0031-3998/93/3305-0469$03.00/0
PEDIATRIC RESEARCH

Vol. 33, No. 5, 1993
Printed in U.S.A.

Natural Killer Cytotoxicity and Antibody-Dependent Cellular Cytotoxicity of Human Immunodeficiency Virus–Infected Cells by Leukocytes from Human Neonates and Adults

MORGAN JENKINS, JOHN MILLS, AND STEVE KOHL

Division of Pediatric Infectious Diseases, Department of Pediatrics [M.J., S.K.], and Division of Infectious Diseases, Departments of Medicine, Microbiology, and Laboratory Medicine [J.M.], San Francisco General Hospital Medical Center, University of California, San Francisco, California 94110

ABSTRACT. In infants born to mothers infected with the human immunodeficiency virus (HIV), antibody-dependent cellular cytotoxicity (ADCC) or natural killer cytotoxicity (NKC) may either eliminate infection or ameliorate its course. We developed and standardized an assay for cytotoxicity of HIV-infected cells and studied the capacity of leukocytes from healthy neonates and adults to lyse HIV-infected cells by ADCC and NKC. The chosen target cell line, a T cell line infected with the HXB-2 clone of human T-cell lymphotrophic virus-IIIB, displayed stable surface expression of viral antigens over months of continuous culture and allowed simultaneous assessment of NKC and ADCC of effector cell populations. Conditions for optimal ADCC lysis of target cells were defined for unpurified peripheral blood mononuclear cells and purified lymphocytes and monocytes. Polymorphonuclear neutrophils from healthy adults and neonates exhibited low activity in ADCC of HIV-infected targets. Lymphocytes and monocytes from adults were found to differ in antibody dependence, kinetics, and sensitivity to latex inhibition for ADCC-mediated lysis of HIV-infected targets. Peripheral blood mononuclear cells of healthy neonates and adults displayed equivalent capacity to mediate NKC of HIV-infected targets. However, neonates' peripheral blood mononuclear cells were found to be significantly less active than adults' in ADCC lysis of HIV-infected cells. This pattern of diminished ADCC cytotoxicity with intact NKC is the opposite of that seen in HIV-infected adults. Our findings suggest that therapies designed to enhance ADCC effector cell function in the neonate may help interrupt vertical transmission of HIV. (*Pediatr Res* 33: 469–474, 1993)

Abbreviations

HIV, human immunodeficiency virus
ADCC, antibody-dependent cellular cytotoxicity
NKC, natural killer cytotoxicity
HBSS, Hanks' buffered saline solution
PBMC, peripheral blood mononuclear cell
PMN, polymorphonuclear neutrophil
HSV, herpes simplex virus

Received October 19, 1992; accepted December 29, 1992.
Correspondence and reprint requests: Morgan Jenkins, M.D., Department of Pediatrics, Division of Pediatric Infectious Diseases, San Francisco General Hospital, Room 6E6, 1001 Potrero Ave., San Francisco, CA 94110.
Supported by NIH AIDS Research Training Grant T32 AI07395, University of California Academic Senate Grant 504182–34935, and NIH NIAID Grant R01 AI32384.

Approximately 80% of infants and children infected with HIV acquire the virus through vertical transmission (1). Nevertheless, only a minority of infants born to women infected with HIV develop clinical or laboratory signs of infection when prospectively monitored (2, 3). Resistance to vertical transmission may be due to maternal factors (*e.g.* viral burden, CD4 cell counts), placental factors, or the immune defenses of the fetus and newborn. An inoculum of infected maternal cells, or fetal cells infected by cell-free virus from the mother, could be destroyed either by NKC or by ADCC, which combines fetal or newborn effector cells with transplacentally acquired maternal antibodies. Either mechanism might avert the establishment of infection in the fetus or newborn.

By definition, NKC operates without prior sensitization, and ADCC may occur in the neonate before development of a humoral immune response because of transplacentally acquired maternal antibody. Furthermore, ADCC may operate to destroy virus early in the infection cycle, before viral replication (4). These features make NKC and ADCC attractive candidates for therapeutic immune enhancement to interrupt vertical transmission of HIV. A CD16+ natural killer cell is thought to be the predominant effector cell for both NKC and ADCC of HIV-infected targets (5, 6), although each uses distinct cellular signals and cytotoxic mechanisms (7, 8). Furthermore, cells other than natural killer cells may participate in ADCC (9, 10).

