Deficient Antibody-Dependent Cellular Cytotoxicity against Human Immunodeficiency Virus (HIV)-Expressing Target Cells in Perinatal HIV Infection

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Peripheral blood mononuclear cells (PBMC) of human immunodeficiency virus (HIV)-infected children, age-matched HIV-seronegative controls, and HIV-infected asymptomatic and symptomatic adults were compared for their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) and natural killer (NK) cell-mediated cytotoxicity against target cells expressing HIV or herpes simplex virus (HSV) antigens. Target cells consisted of CD4 lymphocytes purified from PBMC of HIV-seronegative adults and incubated with the IIIB strain of HIV, HUT78 cells chronically infected with IIIB, and HSV-infected human fibroblasts. PBMC of asymptomatic HIV-infected adults were generally able to lyse CD4 cells expressing HIV antigens. Direct correlation was found between the magnitude of lysis and absolute CD4 cell counts in these individuals. In contrast to these results, PBMC from HIV-infected children were generally unable to lyse IIIB-expressing CD4 cells, regardless of the children's clinical status, age, or absolute CD4 cell counts. Cells from HIV-seronegative adults and children did not directly lyse these target cells either, in contrast to cells of HIV-seropositive children, who were able to mediate cell lysis when serum from an HIV-seropositive adult was added. However, effector cells from these HIV-infected children were able to mediate both ADCC against HSV-infected fibroblasts and NK cell-mediated cytotoxicity against IIIB-infected HUT78 cells. Reduced ability of PBMC from vertically HIV-infected children to mediate ADCC against HIV antigen-expressing CD4 cells may contribute to rapid progression to AIDS.

Materials and Methods

Subjects. Subjects consisted of HIV-infected adults, monitored at the Hospital of the University of Pennsylvania; children with perinatal HIV infection, monitored in the Special Immunology Clinic at The Children’s Hospital of Philadelphia; and age-matched HIV-seronegative healthy volunteers. HIV infection was diagnosed on the basis of at least two positive PCRs and PBMC cultures for HIV. According to Centers for Disease Control and Prevention (CDC) criteria for children and adults (8, 9), HIV-infected children were classified as asymptomatic with normal (P1-A) or abnormal (P1-B) immune function or as symptomatic with nonspecific findings (P2-A) or HIV-related conditions (P2-B-F), whereas adults’ stages were classified as asymptomatic (CDC stage A), symptomatic conditions (stage B), or AIDS-defining conditions (stage C). Patients receiving intravenous immunoglobulin were excluded from the study, since repeated administration of intravenous immunoglobulin may lead to reduced NK cell-mediated cytotoxicity (11) and might affect the ability of PBMC to mediate ADCC. This study was approved by the Institutional Review Boards of the University of Pennsylvania and The Children’s Hospital of Philadelphia.

Effector cells. PBMC were separated from heparinized venous blood by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) gradient centrifugation. Monocytes were removed by adherence on plastic surfaces coated with fetal bovine serum (FBS; HyClone, Logan, Utah) as previously described (23). PBMC were used in cytotoxicity assays within 4 h after the blood drawing.

Experiments in which NK cells were depleted from PBMC by incubation with monoclonal antibody anti-Leu 11B (Becton Dickinson, Mountain View, Calif.), which reacts with the FcγII receptor (CD16) on NK cells, as previously described (3, 39) followed by incubation with baby rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada) to destroy antibody-bound cells were performed. The surviving PBMC were used as effector cells in cytotoxicity assays. Arming of effector cells was accomplished by incubating PBMC for 12 h at 37°C with uninduced heat-inactivated heterologous sera obtained from HIV-infected patients and seronegative controls (58). The cells were washed five times before use as effector cells in cytotoxicity assays. To elute putative cytophilic antibodies, freshly isolated PBMC were incubated at 37°C for 12 h and then washed three times (57).

