Expression of Chemokine and Inhibitory Receptors on Natural Killer Cells: Effect of Immune Activation and HIV Viremia

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We examined the cell-surface expression of chemokine and natural killer (NK) cell inhibitory receptors (iNKRs) on NK cells from individuals with human immunodeficiency virus (HIV) infection, chronic hepatitis C infection, and Wegener’s granulomatosis (WG), an inflammatory, granulomatous vasculitis. The expression of CCR5 on NK cells was up-regulated in individuals with HIV viremia and in individuals with active WG, indicating that expression of this receptor is modulated by states of immune activation associated with viral infection and inflammatory or immune-mediated diseases. In contrast, iNKRs were shown to be up-regulated only on NK cells of individuals with HIV viremia, and they returned to a normal level when viremia was controlled with effective antiviral therapy. In individuals with HIV-1 viremia, there was a direct correlation between the level of expression of p58.1, p58.2, and CD94 receptors and plasma HIV viremia, suggesting that ongoing active HIV replication has an effect on the expression of such receptors on NK cells. These results suggest that immune activation leads to abnormal cell-surface expression of chemokine receptors on NK cells, whereas HIV-specific processes account for the up-regulation of iNKRs on NK cells; this may explain the NK cell–functional defects seen in HIV infection.

Natural killer (NK) cells are bone marrow–derived, large granular lymphocytes that recognize and lyse a variety of transformed and virus-infected cells [1]. Unlike T and B cells, NK cells do not require gene-rearrangement machinery for assembly of their receptor genes [1]; rather, NK cells discriminate potential target cells on the basis of the levels of self–major histocompatibility complex (MHC) class I expression on such cells [2]. Self–MHC class I molecules bind to the NK cell inhibitory receptors (iNKRs) present on NK cells that, in turn, prevent lysis of target cells [3]. However, low or absent expression of self–MHC class I molecules allows NK cells to lyse target cells [3]. Several studies have investigated the expression of iNKRs on NK cells and CD8+ T cells in HIV-infected individuals [4–10]. Whether increased expression of iNKRs contributes to defective NK cell function in vivo in HIV infection is not yet clear.

Chemokine receptors such as CCR5 and CXCR4, expressed on the surface of NK cells, are involved in chemotaxis and recruitment of effector NK cells to sites of active viral replication and thereby potentially contribute to the control of active HIV replication in vivo. It has been shown elsewhere [12, 13] that the chemokine receptor CCR5 is up-regulated on CD4+ T cells of HIV-infected individuals compared with HIV-negative healthy volunteers. NK cells from HIV-negative individuals have been shown to express both CCR5 and CXCR4 receptors on their surfaces [14].

We have shown that HIV viremia affects the ability of NK cells to produce CC-chemokines and thereby suppress HIV replication ex vivo [15]. In the present study, we analyzed the cell-surface expression of chemokine receptors and iNKRs on the surface of NK cells from patients with and without HIV viremia and from healthy volunteers, to understand the effect of active HIV replication on the expression of iNKRs and chemokine receptors on NK cells. We also studied patients with active Wegener’s granulomatosis (WG) and chronic hepatitis...
C infection, to understand how expression of these receptors on NK cells is modulated in the context of a non-HIV chronic viral infection and a noninfectious systemic inflammatory disease.

SUBJECTS, MATERIALS, AND METHODS

Study subjects. Thirty-one HIV-infected individuals were studied. Sixteen patients were receiving various regimens of highly active antiretroviral therapy (HAART), containing at least 1 protease inhibitor and 2 reverse transcriptase inhibitors of HIV, and had plasma HIV viral loads of <50 copies/mL. Mean CD4+ T cell count was 580 cells/mm³ (range, 27–1392 cells/mm³). Fifteen HIV-infected patients were not receiving any antiviral therapy and had detectable levels of plasma HIV viremia at the time of the study (median, 35,333 copies/mL [range, 2900–206,038 copies/mL]; mean total CD4+ T cell count, 445 cells/mm³ [range, 77–939 cells/mm³]). Nineteen individuals with WG were also studied; 9 of them had active disease, either newly diagnosed WG or a relapse during current therapy, with elevated erythrocyte sedimentation rates (ESRs), elevated C-reactive protein (CRP) levels, or both; the remaining 10 patients had normal ESRs and CRP levels at the time of the study and had no clinical evidence of active WG. Nine patients with chronic hepatitis C infection who were seronegative for HIV were also studied, along with 10 HIV- and hepatitis C–seronegative healthy volunteers. All patients were participating in clinical research protocols approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board, and all patients provided written informed consent.