Various assay systems have been used for the measurement of HIV-specific ADCC (5, 6, 9, 11–15). Important differences between these systems include the choice of target cell (Epstein-Barr virus immortalized blasts, tumor cell lines, NKC-resistant clones) and the method of virus infection or antigen presentation (acute or chronic infection, transfection with viral construct, or labeling with HIV surface glycoprotein gp120). Optimally, such an assay should use targets that have natural orientation of the full variety of viral antigens at the target cell surface, stable antigen expression over time, and NKC susceptibility, which allows for determination of effector cell capacity in both NKC and ADCC within a single assay. We therefore characterized an HIV cytotoxicity assay that used a T cell line actively infected with HIV as a target. Such cells are susceptible to ADCC destruction by sera from 100% of HIV-infected individuals (15). We then examined the ability of newborns' leukocytes to mediate NKC and ADCC of HIV-infected cells to ascertain whether the pattern of relative neonatal effector cell deficiency seen in other systems (16–19) was also present for anti-HIV cytotoxicity.

MATERIALS AND METHODS

Cell lines. H9 cells and the HXB-2 clone from H9 cells chronically infected with human T-cell lymphotrophic virus-IIIB

(20) were obtained from the AIDS Research and Reference Reagent Program and maintained in continuous culture in RPMI medium supplemented with 10% FCS, 100 units penicillin per mL, and 100 μg streptomycin per mL (hereafter referred to as complete medium). Cells were subcultured twice a week.

Sera. As a positive standard and interassays control, an HIV-positive reference serum pool was created from serum samples of 120 individuals with HIV antibodies by both ELISA and immunofluorescence. Ten sera lacking HIV antibodies by ELISA were obtained from the same source for the determination of cutoff values for significant ADCC. Negative control sera for use in assays were obtained from healthy adult volunteers at low risk of HIV infection and confirmed negative for HIV antibody by ELISA. All sera were depleted of complement in a 56°C water bath for 30 min before aliquots were taken and stored at −70°C.

Immunofluorescence. Live cell indirect surface antigen immunofluorescence was performed with a modification of the method of Blumberg *et al.* (21). A total of 10^6 H9 or HXB cells were washed in PBS and incubated with 1 mL 1:10 dilution of HIV-negative serum or positive reference serum for 30 min at 4°C. Cells were washed twice in PBS and incubated with FITC-labeled goat anti-human serum (Chemicon, Temecula, CA) diluted 1:40 in PBS for 30 min at 4°C. Cells were washed twice and examined immediately without fixation for fluorescence. Target cells infected with HXB-2 maintained high expression of viral antigen, with 88% of live cells expressing surface antigens by immunofluorescence assay after several months of continuous passage without addition of either virus or feeder cells.

Effector cells. PBMC were obtained from healthy HIV-seronegative adult volunteers or from the umbilical cords of healthy infants born by vaginal delivery not at risk of HIV infection. Whole blood was anticoagulated with acid citrate dextrose (20% vol/vol) or 20 units/mL heparin (Elkins-Sinn, Cherry Hill, NJ) and PBMC isolated by density gradient centrifugation with Ficoll-Hypaque (Pharmacia, Piscataway, NJ). Cells were washed four times in HBSS before use in cytotoxicity assays. When umbilical cord samples were compared with adult PBMC, the adult volunteer was phlebotomized within 3 h of the time of delivery and collection of the umbilical cord samples.

Leukocyte fractions were prepared as follows. PMN were purified by sedimentation of blood after addition of $^1/_{10}$ volume 3% dextran (Sigma Chemical Co., St. Louis, MO) followed by Ficoll-Hypaque density centrifugation. Adherent and nonadherent fractions of PBMC (monocyte-macrophages and lymphocytes) were prepared as described earlier (22). Briefly, PBMC from Ficoll interfaces were washed once in HBSS and enumerated by counting in a hemocytometer. Cells were suspended in RPMI medium supplemented with 10% autologous plasma at a concentration of 5×10^6 cells per mL, and 5 to 10 mL of cell suspension were added to 10-cm plastic Petri dishes (Falcon, Oxnard, CA). Cells were allowed to adhere for 2 h at 37°C in humidified air enriched with 5% CO_2. Nonadherent cells were decanted from the dishes, and the adherent cells were washed three times with HBSS, then removed with a cell lifter (Costar, Cambridge, MA).