Target cells. HUT78 cells, derived from a CD4+ lymphoblastoid T-cell line, uninfected and chronically infected with the HIV-1 strain IIIB (16), were kindly provided by J. A. Hoxie, Hospital of the University of Pennsylvania, Philadelphia. K562 cells, derived from an erythroleukemia cell line and known to be sensitive to NK cell-mediated cytotoxicity, were used as target cells in NK cell assays. SF3 cells, human embryonic foreskin fibroblasts (National Institute of Allergy and Infectious Diseases, Bethesda, Md.), were inoculated with the NS strain of herpes simplex virus type 1 (HSV-1) (kindly provided by H. M. Friedman, Hospital of the University of Pennsylvania) at a multiplicity of infection of 5.0, as previously described (37). After 6 h of incubation at 37°C in 5% CO2, the cells were tryptophanized, washed, and then stored in the vapor phase of liquid nitrogen. Uninfected SF3 cells were prepared simultaneously.

PBMC of healthy seronegative adults were stimulated with phytohemaggluti-
nin (Sigma, St. Louis, Mo.) and then expanded in the presence of human interleukin 2 (IL-2; Schipparelli, Fairfield, N.J.) as described previously (56). Thereafter, CD4 cells were selected from these phytohemagglutinin–IL-2-stimulated PBMC by panning (60) with the monoclonal antibody OKT4. Purified CD4⁺ cells were incubated for 48 h in medium containing 32 U of IL-2 per ml and 20% FBS. The cells were then washed and subjected to low-speed centrifugation. Cell-free supernatant of strain IIIB-infected HUT78 cells was added to pelleted CD4⁺ cells at a final dilution of 1:10,000. After 1 h of incubation at 37°C, the cell surface expression of viral antigens was confirmed by flow cytometric analysis after immunofluorescent staining with HIV-seropositive human serum and fluorescein-conjugated goat F(ab)₂ anti-human immunoglobulin G (IgG) (TAGO, Burlingame, Calif.). After virus inactivation with 4% paraformaldehyde in phosphate-buffered saline, viral antigen expression was quantified by flow cytometry.

Unexposed and HIV-coated target cells were electronically gated to exclude aggregates and nonviable cells from evaluation. Fluorescence intensity thresholds of less than 2% positive cells were established by using uninfected target cells incubated with HIV-1 antibody-positive human serum and HIV-1-infected target cells incubated with HIV-1 antibody-negative human serum. HIV antigens were detected on >90% of target cells after 1 h of exposure to IIIB-containing supernatant.

**NK cell-mediated cytotoxicity.** Target cells, consisting of HUT78 cells chronically infected with the IIIB strain of HIV-1 (HUT78/IIIB), uninfected HUT78 cells, and K562 cells were labeled with Na₂⁵¹CrO₄ (Amersham, Arlington Heights, Ill.), resuspended in RPMI containing 20% FBS, and aliquoted into round-bottomed 96-well microtiter plate wells. Effector cells, prepared as described above, were added to give effector/target (E:T) cell ratios of 100:1 (5 ml supernatant per well) to PBMC to give final E:T ratios of 100:1. Microtiter plates were centrifuged for 3 min) and incubated for 4 h at 37°C in 5% CO₂. ⁵¹Cr release in supernatants was determined as described above.

**ADCC against HIV-expressing CD4 lymphocytes.** HIV-expressing and control CD4 lymphocytes, obtained as described above, were labeled with Na₂⁵¹CrO₄ and suspended in RPMI 1640 containing 20% FBS and 32 U of IL-2 per ml, and added to PBMC to give final E:T ratios of 100:1. Microtiter plates were centrifuged (100 × g for 3 min) and incubated for 4 h at 37°C in 5% CO₂. ⁵¹Cr release in supernatants was determined as described above.

**Quantitation of NK cells.** Immunophenotyping was performed by standard procedures. Heparinized venous blood was incubated with the monoclonal antibody B73.1 (kindly provided by G. Trinchieri, Wistar Institute, Philadelphia, Pa.), which reacts with the FcRIII (CD16) receptor present on NK cells and polymorphonuclear leukocytes (39). HIV was inactivated by incubation in % paraformaldehyde in phosphate-buffered saline for 16 h at 4°C prior to flow cytometric analysis. Mononuclear leukocytes were electronically gated to exclude other leukocytes from evaluation. After accumulation of 5,000 events, a fluorescence complexity threshold of less than 2% positive cells was established by using cells incubated with control mouse IgG.

**Statistical methods.** The data shown in the figures represent means ± standard errors. The two-sample t test was used to compare mean ADCC antibody titers for HIV-1-infected and uninfected infants. n refers to the total number of determinations for a particular condition.