Fluorescence-activated cell sorter (FACS) analysis. Blood was drawn from each individual by venipuncture, in EDTA tubes (Becton Dickinson), for FACS analysis. All samples for FACS analysis were prepared <2 h after blood was drawn. For flow-cytometric analyses, the following combinations of fluorochrome-conjugated antibodies were used: CD3 (perdinine chlorophyll protein), CD56 (phosphatidylethanolamine [PE]) with CCR5 (fluorescein isothiocyanate [FITC]), p58.2 (FITC), CD94 (FITC), CD161 (FITC), 2B4 (FITC), and p58.1 (FITC). For evaluation of the expression of CXCR4 on NK cells, CD56 (FITC) and CXCR4 (PE) were used. All antibodies and appropriate isotype controls were obtained from BD Biosciences.

For each stain, 100 µL of whole blood was washed with 1% fetal calf serum and 0.02% sodium azide in PBS. After being washed, whole blood was incubated with appropriate antibodies for 30 min at 4°C. The blood was washed, red blood cells were lysed by FACS lysis buffer (Becton Dickinson, catalog number 349202), and cells were fixed and suspended in 1% paraformaldehyde in PBS. Three-color flow-cytometric analysis was performed on an FACS Caliber (Becton Dickinson). For subset analysis, lymphocyte gate and gates uniquely identifying CD3+ cells were applied, ~100,000 events were collected, and the frequency of CD56+ CD3+ NK cells expressing each receptor was analyzed with CELL QUEST software (Becton Dickinson). For example, CCR5 expression on NK cells was obtained after gating for lymphocytes, on the basis of forward and side scatter, and then gating for CD3+ CD56+ cells.

Statistical analysis. Comparisons of the 6 groups of individuals were made by analysis of variance with Tukey’s multiple-comparison test; data were recorded. Student’s t test was used for 2-group comparisons; percentage of NK cells expressing various receptors were recorded. Spearman rank correlation was used to determine the association between viral load and each of the chemokine- and NK-receptor variables. The Bonferroni method was used to adjust P values for multiple testing.

RESULTS

Relationship between expression of chemokine receptors on the surface of NK cells and the state of immune activation. To study the relationship between the state of active HIV replication and expression of chemokine receptors, we determined, in the 6 different groups of individuals, the proportions of NK cells expressing CCR5 and CXCR4 receptors. The percentage of NK cells expressing CCR5 on their surfaces was significantly higher in individuals with HIV viremia (geometric mean, 15% [range, 7%–28%]) and individuals with active WG (mean, 15% [range, 9%–22%]) compared with the percentage among HIV-seronegative healthy volunteers (mean, 4% [range, 2%–7%]; P = .001) (figure 1A). However, the level of expression of CCR5 on NK cells was not significantly different in individuals without HIV viremia, patients with inactive WG, and patients with chronic HCV, compared with the level in HIV-seronegative healthy volunteers (P > .5). There was no statistically significant difference in the expression of the CXCR4 receptor on NK cells among the 6 different groups (P > .5; figure 1). These results indicate that expression of the CCR5 receptor on the surface of NK cells is modulated by states of immune activation, particularly in HIV-infected individuals with uncontrolled viremia and active immune-mediated conditions such as WG. However, control of immune activation, either by effective anti-HIV therapy or by immunosuppression, results in the normalization of expression of this receptor on the surface of NK cells. In addition, the expression of CXCR4 is not modulated by similar states of immune activation to the extent seen with CCR5 expression on NK cells.