The resulting nonadherent (predominantly lymphocyte) and adherent (predominantly monocyte-macrophage) cell populations were further characterized by latex bead ingestion as described (22). Ten μL of 10% latex particles (Dow Diagnostics, Indianapolis, IN) or medium control were added to 1.5×10^6 cells in 1 mL of complete medium. After 2 h of incubation, the cells were washed and added to cytotoxicity assays or examined microscopically for the ingestion of beads. Of the adherent cells, 88% ingested latex beads, whereas fewer than 5% of nonadherent cells ingested latex.

Cytotoxicity assay. ADCC and NKC of HIV-infected cells were measured using a modification of the assay of Blumberg *et al.* (11). Approximately 10^6 H9/HXB-2 target cells were labeled with 100 μCi of ^{51}Cr (sp act 250 mCi/mg chromium) (Amersham, Arlington Heights, IL) in a volume of 100 μL at 37°C for 1 h and then washed four times in complete medium. Because H9/HXB-2 cells internalized 1–2 cpm of ^{51}Cr per cell, we were able to use only 2000 target cells per well in cytotoxicity assays. Target cells were consistently greater than 95% viable by trypan blue vital staining before addition to assays. A total of 2×10^3 target cells suspended in 50 μL of complete medium were added to each well of round-bottom microtiter trays (Corning, Corning, NY).

Effector cells (PBMC or subsets) were then added at various effector-to-target ratios in 100 μL of complete medium. Serum or medium blanks were then added in a volume of 50 μL and plates were centrifuged at $300 \times g$ for 3 min before incubation. Control conditions included target cells alone (for spontaneous release), target cells and effector cells alone (for NKC), and target and effector cells plus HIV antibody or negative sera (for ADCC). Triplicate wells were used for each condition. After incubation (4 h for lymphocytes and PBMC and 18 h for monocyte-macrophages unless otherwise specified), 100 μL of supernatant were harvested from each well (sample A), and the remaining 100 μL and cell pellet were separately collected after decontamination and solubilization with 50 μL of 50% sodium hypochlorite (sample B). Percent of radioactivity released was calculated as follows:

$$\% \text{ release} = \frac{(2 \times \text{cpm A})}{(\text{cpm A} + \text{cpm B})} \times 100$$

NKC and ADCC of the HXB targets were calculated as follows:

$$\% \text{ NKC} = \frac{\% \text{ release by targets and effector cells} - \% \text{ release by targets alone}}{100 - \% \text{ release by targets alone}} \times 100$$

$$\% \text{ ADCC} = \frac{[(\% \text{ release by targets + effector cells + HIV-positive antiserum}) - (\% \text{ release by targets + effector cells + HIV-negative antiserum})]}{100 - (\% \text{ release by targets + effector cells + HIV-negative antiserum})} \times 100$$

Four h of incubation was found to be optimal for demonstration of ADCC and NKC by PBMC. In 4-h assays, spontaneous release by target cells averaged 7.5% (SD 2.4%), and at 18 h, 32.6% (SD 5.3%). NKC and ADCC of HXB-infected cells were approximately log linear with respect to effector-to-target ratio when tested in the range of 25:1 to 100:1 (data not shown). A ratio of 50:1 was used in further experiments examining PBMC subsets.

Statistical analysis. Significant ADCC was defined as lysis by effectors and positive sera that exceeded the mean + 2 SD of the lysis value by effectors and HIV-negative sera in triplicate wells for each experimental condition. This value corresponded to approximately 6% ADCC when 10 HIV-negative sera were assayed simultaneously with PBMC effector cells. Because cytotoxicity values for each group compared were not in every case normally distributed, the Wilcoxon signed rank test was used as a nonparametric test for the comparison of effector cell capacity of PBMC from adults and neonates. The *t* test was used to compare the relative percentage of ADCC values by neonatal and adult effectors.

