHC)-devoid target cells for 6 h. The correlation between the level of viral replication and the level of NK cell degranulation and IFN-γ secretion after stimulation with MHC-devoid target cells is shown in panels C and D, respectively.

and slower HIV-1 disease progression [9], suggests that NK cells may play an important role in the control of HIV-1 disease. Here, we have characterized the kinetics of the changes in NK and HIV-1–specific CD8+ T cell numbers and function during the earliest period of acute HIV-1 infection and have demonstrated that the NK cell response dominates initially, at a time when the virus-specific CD8+ T cell response is just emerging, but declines once significant virus-specific T cell responses and antibody responses have developed. In contrast to the inverted kinetics of NK and HIV-1–specific CD8+ T cell responses during acute HIV-1 infection, these cell populations expanded with similar kinetics during chronic infection after reexposure to HIV-1 during STI. Taken together, these analyses of the kinetics by which cytolytic effector cells respond to HIV-1 viremia suggest that NK cells represent the dominant cytolytic effector population during the earliest phase of HIV-1 infection, whereas both NK cells and virus-specific CD8+ T cells cooperatively expand during the chronic phase of infection in response to exposure to HIV-1 antigen.

Several lines of evidence have suggested an important role for virus-specific CD8+ T cells in the control of HIV-1 infection, including the temporal correlation between the induction of CD8+ T cell responses and a reduction in plasma viremia [23, 24] and the evolution of sequence mutations in regions targeted by virus-specific CD8+ T cell responses [25]. However, to date the strongest direct evidence to support a role for CD8+ T cells in the control of HIV-1 replication has been drawn from studies in which CD8+ cells were depleted in rhesus macaques during primary SIV infection, which resulted in the failure to control viral replication [7, 26]. An important caveat of these studies, as highlighted by the authors, is that the antibody used to deplete CD8+ T cells, CM-T807, binds to all cells expressing CD8 on their surface. Although only ∼30% of NK cells express CD8 in humans, 90% of macaque NK cells express this marker.
Figure 4. Synchronous increase in NK and HIV-specific CD8+ T cell numbers and activity after discontinuation of highly active antiretroviral therapy (HAART). Primary flow data shows the percentage of NK cells (A) and killer immunoglobulin-like receptor (KIR)+ NK cells (B) in a representative subject when viral replication was undetectable during HAART and at peak viremia during structured treatment interruption (STI). The dot plots depict the overall change in NK cell numbers (A), KIR+ NK cells (B), degranulating NK cells (C), interferon (IFN)-γ+ NK cells (D), degranulating HIV-1-specific CD8+ T cells (E), and IFN-γ+ HIV-1-specific CD8+ T cells (F) in subjects at a time when viral load was undetectable (<50 RNA copies/mL) during HAART before STI, at peak viremia during STI, and after reduction of viral load back to undetectable numbers with treatment reinitiation.

[27], making it likely that this antibody depleted both CD8+ T cells as well as NK cells during these experiments [27]. It is therefore difficult to determine whether the depletion of CD8+ T cells alone, NK cells alone, or both contributed to the observed loss of control over viral replication.

The innate immune response—and NK cells in particular—is critical in the early control of various viral infections [2, 18, 28, 29] and works in synergy with CD8+ T cells to clear infection. Work in the herpes simplex virus type 1 (HSV-1) model has demonstrated that NK cells are the largest fraction of the lymphocyte infiltrate during the first 1–5 days of infection but decline as CD8+ T cell numbers expand by day 5 of infection. The critical role played by NK cells in the containment of HSV-1 infection early during infection was further confirmed by the demonstration that NK cell–depleted mice exhibited 1–2 log higher viral loads than control mice [30], whereas depletion of NK cells at day 5 after infection had no impact on the clearance of the virus. Previously, data from our group demonstrated a
significant expansion of the NK cell population during primary HIV-1 infection, with a preferential increase in cytolytic CD56dim NK cells [13]. Here, we have shown that the most dramatic expansion of the NK cell population occurs during the earliest window of acute HIV-1 infection, when both virus-specific T cells and antibodies are not yet detectable. Similar to observations in other viral models, NK cell numbers declined once the adaptive HIV-1–specific immune responses became detectable. These data demonstrate, for the first time, the inverse kinetics of NK and virus-specific CD8+ T cell responses during acute HIV-1 infection, indicating that NK cells represent the dominant cytotoxic effector population during the initial phase of infection, potentially playing a critical role in the control of peak viremia.

Interestingly, NK cell activity was directly associated with the level of viral replication during acute HIV-1 infection and declined with persisting infection. Similarly, the decline in NK cell activity was most pronounced in subjects who initiated therapy and achieved viral suppression, suggesting that active viral replication sustains the observed NK cell expansion and activity in vivo. In parallel, the HIV-1–specific CD8+ T cell population expands with progressive infection and broadens in specificity and function in subjects who continue to not receive HAART, whereas the breadth and strength of the HIV-specific CD8+ T cell response is constrained with early therapeutic intervention [15, 20, 21]. Although the kinetics of NK and CD8+ T cells appear to follow opposite trajectories during acute infection, discontinuation of therapy during chronic infection was associated with a synchronous expansion of the 2 effector subsets. These data demonstrate that, although NK cells and CD8+ T cells exhibit diverging kinetics during acute infection, effector cells of both the innate and adaptive immune systems respond rapidly to fluctuations in viremia during chronic infection.

Taken together, the present investigation of the NK cell compartment during acute HIV-1 infection has demonstrated that the population of these innate effector cells rapidly expands in the peripheral blood before the induction of detectable virus-specific CD8+ T cell or antibody responses, dominating the initial effector cell response to HIV-1. However, both the NK cell population and the HIV-1–specific CD8+ T cell population rapidly expand in response to viral replication in subjects who undergo STIs during chronic infection. Cumulatively, these data highlight the differential role that cytotoxic effector cells of the innate and adaptive immune systems may play during different phases of HIV-1 infection.

References
22. Bonaparte MI, Barker E. Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is de-
pendent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. Blood 2004; 104:2087–94.