**RESULTS**

Cell-mediated cytotoxicity against HIV-expressing CD4 cells. Forty-six children, 3 months to 10 1/2 years of age, with perinatally acquired HIV infection (19 patients at CDC classification stage P1, 15 at stage P2-A, and 12 at stage P2-B-D), were tested for cytotoxic activity against HIV-expressing CD4 cells. As shown in Fig. 1, PBMC from 31 of 46 children failed to lyse HIV-1-expressing CD4 cells, and PBMC of only four children gave >3% lysis. Even for these four, corresponding levels of lysis of control CD4 cells were not significantly different (data not shown). The lack of cytotoxic activity against HIV-expressing CD4 cells did not correlate with the patients’ stage of infection, age, or absolute CD4 lymphocyte counts.

Concurrently with pediatric samples, we tested the ability of PBMC isolated from 45 HIV-seropositive adults to lyse HIV-

**TABLE 1. Effect of NK cell depletion on cytotoxic activity**

| Treatment of effector cells | HUT | HUT-IIIB | CD4 | CD4-IIIB |
|----------------------------|-----|---------|-----|---------|
| None                       | 25.6 ± 9.2 | 58.0 ± 5.6 | 0.4 ± 2.6 | 16.0 ± 7.5 |
| Complement                 | 18.9 ± 8.4 | 41.9 ± 4.8 | 1.4 ± 1.9 | 12.7 ± 6.2 |
| Leu 11B plus complement    | 9.0 ± 3.2 | 15.7 ± 1.2 | 0.0 ± 0.0 | 0.0 ± 0.0 |

*PBMC of three HIV-infected adults were treated with Leu 11B and complement or complement alone, as described in Materials and Methods, and then used as effector cells in ⁵¹Cr release assays.

*Means ± standard errors.
confirm that lysis of HIV-expressing CD4 cells was mediated primarily by NK cells.

After incubation for 12 h at 37°C, PBMC of HIV-seropositive adults lost their ability to lyse HIV antigen-expressing CD4 cells (Table 2). The ability of such PBMC to lyse HIV-expressing CD4 cells was restored when autologous HIV antibody-containing serum was added to assays at a final concentration of 1:100. These findings indicate that decreased cytotoxicity against HIV-expressing CD4 cells after incubation of PBMC is due to elution of cytophilic antibodies from NK cells rather than to defective effector cells. Thus, our data confirm a previous report that lysis of HIV-expressing CD4 lymphocytes occurs via an antibody-dependent mechanism, mediated by NK cells armed in vivo with cytophilic HIV antibodies (62).

**Mechanism and specificity of the deficient cytotoxicity against HIV-expressing CD4 cells in HIV-infected children.** To determine whether the inability of PBMC from perinatally HIV-infected children to lyse HIV-expressing CD4 cells was due to a reduced number of effector cells, we used flow cytometry to determine the percentage of NK cells in PBMC populations. Seventeen HIV-infected pediatric patients, 14 weeks to 8 years of age (stage P1 to P2-D1) had NK cell percentages within the normal range for age-matched seronegative children (range, 11.5 to 22.8%; mean, 17.6%).

To determine whether decreased cytotoxic activity of PBMC from perinatally HIV-infected children was specific for HIV-expressing CD4 cells, we tested PBMC of HIV-infected and uninfected children and adults for their ability to lyse FS4 fibroblasts, infected with HSV-1 strain NS, as previously described (37). Cytotoxicity against HIV-expressing CD4 lymphocytes was studied in parallel. Similar to the results reported above, effector cells from HIV-infected children were unable to lyse HIV-expressing CD4 lymphocytes (Table 3). Addition of HIV-seropositive serum from an adult to the effector cells of HIV-infected children did not significantly increase lysis of HIV-expressing CD4 cells. Effector cells of age-matched HIV-seronegative children were able to kill these targets to a significantly greater extent in the presence of serum from the same HIV-seropositive adult than in the presence of serum from a seronegative adult (P < 0.05).