RESULTS

Characterization of HIV-specific cytotoxicity assay. With PBMC from normal adults, the HIV-seropositive reference pool mediated significant ADCC against HXB-infected cells at final dilution as high as 1:10 000 (Fig. 1). A prozone effect—diminished cytotoxicity with more concentrated sera—was observed in most experiments with dilution of the reference pool less than 1:100 (Fig. 1). When the serum pool was tested with uninfected

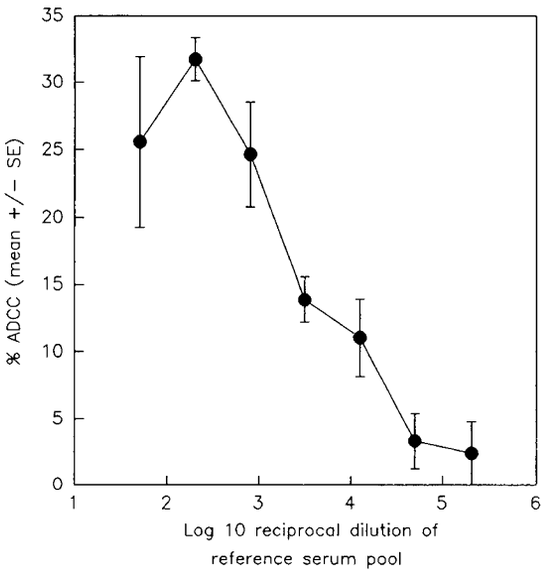

Fig. 1. Antibody dependence of PBMC for ADCC toward HIV-infected cells. ADCC was tested with serial 4-fold dilution (from 1:50 to 1:204 800) of the reference seropositive pool or HIV-negative serum with PBMC from healthy adults at an effector-to-target ratio of 50:1 and 4 h of incubation. The mean ± SEM of four experiments is plotted for each point.

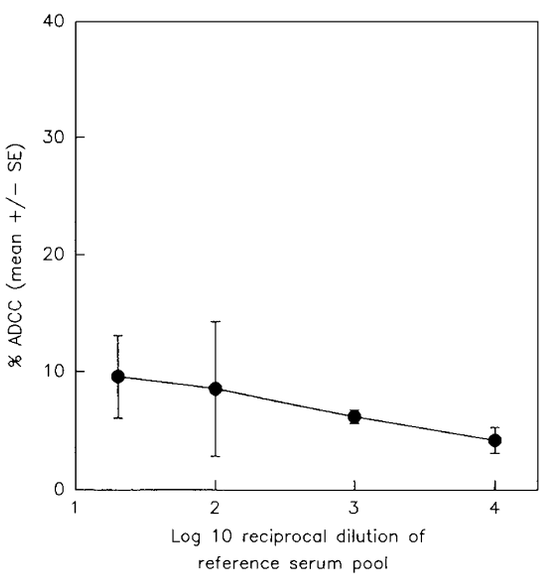

Fig. 2. Activity of PMN in ADCC of HIV-infected cells. PMN from healthy adults were added at an effector-to-target ratio of 100:1 in 18-h cytotoxicity assays. Reference serum was added at 1:20 to 1:10 000 dilution. The mean ± SEM of four experiments is plotted for each point.

H9 cells, no enhancement of lysis was seen when compared with HIV-negative sera.

Characterization of HIV-specific ADCC by effector cell subsets. Because the cytotoxic activities of leukocyte subsets have been found to differ in other systems (9, 10, 22), we characterized the cells responsible for HIV-specific ADCC. PMN from adults mediated only weak ADCC and NKC cytotoxicity of the HXB targets, with ADCC values consistently less than 10% even at effector-to-target ratios up to 100:1 and with antiserum dilutions as low as 1:20 (Fig. 2). PMN from neonates displayed similarly low capacity for ADCC of the HXB-2 cells (data not shown). Mononuclear cell fractions were therefore studied more intensively.

