In Vivo Natural Killer Cell Depletion during Primary Simian Immunodeficiency Virus Infection in Rhesus Monkeys

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The contribution of natural killer (NK) cells to the immune containment of human immunodeficiency virus infection remains undefined. To directly assess the role of NK cells in an AIDS animal model, we depleted rhesus monkeys of >88% of CD3− CD16+ CD159a+ NK cells at the time of primary simian immunodeficiency virus (SIV) infection by using anti-CD16 antibody. During the first 11 days following SIV inoculation, when NK cell depletion was most profound, a trend toward higher levels of SIV replication was noted in NK cell-depleted monkeys compared to those in control monkeys. However, this treatment did not result in significant changes in the overall levels or kinetics of plasma viral RNA or affect the SIV-induced central memory CD4+ T-lymphocyte loss. These findings are consistent with a limited role for cytotoxic CD16+ NK cells in the control of primary SIV viremia.

Natural killer (NK) cells are a component of the innate immune system that plays a central role in host defense against viral infections and tumor cells. Much of the evidence for a role for NK cells in controlling viral infections has come from experiments with mice that were genetically modified (9) or treated with NK cell-depleting antibodies (8) or from the study of humans with inherited NK cell deficiencies (2, 11). The effect that NK cells may exert on the pathogenesis of human immunodeficiency virus (HIV) infection is less certain. A number of studies have indirectly implicated NK cells in affecting HIV replication or AIDS progression by potentially acting through the mechanisms of direct cytotoxicity, antibody-dependent cellular cytotoxicity, or the release of β-chemokines (reviewed in reference 1). However, direct experimental evidence defining the contribution of NK cells to the control of HIV replication and AIDS is lacking.

The simian immunodeficiency virus (SIV)-infected rhesus monkey is a powerful animal model for studying the immune control of HIV infection. We and others have used monoclonal antibodies to target and deplete T- or B-lymphocyte populations to define the contribution of cellular and humoral immunity to the control of SIV in monkeys (7, 14, 15). We recently developed and validated a nonhuman primate model of in vivo depletion of cytotoxic NK cells by antibody targeting of CD16 (3). Uniquely, the expression of CD16 on macaque monkey leukocytes is almost exclusively limited to a subset of lymphocytes and monocytes and is not expressed on neutrophils (13). CD16 is expressed on the majority of blood NK cells in rhesus macaques (17). The present study was initiated to investigate the role of NK cells in controlling SIV during primary infection.

To explore the role of NK cells in the control of virus replication during primary SIV infection in rhesus monkeys, we slowly administered an anti-human CD16 monoclonal antibody (clone 3G8; a murine immunoglobulin G(κ) [IgG(κ)] antibody) by the intravenous route to five monkeys at a dose of 50 mg/kg. Five additional rhesus monkeys received an equivalent dose of the irrelevant control mouse IgG(κ) monoclonal antibody MOPC-21. One day after antibody administration, all monkeys were infected intravenously with uncloned SIVmac251 at a dose equivalent to 6.12 log_{10} SIV RNA copies. All monkeys were negative for the major histocompatibility complex class I allele Manu-A*01 and were maintained in accordance with the guidelines of the Committee on Animals for the Harvard Medical School, federal and state laws, American Association for Accreditation of Laboratory Animal Care regulations, and the Guide for the Care and Use of Laboratory Animals (9a).

Changes in leukocyte subsets were monitored in EDTA-anticoagulated blood specimens obtained from these monkeys. Whole blood was incubated with anti-CD3 (SP34; BD Biosciences), anti-CD4 (L200; BD Biosciences), anti-CD16 (DJ130c, not cross-blocked by 3G8; DakoCytomation), anti-CD159A (NKG2A, Z199; Beckman Coulter), anti-CD8-alpha (SKI1; BD Biosciences), anti-CD14 (M5E2; BD Biosciences), anti-CD28 (L293; BD Biosciences), and anti-CD95 (DX2; BD Biosciences) antibodies. Erythrocytes were then lysed using an ImmunoPrep system (Beckman Coulter), washed with phosphate-buffered saline, and resuspended in phosphate-buffered saline–1% formalin. Samples were analyzed using an LSRII flow cytometer (BD Biosciences), and data were reanalyzed using FlowJo software (Tree Star). Complete blood counts were performed on an automated hematology analyzer with rhesus-monkey-specific automated leukocyte differential software (ADVIA 120; Bayer).