In contrast to results with HIV-expressing CD4 lymphocytes, effector cells from HIV-infected children lysed HSV-infected FS4 target cells to a significantly greater extent in the presence of an HSV-seropositive serum sample than in the presence of an HSV-seronegative serum sample (P < 0.05). In addition, effector cells from HIV-infected children were able to lyse HSV-infected targets to the same extent as did effector cells expressing CD4 cells (Fig. 2). Most of these patients were male homosexuals; 28 were asymptomatic (CDC classification stage A), and 17 were symptomatic (CDC stage B or C). In contrast to the results obtained for HIV-seropositive children, cytotoxicity was observed for the majority of adults tested. The magnitude of target cell lysis correlated positively with absolute CD4 cell counts: the highest magnitude of cytotoxicity was observed when the CD4 cell count exceeded 500/mm³ (mean, 16.3%; range, 5.1 to 36.9%). PBMC of patients with CD4 cell counts between 200 and 500 cells/mm³ was significantly higher than that of patients whose CD4 cell counts were <200 cells/mm³ (P < 0.05). Cytotoxicity was not detected against uninfected CD4 cells (data not shown). PBMC of HIV-seronegative adults (n = 30) and children (n = 21) failed to lyse HIV-expressing CD4 lymphocytes (data not shown).

**Characterization of effector cells and requirement for HIV-specific antibodies.** A series of experiments was carried out to investigate the mechanism of cytotoxicity against HIV-expressing CD4 lymphocytes in adults. CD16⁺ NK cell-depleted effector cells were tested for their ability to lyse a variety of target cells in ⁵¹Cr release assays (Table 1). After depletion of CD16⁺ cells, specific ⁵¹Cr release from all target cells, except from uninfected fibroblasts, was significantly decreased (P < 0.05). When the effector cells were treated with complement alone prior to being added to cytotoxicity assays, only a slight loss of lytic activity was observed. These experiments TABLE 2. Effect of incubation on the ability of PBMC to mediate lysis in the absence and presence of autologous serum

| PBMC incubation | % Lysis of different target cells | CD4 | CD4/HIB | HUT78 | HUT78/IIIIB |
|-----------------|----------------------------------|-----|---------|-------|------------|
| 2 h             | −1.1 ± 0.6                       | 23.5 ± 4.9 | 26.6 ± 5.1 | 49.6 ± 1.8 |
| 12 h            | −1.0 ± 1.1                       | 6.2 ± 1.1  | 23.2 ± 3.9 | 36.2 ± 1.1 |
| 12 h and autologous serum added to assay | −1.0 ± 1.7 | 26.4 ± 0.6 | 19.1 ± 4.2 | 37.5 ± 0.7 |

a PBMC were obtained from HIV-infected adults with mild or no symptoms. Incubation was in RPMI 1640 containing 20% FBS.
b Means ± standard errors.
c Autologous serum was added at a final dilution of 1:100.

table 3. Cytotoxicity against HIV-coated CD4 cells and HIV-infected FS4 cells

| Source of effector cells (n) | Value for target cells in the presence of serum
|-----------------------------|----------------------------------|
|                             | % Lysis | P value     | % Lysis | P value     |
|                             | HIV seronegative | HIV seropositive | HSV seronegative | HSV seropositive |
| HIV-infected children (12)  | 0       | 4.4 ± 2.3   | 0.1643  | 11.4 ± 1.6  |
| Uninfected children (10)    | 0       | 8.4 ± 1.3   | <0.05   | 9.0 ± 0.7   |
| HIV-infected adults (6)      | 13.4 ± 5.6 | 16.5 ± 4.0  | 0.3494  | 16.0 ± 1.6  |
| Uninfected adults (6)        | 0.8 ± 1.3 | 15.6 ± 3.4  | <0.05   | 16.6 ± 1.0  |

a PBMC were prepared as described in Materials and Methods.
b HIV- and HSV-seropositive and -seronegative sera obtained from adults.
c Means ± standard errors.
d Data were analyzed by Student’s t test.
from HIV-seronegative children. These results suggest that NK cells from HIV-infected children are capable of mediating ADCC against target cells infected with a virus other than HIV.

