Short Communication

HIV-1-Specific T Cell-Dependent Natural Killer (NK) Cell Activation: Major Contribution by NK Cells to Interferon- γ Production in Response to HIV-1 Antigens

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Abstract

Natural killer (NK) cells can directly recognize virus-infected cells. Here, we demonstrate that NK cells also produce interferon (IFN)- γ in an HIV-1-specific, T cell-dependent manner. After stimulation of peripheral blood mononuclear cells (PBMCs) from HIV-1-infected individuals with HIV-1-derived peptides, up to half of the IFN- γ -producing PBMCs are NK cells. These results indicate that T cell-dependent NK cell IFN- γ production can be important for immune control of HIV-1, and have implications for the interpretation of data from vaccine trials using ELISPOT and ELISA.

NATURAL KILLER (NK) CELLS operate on the border between innate and adaptive immunity. It is well established that they play an important role in the early response against certain viral infections, and they can set the stage for subsequent adaptive immune responses through the production of cytokines.¹ In addition, it was demonstrated that NK cells respond to influenza and tetanus antigens in a T cell-dependent manner,^{2,3} indicating that NK cells play an important role after the onset of adaptive immune responses.

There has been increasing evidence of a role for NK cells in the control of HIV-1 infection.^{4–8} In this study, we investigated whether T cell responses to HIV-1-derived antigens can activate NK cells, and to what extent NK cells contribute to IFN- γ production after stimulation of peripheral blood mononuclear cells (PBMCs) with HIV-1-derived antigens. IFN- γ production by T cells and NK cells was analyzed by cytokine flow cytometry in PBMCs from five viremic HIV-1-infected subjects (mean plasma viral load, 20,198 copies/ml; range, 3270–40,700 copies/ml) and five healthy controls. All subjects gave written informed consent, and the UCSF Committee for Human Research approved the study.

To assess IFN- γ production by T cells and NK cells, frozen PBMCs were thawed and stimulated for 24 hr with 5 μ g/ml of

HIV-1-derived peptides (15-mers overlapping by 11, spanning the full length of HIV-1 Gag and Nef), with brefeldin A present during the last 6 hr of culture. We could readily detect HIV-1-specific CD8⁺ and CD8[–] T cell responses in the HIV-1 seropositive subjects (Fig. 1a, left and middle, respectively), whereas no responses above background were detected in the HIV-1-seronegative subjects (data not shown). Concomitantly, we could detect a strong NK cell response after stimulation with HIV-1-derived antigens in the HIV-1-seropositive subjects (Fig. 1a, right), but not in HIV-1-seronegative, healthy control subjects (data not shown). In addition, depletion of CD3⁺ T cells in PBMCs from HIV-1-seropositive subjects abrogated the NK cells response to the HIV Gag-derived peptides (data not shown). The absence of responses in PBMCs from HIV-1-seronegative subjects, and in PBMCs from HIV-1-seropositive subjects depleted of T cells, indicates that the NK cell activation is T cell dependent, and that the HIV-1-derived peptides per se do not activate human NK cells. A higher frequency of CD56^{bright} NK cells compared with CD56^{dim} NK cells produced IFN- γ after stimulation of PBMCs with HIV Gag-derived peptides (data not shown), consistent with reports on influenza-specific T cell activation of NK cells.³ However, because of the low frequency of CD56^{bright}