In the herpes simplex virus model, lymphocytes and monocytes differ in their kinetics and dependence on antiserum concentration for efficient ADCC (22). A kinetic analysis of HIV-specific ADCC by adult lymphocytes and monocytes was performed by measuring ADCC after incubation periods of 3 to 18 h with optimal antibody concentrations for each cell type (1:20 dilution for adherent cells and 1:100 for nonadherent cells, see below) (Fig. 3). The lymphocytes obtained from healthy adults showed maximal ADCC after 6 h of incubation, with a plateau thereafter (Fig. 3*A*). This time curve resembled that of ADCC by bulk PBMC (data not shown). In contrast, ADCC mediated by monocytes increased gradually through 18 h of incubation (Fig. 3*B*). Longer incubation periods could not be tested because the spontaneous release of chromium from target cells exceeded 20%. For this reason, 18 h of incubation was chosen for further monocyte-macrophage ADCC experiments.

The antibody dependence of lymphocytes for ADCC of HIV-infected cells was found to be similar to bulk PBMC: significant ADCC was seen at dilutions of the reference serum as high as $1:10^4$, and a prozone effect was regularly seen with reference serum more concentrated than a $1:10^2$ dilution (Fig. 4*A*). In

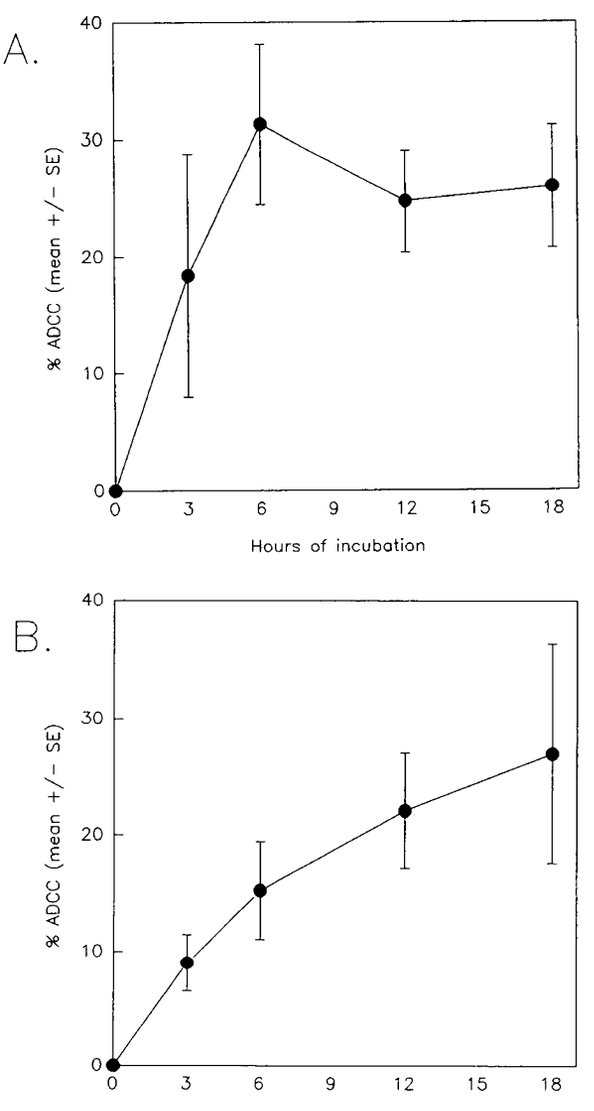

Fig. 3. Kinetics of HIV ADCC by lymphocytes and monocytes of adults. Lymphocytes (*A*) or monocytes (*B*) were tested in ADCC assays with an effector-to-target ratio of 50:1. A 1:100 dilution of the reference serum pool was used for nonadherent cells and a 1:20 dilution for adherent cells. Replicate plates were harvested after 3, 6, 12, and 18 h of incubation. The mean ± SEM of four experiments is plotted for each point.