In animals that received the control antibody, the absolute number and percentage of peripheral blood NK cells,
defined by immunophenotypic characterization as CD3+CD16+CD159A-, remained stable or increased during primary SIV infection (Fig. 1A and B, respectively). In contrast, animals that received the anti-CD16 antibody showed a sudden decline in the number and percentage of peripheral blood NK cells by day 1 following antibody treatment (Fig. 1A and B). At the point of maximum depletion, nearly 90% of NK cells were removed from the blood and the median number of NK cells remained less than 30% of baseline values through day 12. Anti-CD16 treatment also resulted in a 16 to 55% loss of CD16-bearing monocytes, whereas the monocyte numbers remained constant in the control antibody-treated group.

Plasma SIV RNA levels were monitored prospectively using an ultrasensitive branched-DNA amplification assay with a detection limit of 125 copies per ml (Siemens, Berkeley, CA). Plasma viremia peaked between days 8 and 13 in all animals and then declined to a stable set point after 4 weeks (Fig. 2A). Over the 14 weeks that the plasma viral loads were monitored post-SIV inoculation, the median level of plasma virus was higher in the NK cell-depleted group than in the controls at 12 of the 15 time points assessed. However, significant differences in plasma viral load were not detected between the NK cell-depleted and control groups at any time point measured through day 99 (Kruskal-Wallis/Dunn’s multiple-comparison posttest, P < 0.05).

To more carefully assess virus replication early in infection, we also measured viral load by integrating the areas under the curve for plasma viral RNA levels over days 0 to 11. This time corresponded to the period of most-profound NK cell depletion and preceded the emergence of acquired immune responses to SIV. As shown in Fig. 3A, the median cumulative viral load during days 0 to 11 was higher in the NK cell-depleted group than in the control group (median AUC viral load, 7.24 versus 6.88 log_{10} copies, respectively). However, this difference failed to reach statistical significance (P = 0.10, Mann-Whitney U test). For comparison, integrated measurements of viral load were also made over days 11 to 27 (Fig. 3B). This time corresponded to the period when plasma virus was declining to the set point, acquired immune responses were emerging, and NK cells in the depleted group were recovering.
in number. During this time, the experimental and control groups showed nearly identical levels of viremia (8.04 versus 8.05, Mann-Whitney U test). Power calculations have shown that a sample size of five animals per group has 80% power to detect 0.75 to 1.0 log10 differences in peak SIV viremia (12). Thus, this study was not powered to detect small differences in plasma viral load.

Since the extent of early central memory CD4+ T-cell loss in rhesus monkeys is associated with the level of SIV replication during primary infection (16), we evaluated this clinical consequence of primary infection in the monkeys with and without CD16+ NK cells. As shown in Fig. 2B, the two groups showed similar kinetics of central memory CD4+ T-cell loss and similar nadir values for this lymphocyte population (P = 1.0, Mann-Whitney U test).

The observations in the present study were unable to demonstrate that CD16+ NK cells contribute significantly to controlling SIV replication during the period of primary infection. However, several features of the NK cell depletion model need to be considered when these results are interpreted. The mouse anti-CD16 monoclonal antibody targets the CD16+ subset of the NK cells that is responsible for cytolytic function (4). However, the CD16+ NK cell subset, which includes most NK cells in human lymph nodes (6), has been shown to control the replication of viruses, including HIV, through chemokine secretion (5, 10). Therefore, this model’s feature of sparing the CD16+ noncytolytic subset of NK cells might preserve a portion of the NK cell-mediated antiviral activity. The depleting activity of the mouse anti-CD16 activity is limited in duration by the emergence of anti-Ig antibodies, which we have shown to appear during the second week following antibody administration (3). In addition to a more-complete depletion of all subsets of NK cells, a longer duration of depletion that would be possible through the use of a recombinant anti-CD16 designed for use in primates would provide a more-rigorous evaluation of the role of NK cells in the SIV model. Finally, a mucosal route of virus inoculation, instead of intravenous infection, could have provided a setting where innate host defenses might have made a greater contribution. However, due to the transient nature of depletion of the target (NK) cell population, a low-dose, repeated mucosal challenge, which might have been more sensitive to changes in innate immunity, was not possible. Nevertheless, these studies do not support a prominent role for CD16+ cytolytic NK cells in contributing to the control of an AIDS virus infection.

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