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FIG. 1. NK cells are a major contributor to IFN- γ production after stimulation of PBMCs from HIV-1-infected subjects with HIV-1-derived peptides. PBMCs from HIV-1-infected subjects were stimulated with HIV-1-derived peptides or recombinant human IL-12 (hIL-12) for 24 hr, followed by analysis by intracellular cytokine flow cytometry. Cells were stained on with anti-CD56-PE-Cy7, anti-CD8-Pacific Blue, and ethidium monoazide (EMA). After fixation and permeabilization, cells were stained with anti-CD3-ECD and anti-IFN- γ -allophycocyanin. The PBMCs were stained with anti-CD3 intracellularly to detect T cells that have downregulated CD3 in response to the peptide stimulation. In the analysis, a forward scatter (FSC)-versus-side scatter plot was used to identify lymphocytes. Single cells were identified by an FSC-Area versus FSC-Height gate, and dead cells were excluded by gating on EMA⁻ cells. T cells were identified by expression of CD3, and NK cells were identified as CD3⁻CD56⁺. (a) Representative example of production of IFN- γ by CD8⁺ T cells (left), CD8⁻ T cells (middle), and by CD3⁻CD56⁺ NK cells (right). Percentages indicate the percentage of each population expressing IFN- γ . (b) Frequencies of IFN- γ ⁺ cells among CD8⁺ T cells, CD8⁻ T cells, and NK cells after stimulation of PBMCs with HIV-1 Gag/Nef (left) and hIL-12 (right) from five HIV-1-infected subjects. The background responses (medium alone) have been subtracted. The horizontal bar indicates the average frequency of IFN- γ ⁺ cells. Note that the y axis is log-transformed. (c) Relative contribution of T cells and CD3⁻CD56⁺ NK cells in response to HIV-1 Gag/Nef (left) or hIL-12 (right) after 24 hr of stimulation. The percentages indicate the frequency of T cells, NK cells, or other cells among all IFN- γ ⁺ PBMCs.

NK cells, CD56^{dim} NK cells still made up the majority of the IFN- γ -producing NK cells (data not shown). A summary of the IFN- γ responses to HIV-1-derived peptides by T cells and NK cells in PBMCs of the five HIV-1-infected subjects is depicted in Fig. 1b (left). Stimulation of PBMCs with IL-12, which was used as a positive control for NK cell stimulation, resulted in a strong IFN- γ response by the NK cells, and to a lesser extent by T cells (Fig. 1b, right).

T cells are the largest population of lymphocytes in human peripheral blood, making up more than 70% of the lymphocytes. In contrast, the frequency of NK cells is lower, and variable, making up only 5–25% of the lymphocytes. A low frequency of responding T cells could thus still constitute a majority of the IFN- γ -producing cells detected in these assays. To assess the contribution of NK cells to the total pool of IFN- γ ⁺ cells after stimulation with HIV-1-derived antigens, we gated on all IFN- γ ⁺ cells among the lymphocytes, and then analyzed the relative contribution of T cells, NK cells, and other cells to the total pool of IFN- γ ⁺ cells. As expected, T cells made up a large part of the IFN- γ -producing cells after stimulation with HIV-1-derived peptides (mean, 49.5%; range, 34–71%) (Fig. 1c, left). Strikingly, NK cells made up half of the IFN- γ -producing cells after stimulation with HIV-1-derived peptides (mean, 47%; range, 27–62%) (Fig. 1c, left), thus providing a major contribution to IFN- γ production. Together, NK cells and T cells made up the vast majority of all IFN- γ -producing cells after stimulation with HIV-1-derived antigens, and other cells contributed minimally (mean, 3.1%; range, 1.8–5.3%) (Fig. 1c, left). As a comparison, NK cells made up more than 70% of the IFN- γ -producing cells after stimulation with IL-12 (mean, 73%; range, 51–84%) (Fig. 1c, right).

In conclusion, we show that stimulation of HIV-1-specific T cells results in a strong activation of NK cells. NK cells constitute up to half of the IFN- γ -producing cells after stimulation with HIV-1-derived peptides. NK cells can thus be a major contributor to the immune response against HIV-1, also after the onset of adaptive immune responses. Our results highlight the need to study complex interactions between different immune cells, in order to reveal the basis for protective immunity in HIV-1 infection. In addition, the results have important implications for the interpretation of data generated by, for example, ELISPOT assays and ELISAs in vaccine trials, as a substantial component of the IFN- γ in these assays is likely to be produced by NK cells, and not by T cells.

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Author Disclosure Statement

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