Relationship between expression of NK cell receptors and the state of immune activation. To study the relationship between the expression of INKR and the state of active HIV replication, we determined the percentage of NK cells expressing the following receptors: p58.1, p58.2, CD161, CD94, and 2B4. Of these, p58.1, p58.2, and CD161 are inhibitory receptors expressed on both NK cells and CD8+ T cells. CD94 can function as either an
Figure 1. Percentage of NK cells expressing chemokine and NK cell receptors on their surfaces, from patients with or without HIV viremia, patients with active or inactive WG, and patients with chronic hepatitis C. Flow cytometry was performed as described in Subjects, Materials, and Methods. Expression of CCR5 (A), CXCR4 (B), p58.1 (C), p58.2 (D), CD94 (E), 2B4 (F), and CD161 (G) is shown, with the respective geometric mean values indicated by thick black bars. Statistically significant differences are indicated, with respective P values. Wegener’s, Wegener’s granulomatosis.
inhibitory or an activating receptor by forming heterodimers with NKG2A or NKG2C, respectively. 2B4 is a coreceptor involved in augmenting the cytotoxic function of NK cell–activating receptors. The expression of p58.1, p58.2, and CD94 was significantly greater on NK cells from individuals with HIV viremia (geometric means, 18.9%, 18%, and 70%, respectively) than on NK cells from HIV-seronegative healthy volunteers (5%, 4.8%, and 59%, respectively) (P < .001 for p58.1, P = .005 for p58.2, and P = .05 for CD94) (figure 1C–1E). The expression of these receptors was not statistically different in the other 5 groups of subjects. In addition, the levels of expression of 2B4 and CD161 were not statistically different between the 6 groups of individuals studied. These results suggest that HIV viremia induces the up-regulation of cell-surface expression of major inhibitory receptors on NK cells. However, since this increase in expression of iNKRs on NK cells was restricted to patients with HIV viremia and was not observed in other groups, up-regulation of iNKRs may occur as a result of active HIV replication.

**Relationship between expression of chemokine and NK cell receptors on NK cells and the level of plasma HIV viremia.** To understand the relationship between the expression of chemokine and NK cell receptors and active HIV replication, we performed statistical analyses using the percentage of NK cells expressing each receptor and the level of plasma HIV viremia at the time of the study. There was a statistically significant correlation between the percentage of NK cells expressing CCR5 (r = 0.96; P < .001), p58.1 (r = 0.75; P = .004), p58.2 (r = 0.93; P = .004), and CD94 (r = 0.82; P = .004) and the level of plasma viremia in the 15 individuals with HIV viremia (figure 2). In contrast, there were no statistical correlations between the expression of 2B4, CD161, and CXCR4 on NK cells and the level of plasma viremia at the time of the study. These results indicate that the increased expression of CCR5 and iNKRs p58.1, p58.2, and CD94 is a consequence of HIV viremia. In addition, neither the state of immune activation nor the level of HIV viremia influences the expression of CXCR4 and the coactivating receptor 2B4 on NK cells.

**DISCUSSION**

In the present study, we have demonstrated that active HIV replication, as manifested by detectable plasma HIV viremia, up-regulates the expression of the chemokine receptor CCR5 and iNKRs p58.1, p58.2, and CD94 on NK cells. Modulation of CCR5 expression on the surface of NK cells is influenced by the state of immune activation associated with HIV infection rather than being a direct effect of HIV viremia, given that elevated CCR5 expression on NK cells of individuals with active WG was also observed. However, the up-regulation of iNKRs p58.1, p58.2, and CD94 seems to be a consequence of a direct effect of HIV viremia on NK cells, since such up-regulation was not seen in individuals with either active or inactive WG or chronic hepatitis C infection. Moreover, the levels of expression of CCR5, p58.1, p58.2, and CD94 correlate with the levels of HIV viremia at the time of the study, reinforcing the notion that HIV viremia has an impact on the expression of both chemokine receptors and iNKRs on the surface of NK cells. Given that the degree of immune activation correlates with the level of plasma HIV viremia [15], it is likely that the effect of HIV viremia on the expression of CCR5 is due to the immune activation seen in individuals with HIV viremia.

Apart from being coreceptors for HIV entry, chemokine receptors play an important role in chemotaxis and inflammatory processes in vivo [12, 13]. In the case of NK cells, the expression of these receptors may play a significant role in both chemokinesis of these cells in response to cytokines and their redistribution to sites of active viral replication [14]. In this regard, in HIV infection, these receptors could play a role in the interaction between NK cells and HIV envelopes and/or virions, thereby interfering with the ability of NK cells to control ongoing viral replication. CCR5 up-regulation seen in HIV infection could thereby play a significant role in NK cell–HIV interactions in vivo. Moreover, this increase in CCR5 expression is consistent with that seen in CD4+ and CD8+ T cells [12, 13], suggesting a global modulatory effect of HIV viremia on the expression of chemokine receptors on all lymphocyte subsets.