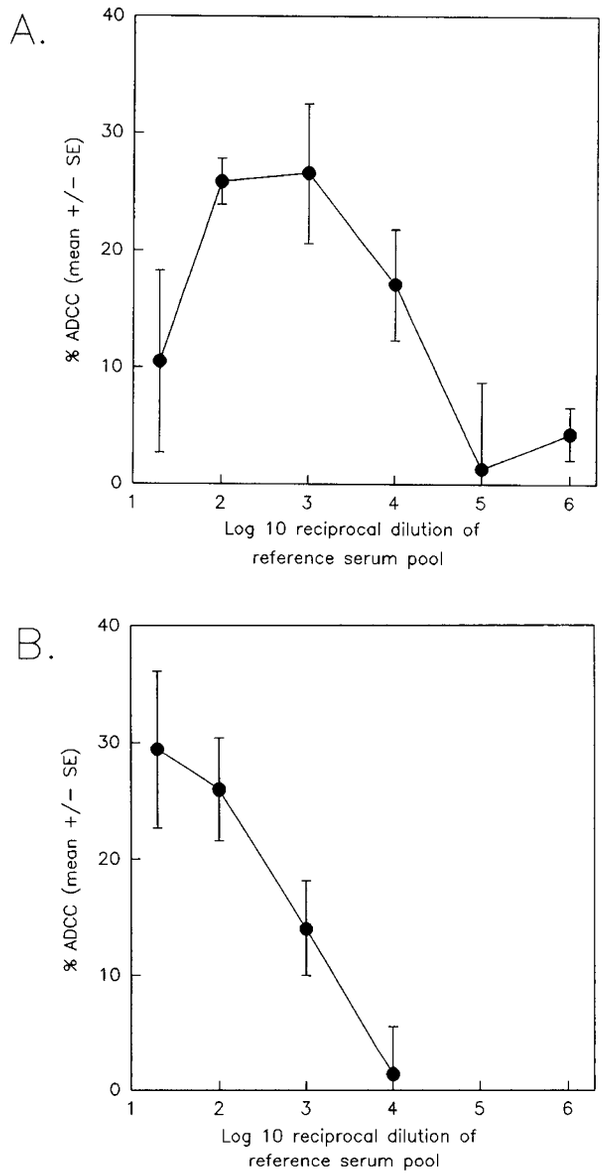

Fig. 4. Antibody dependence of HIV ADCC by lymphocyte or monocytes from healthy adults. Lymphocytes (*A*) or monocytes (*B*) were tested in 18-h cytotoxicity assays at an effector-to-target ratio of 50:1 with dilutions of reference serum from 1:20 to 1:1 000 000. The mean ± SEM for six experiments is plotted for each point.

contrast, monocytes showed much less ADCC at high dilutions of serum but demonstrated increased ADCC in the most concentrated sera tested (Fig. 4*B*).

Latex beads are reported to efficiently inhibit ADCC by monocyte-macrophages. When latex was added to effector cell subsets before incubation with targets, significant inhibition of ADCC was seen with purified monocyte-macrophages, whereas lymphocyte ADCC was little affected (Table 1).

Defective HIV-specific ADCC by cord blood PBMC. Because developmental defects in antiviral cytotoxicity have been observed toward cells infected with viruses other than HIV (17, 23), we compared the effector cell capacity of leukocytes from healthy infants and adults in the NKC and ADCC HIV assay. Umbilical cord blood samples collected at the same time as blood samples from healthy adult volunteers were tested in pairs in the cytotoxicity assay to minimize artifacts from interassay variability. Standard sera were diluted 1:200 for determination of ADCC because this dilution yielded the highest values for ADCC by PBMC from both adults and neonates. NKC of HIV-infected target cells by adult and cord blood PBMC was comparable at each effector-to-target ratio from 25:1 to 100:1 (Fig. 5*A*). However, when ADCC of HIV-infected targets by PBMC from neonates and adults were compared, a significant deficit in the cytotoxic capacity of the neonatal effectors was seen ($p < 0.054$ by Wilcoxon signed rank test) (Fig. 5*B*). Despite a wide variability between individuals for ADCC activity, differences in percentage of ADCC by PBMC obtained from umbilical cord and adult blood were statistically significant at each of the effector-to-target ratios studied. When neonatal cytotoxic capacity was calculated as a percentage of the adult value and data from all effector-to-target ratios were pooled, neonates' NKC capacity was 101% (± 11% SEM) of that of adults ($p = 0.932$ by t test) and neonates' mean ADCC activity was 51 ± 15% of that of adults ($p = 0.003$ by t test).