PBMC from HIV-infected adults lysed HIV-expressing lymphocytes equally in the presence and in the absence of HIV-seropositive serum ($P = 0.05$). Specific cytotoxicity was mediated by PBMC of HIV-seronegative controls when an HIV-seropositive serum sample was added to assay mixtures ($P < 0.05$). These results were consistent with previous reports that in vitro lysis of HIV-expressing CD4 lymphocytes is mediated by HIV patients’ effector cells armed in vivo with cytotoxic antibodies (57, 62). Similar to the observations with children, PBMC from HIV-infected adults and those from HIV-seronegative adults were equally effective in lysing HSV-infected targets in the presence of anti-HSV antibody-containing sera ($P > 0.05$).

To further characterize the functional defect in HIV-infected children, we tested the ability of their PBMC to lyse HUT78 cells chronically infected with the IIIB strain of HIV and uninfected HUT78 cells. PBMC from HIV-infected children and those from uninfected children gave similar results with respect to the ability to lyse uninfected or infected HUT78 cell targets (Table 4). These observations, along with the results for ADCC against HSV-infected targets, indicate that antibody-dependent and -independent NK cell-mediated cytotoxicity is generally intact in perinatally HIV-infected children.

To determine whether deficient cytotoxicity against HIV-expressing CD4 cells in HIV-infected children was due to deficient production or to effector cell binding of ADCC-mediating antibodies, assays were carried out by addition of serum from an HIV-seropositive adult throughout the 4-h incubation of the ADCC assay. The presence of seropositive serum was considered to increase the magnitude of cytotoxicity if the mean lysis was higher than the mean cytotoxicity plus 2 standard deviations in the presence of HIV-seronegative serum. For 7 of the 15 HIV-infected children tested, addition of HIV-seropositive serum from an adult had a positive effect on the magnitude of cytotoxicity (Table 5). The presence of adult HIV-seropositive serum also resulted in increased lysis mediated by effector cells of uninfected children.

In further studies, we added sera from children with perinatal HIV infection to ADCC assays in which PBMC from HIV-seropositive adults were tested for their ability to lyse HIV-expressing CD4 lymphocytes. As shown in Fig. 3a, the addition of individual sera from seven pediatric HIV patients in seven experiments each time inhibited cytotoxicity mediated by adult PBMC ($P < 0.05$). In contrast, the presence of sera from

### Table 4. NK cell-mediated cytotoxicity against HUT78 cells chronically infected with the HIV-1 strain IIIB

| Source of effector cells (n) | % Lysis of target cells |
|-----------------------------|-------------------------|
|                            | Uninfected HUT78 | IIIB-infected HUT78 |
| HIV-infected children (34)  | 14.8 ± 1.8 | 27.6 ± 2.2 |
| Uninfected children (16)    | 12.6 ± 1.6 | 26.2 ± 2.3 |
| HIV-infected adults (28)    | 13.8 ± 1.8 | 24.2 ± 3.4 |
| Uninfected adults (19)      | 20.5 ± 2.8 | 44.0 ± 3.1 |

* PBMC were obtained as described in Materials and Methods.
* Means ± standard errors.

### Table 5. Lysis of IIIB-coated CD4 lymphocytes mediated by PBMC of HIV-infected children in the presence of adult serum

| HIV status of children | Characteristic of children | % Lysis of CD4-IIIB in the presence of adult serum |
|------------------------|---------------------------|--------------------------------------------------|
|                        | Age | Stage (CDC classification) | Absolute CD4 cell count/mm³ | HIV seronegative | HIV seropositive |
| Seropositive           | 3 mo | P1-A | 3,000 | 0.6 | 0 |
|                        | 6 mo | P2-C | 950  | 0  | 0 |
|                        | 7 mo | P1-B | 950  | 1.8 | 1.9 |
|                        | 1 yr | P1-A | 3,000 | 0  | 0 |
|                        | 1 yr | P1-A | 5,000 | 4.0 | 12.6 |
|                        | 1 yr | P2-A | 1,000 | 0  | 0 |
|                        | 1 yr | P2-A | 1,550 | 1.0 | 8.9 |
|                        | 1 yr | P2-D1 | 280  | 0  | 6.8 |
|                        | 1 yr | P2-D1 | 790  | 0  | 20.3 |
|                        | 2 yr | P2-A | 500  | 0.9 | 0.3 |
|                        | 2 yr | P2-D1 | 1,200 | 0  | 0 |
|                        | 2 yr | P2-D1 | 1,000 | 0  | 0 |
|                        | 4 yr | P2-C | 200  | 0  | 15.5 |
|                        | 10 yr | P1-B | 185  | 0  | 10.1 |
|                        | 10 yr | P2-A | 100  | 0  | 13.1 |
| Seronegative           | 15 mo | NA  | ND   | 0  | 1.3 |
|                        | 2 yr  | NA  | ND   | 0  | 6.9 |
|                        | 3 yr  | NA  | ND   | 0  | 16.6 |
|                        | 5 yr  | NA  | ND   | 0  | 10.8 |
|                        | 5 yr  | NA  | ND   | 0  | 6.5 |
|                        | 5 yr  | NA  | ND   | 0  | 8.9 |
|                        | 5 yr  | NA  | ND   | 0  | 7.4 |
|                        | 8 yr  | NA  | ND   | 0  | 3.7 |
|                        | 11 yr | NA  | ND   | 0  | 10.2 |