iNKRs have been associated with NK cells and subsets of CD8+ T cells. They play a significant role in regulating NK cell–mediated cytotoxicity against cells transformed by tumors or viruses. Recent studies have shown that certain iNKRs are up-regulated in HIV infection [10, 11, 16]. The present study has addressed the role of HIV viremia in the expression of iNKRs on NK cells. We found that there was a significant up-regulation of p58.1, p58.2, and CD94 on the surface of NK cells of individuals with HIV viremia but not on NK cells from patients with either active WG or chronic hepatitis C infection. These results indicate that the increase in the expression of iNKRs seen in HIV infection is likely due to a direct effect of HIV on NK cells rather than to a nonspecific effect of either the state of immune activation or chronic infection. Thus, these data suggest that HIV viremia has a unique effect on NK cells, up-regulating the expression of iNKRs, which could interfere with the ability of NK cells to lyse virus-infected target cells. Various studies have shown different iNKRs up-regulated in association with HIV viremia; several discrepancies regarding the expression of iNKRs including p58.1 and CD94 have been noted in these studies [4–10]. The difference in the disease states of individuals with HIV viremia, in the type of samples used (whole blood vs. purified NK cells), and in the methods used in analyses of receptor expression could be responsible for the variability in the expression of these receptors [4–10]. To our knowledge, however, all studies have reported that iNKRs are up-regulated in individuals with HIV viremia,
Figure 2. Relationship between the percentage of NK cells expressing chemokine and NK cell receptors on their surfaces and the level of plasma HIV viremia at the time of the study. Expression of CCR5 (A), CXCR4 (B), p58.1 (C), p58.2 (D), CD94 (E), 2B4 (F), and CD161 (G) is shown. Statistically significant differences are indicated, with respective \( P \) values, and the strength of correlation is shown as \( r \) values.
indicating that HIV has an effect on the expression of iNKRs on NK cells and CD8+ T cells [4–10].

WG is an immune-mediated disease of unknown etiology and is characterized by granulomatous and vasculitic lesions involving the respiratory tract and kidneys [17]. Analyses of chemokine receptors on the surface of NK cells isolated from patients with active WG have shown an increased CCR5 expression similar to that seen in individuals with HIV viremia; however, the expression of CCR5 in patients with inactive (treated) WG is similar to that seen in healthy volunteers, indicating that the modulation of CCR5 expression is dependent on the state of disease activity and thus on the level of immune activation. A recent study has shown increased expression of CCR5 and CCR3 on the effector T cell–memory population (CD45RO+) isolated from patients with WG [18], consistent with the results seen for NK cells in the present study. However, the expression of iNKRs on the surface of NK cells from patients with WG was not statistically different from that seen in healthy volunteers, indicating the minimal role of immune activation in the modulation of iNKRs. These findings indicate that the expression of CCR5 is modulated by the state of immune activation, whereas the expression of iNKRs seems to be relatively specific to HIV viremia.

Patients with chronic hepatitis C who had viremia at the time of the present study served as controls for a systemic infectious disease similar to HIV infection. However, the levels of expression of either chemokine receptors or iNKRs were not statistically different from the levels in healthy volunteers, indicating that chronic hepatitis C infection has a minimal effect on the expression of iNKRs and chemokine receptors on the surface of NK cells.

The present study clearly demonstrates that active HIV replication modulates the expression of CCR5 and certain iNKRs on the surface of NK cells, which explains in part the functional defects seen in NK cells from individuals with HIV viremia. However, such effects seem to be dependent on the presence of HIV viremia, since HIV-infected individuals in whom viral replication was suppressed by HAART had normal expression of iNKRs and CCR5 on NK cells. The underlying mechanisms for this effect of HIV viremia on NK cell–receptor expression is unclear at present. It has been shown that HIV viremia impairs the ability of NK cells to secrete CC-chemokines [15]. Whether this effect is related to the CCR5 up-regulation seen in immune activation is not clear. However, in vitro studies have shown that HIV envelope proteins are capable of inhibiting NK cell functions through their binding to chemokine receptors, suggesting that ongoing HIV replication has the deleterious effect of inhibiting NK cell functions (authors’ unpublished data). Furthermore, NK cell–mediated redirected cytotoxic assays have demonstrated the inability of NK cells from patients with viremia to lyse targets in vitro, corresponding to increased expression of iNKRs [19]. Further studies designed to address the interactions between the HIV envelope and NK cells should prove helpful in delineating the underlying mechanisms of the receptor dysregulation.

References