To be certain that the intact NKC was not due to the effects of delivery and possible up-regulation on NKC, a small number of experiments were performed using PBMC obtained from healthy babies in the first days of life. There was no difference in neonates' NKC (mean ± SEM in six or seven experiments at an effector-to-target cell ratio of 25:1 and 50:1, respectively = 15.0 ± 4.4 and 23.0 ± 4.8) compared with adults' NKC (14.8 ± 3.6 and 23.0 ± 3.9 at the same cell ratios).

In a limited number of studies, effector cells were tested against uninfected parent cells (H9) of the HXB-IIIB–infected cells. At an effector-to-target cell ratio of 25:1, higher NKC to H9 cells than to HXB-IIIB–infected cells was seen using both cord PBMC (55.8 ± 6.9 *versus* 44.8 ± 10.7, respectively) and adult cells (36.0 ± 4.9 *versus* 18.9 ± 6.1, respectively).

DISCUSSION

Using a well-characterized and reproducible assay, we have shown a marked defect in normal neonates' capacity for ADCC of HIV-infected cells. NKC of HIV-infected cells by PBMC from neonates, however, was comparable to that of cells from adults. This was surprising because several studies have documented deficiencies in neonatal NKC of tumor cell lines (16, 18, 19) or virus-infected cells (17). Our findings may result from altered susceptibility to natural killer lysis of target cell lines after infection with HIV (24, 25). Alternatively, interferon-α production induced by HIV-infected targets (26) may enhance NKC selectively in the neonate (16). Indeed, neonates' PBMC have been found to be as effective as adults' in NKC of human T-cell lymphotrophic virus-1–infected target cells (23). However, the striking difference we observed between adults and neonates in ADCC capacity suggests that a developmental defect in this mode of antiviral activity may exist in the human neonate.

Table 1. *Inhibition of ADCC activity by latex beads*

	% ADCC						
	Experiment 1		Experiment 2		Experiment 3		
	Control	Latex	Control	Latex	Control	Latex	% Inhibition
Nonadherent	22.7	14.9	38.3	33.2	13.4	13.5	15.6*
Adherent	14.1	1.7	12.9	3.4	26.6	8.7	76.3*

* $p = 0.007$ for difference between adherent and nonadherent effectors.