* Sera obtained from HIV-seronegative adults.
* Sera obtained from HIV-infected asymptomatic adults.
* NA, not applicable; ND, not determined.
PBMC of HIV-infected adults (children on cytotoxicity against IIIB-coated CD4 lymphocytes mediated by EHIV-seronegative adult (were used as targets. Different results among these studies may

globulin was added to the assay and HIV-infected T-cell lines remained constant throughout the course of disease (19). In all around the time of first symptoms, and ADCC activity, which declined NK cell-mediated cytotoxicity, which was observed to decline HIV infection (26–28). Others described a disparity between any correlation between cytotoxic activity and clinical stage of

diseases. Other investigators of pediatric cell-mediated immune

tissues. Studies on the role of cell-mediated cytotoxicity in HIV-infected adults have yielded contradictory results (5, 13, 14, 20, 21, 28, 38, 42, 46, 53, 58). Several recent publications have addressed the correlation between clinical stages of infection and various types of cytotoxic activity detected in vitro, including cytotoxic T-lymphocyte-mediated cytotoxicity, NK cell-mediated cytotoxicity, and ADCC (1, 20, 26–28, 38, 58, 61). In some studies, reduced ADCC (1, 45, 58) and NK cell-mediated cytotoxicity correlated with disease progression (20, 52, 58, 61), whereas in another study increased ADCC activity was detected in patients with AIDS compared to those at earlier stages of infection (38). Some investigators did not find any correlation between cytotoxic activity and clinical stage of HIV infection (26–28). Others described a disparity between NK cell-mediated cytotoxicity, which was observed to decline around the time of first symptoms, and ADCC activity, which remained constant throughout the course of disease (19). In all these ADCC studies with PBMC from adults, HIV immune globulin was added to the assay and HIV-infected T-cell lines were used as targets. Different results among these studies may be explained by the different virus strains, target cell lines, or test conditions employed in the cytotoxicity assays. Even subclones of the same cell line may vary significantly in their susceptibility to ADCC (48). For this reason, we decided to use effector cells, armed in vivo with ADCC-mediating antibodies, to measure cytotoxicity against target cells which appear to be one of the major reservoirs for HIV in infected humans, namely, CD4 lymphocytes.

We showed that PBMC of vertically HIV-infected children were impaired in their ability to lyse HIV-expressing CD4 lymphocytes. In contrast, PBMC of HIV-infected adults lysed such target cells, and the magnitude of lysis correlated with absolute numbers of circulating CD4 lymphocytes. This cytotoxicity was shown to be mediated by NK cells armed in vivo with cytophilic HIV antibodies, confirming previous reports (57, 62). We found normal percentages of CD16+ NK cells in HIV-infected children, confirming previously published data (34).

The inability of PBMC from children with vertical HIV infection to mediate such ADCC against CD4 lymphocytes expressing HIV antigens was a specific and functional, rather than a quantitative, deficiency: effector cells from the same children lysed HSV-infected fibroblasts in ADCC assays and killed HIV-infected HUT78 cells and K562 cells in NK cell-mediated cytotoxicity assays to the same extent as did PBMC from HIV-seronegative children.

Attempts to induce ADCC against HIV-expressing CD4 lymphocytes, by using effector cells of HIV-infected children and serum of an HIV-seropositive adult, gave variable results. PBMC of about one-half of the children showed ADCC-mediated lysis when antibody-containing serum of an HIV-infected adult was added directly to the assays. Furthermore, sera from HIV-infected children interfered with the ability of PBMC from infected adults to mediate ADCC against CD4 lymphocytes. Thus, factors capable of blocking ADCC against HIV-expressing CD4 cells may be present in sera of perinatally HIV-infected children.

NK cells are present in human fetal liver mononuclear cells by the 8th week of gestation (40), mediate cytotoxicity as early as the 9th gestational week (59), and are functionally mature by the 32nd week (47). However, decreased NK cell activity against HIV-expressing targets, as reported for premature (<35 weeks of gestational age) neonates (35), may be due to antenatal glucocorticoids, fetal stress, or other critical care issues. Other investigators of pediatric cell-mediated immune responses reported normal NK cell activity and decreased ADCC activity against HIV-expressing target cells in HIV-seropositive neonates (17, 41). Interestingly, HIV-1 gag–cytotoxic T-lymphocyte responses were reported to be also deficient in vertically HIV-infected children (30). Our data indicate that vertical HIV infection does not interfere with maturation of functional NK cells. PBMC of HIV-infected children mediated cytotoxicity against IIIB-infected HUT78 cells in NK cell assays as well as against HSV-infected fibroblasts in ADCC assays. The levels of cytotoxicity observed were comparable to those obtained with PBMC from age-matched healthy peers.

It has been previously shown that decreased NK cell-mediated lysis in AIDS patients could be restored in vitro by addition of cytokines to cytotoxicity assays (1, 24, 25, 44). Bonavida et al. (6) reported that IL-2 triggered release of NK cell cytotoxic factors from PBMC of HIV-infected adults. Ahmad et al. (1) showed in vitro that the addition of IL-2 or gamma interferon enhanced ADCC activity significantly in PBMC from AIDS patients (CD4 counts of <200), which had without cytokine addition significantly lower target lysing ability than

![Diagram](image-url)
those from patients with CD counts of >400. Positive in vitro effects of other cytokines, such as IL-12 and IL-15, on ADCC-mediated lysis in pediatric HIV patients have been reported elsewhere (25). Other cytokines are still under investigation. However, addition of IL-2 to effector cells of HIV-infected children did not enhance their ability to mediate lysis of HIV-expressing CD4 cells (data not shown).

ADCC against HIV-expressing CD4+ lymphocytes is mediated by NK cells of HIV-infected adults, linked in vivo to antibodies of the subclass IgG1 (27). Such antibodies are directed against the viral envelope glycoproteins gp120 and gp41 (12, 15, 22, 31, 33, 51, 55, 60, 62, 64) and are distinct from virus-neutralizing antibodies (7, 32, 50). Both ADCC-mediating antibodies and neutralizing antibodies were shown to be present in sera of infants born to HIV-infected mothers (19, 29). The presence of such antibodies, most likely of maternal origin, correlated with a better clinical outcome in one study (32) but had no clinical significance in another investigation (18). However, both reports confirm that the presence of ADCC antibodies failed to prevent vertical transmission of HIV infection. Hypergammaglobulinemia with high levels of IgG1 and IgG3 has been detected in perinatally infected children, but such children frequently have functional hypogammaglobulinemia and reduced defenses against bacterial opportunistic infections (43). A similar functional inability of antibodies to mediate ADCC against HIV-expressing CD4 lymphocytes might explain the results observed in the present study.

Blocking factors in sera of HIV-infected children might inhibit the arming of effector cells or the contact between effector and target cells. Immune complexes, known to be present more frequently and in higher concentrations in HIV-infected children (10, 27) than in adults (36), might act as such blocking factors. Differences in non-HIV-specific immunoglobulin, HIV-specific noncytophilic antibody, or cytokine profiles between sera of pediatric patients and sera of adult patients may also be accountable for these inhibitive effects.

In summary, our results are similar to several reports about deficient ADCC activity against HIV-expressing target cells in pediatric HIV-infected populations (17, 35, 54) and noncompromised NK cell activity (17, 35). In addition, we demonstrate that the deficit in ADCC activity in HIV-infected children is not due to defective effector cells, appearance to be specific to HIV-infected ADCC target cells, and may be caused by undefined serum blocking factors. This deficiency may be a contributing factor to the rapid disease progression often observed with this patient population.

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