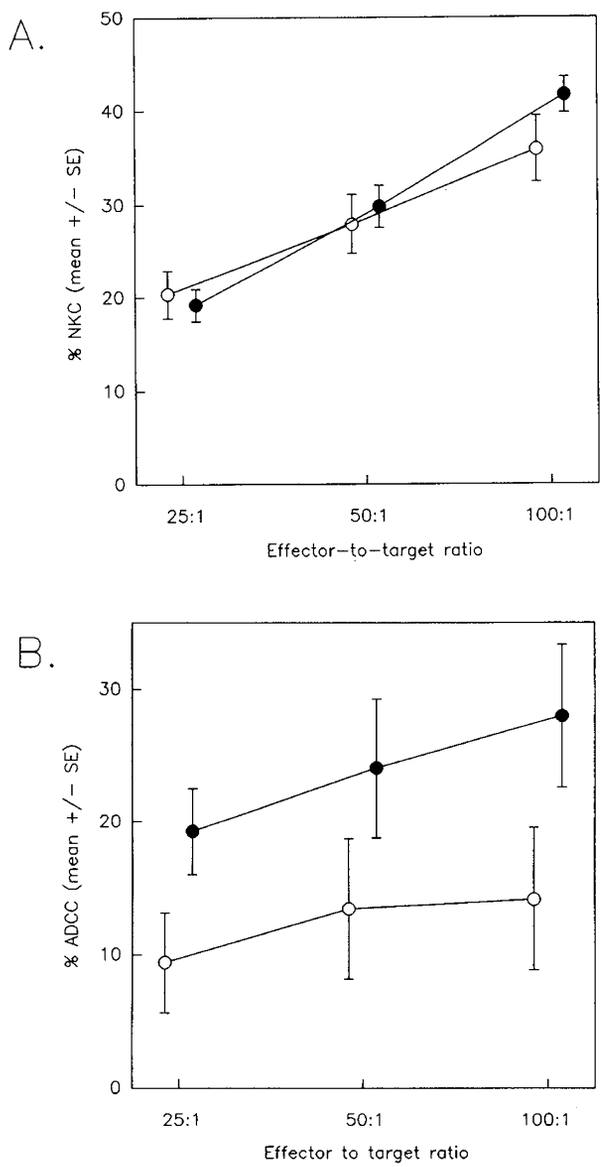

Fig. 5. Comparison of adult and neonatal NKC (*A*) and ADCC (*B*) toward HIV-infected cells. Eight pairs of PBMC simultaneously collected from adults (●) and umbilical cord blood (○) were tested at the effector-to-target ratios shown. For ADCC, reference positive and negative sera were used at 1:200 dilution. Data represent mean ± SEM of eight experiments.

Natural killer cells appear to be the chief effector cells for both NKC and ADCC of HIV-infected targets (5, 6), and these two immune responses are initiated by the same cell receptor complex (27). However, NKC and ADCC may use different cell signal transduction mechanisms (7) and different cytotoxic mechanisms (8, 28). NKC and ADCC also may play different roles in the immune defense against, and pathogenesis of, HIV infection at different stages of illness or in different hosts. Leukocytes from HIV-infected adults have been reported to have defects in NKC against HIV-infected target cells, although ADCC capacity in these individuals was normal (29–31). Our data suggest that a different defect in immune defense against HIV may exist in the normal newborn. Studies of HIV-infected children have emphasized cellular defects in ADCC lysis of HIV-infected cells (10, 32). It has been hypothesized that HIV-specific ADCC may be deleterious to the adult host with established HIV infection by contributing to the destruction of infected CD4 lymphocytes (31). However, in an HIV-infected child, augmentation of anti-HIV ADCC through administration of HIV hyperimmune globulin has been observed to result in clinical improvement (33).

In the fetus and neonate at risk of vertical transmission of HIV, ADCC may play a protective role. In models of vertical transmission of murine leukemia virus and feline leukemia virus, providing antiserum to viral envelope glycoprotein to newborns protects against viremia and the development of leukemia (34, 35). The single study to date of anti-HIV ADCC titers in newborns at risk of infection included only a few neonates but suggested a possible correlation between transplacental HIV ADCC titers at birth and protection from infection (36). If this correlation is proven, it would agree with the HSV model (37) and other retroviral systems (34, 35) in which antiviral antibody in neonates protects against infection or severe disease.

For such immunotherapy to succeed, however, naive neonatal effector cells must be competent for lysis. The neonatal ADCC deficit we demonstrated against HIV-infected cells is also seen in the HSV system (17, 37). Rational therapies to specifically augment this effector cell function must await the delineation of the specific defect, which may include effector-target binding, competence for lysis, or recycle steps (17, 38).

The cell that predominantly directs anti-HIV ADCC *in vivo* is not yet identified. The antibody dependence, kinetics, and latex-inhibition sensitivity of ADCC in the HIV system are notably similar to what has been reported for HSV (22). Lymphocyte ADCC of HIV-infected cells was not inhibited by latex incubation, which ablated monocyte ADCC (Table 1). Lymphocytes also mediated faster responses, and at lower antibody concentrations, than did monocytes (Figs. 3 and 4). These data suggest that, under conditions that may be present *in vivo*, monocytes may be as competent as lymphocytes in anti-HIV ADCC. This finding is at variance with the report from another group that found that normal individuals' monocytes mediated only low levels of ADCC to HIV targets (9). This discrepancy is likely due to methodologic differences, including the use of tumor necrosis factor–resistant target cells, labeling of cells with killed virus rather than active infection, and overnight incubation of effectors before use in cytotoxicity assay. The important differences in the conditions necessary for observation of optimal *in vitro* ADCC by each effector subset make direct comparison of the relative contribution of each impossible. Furthermore, the determination of each cell type's contribution to protective immune responses to HIV *in vivo* is even more speculative, owing to their different egress from the circulation into lymphoid tissue, reticuloendothelial cell organs, and the CNS, each of which may be a reservoir for HIV in infected children.

Our finding of a selective ADCC effector cell deficit in healthy neonates suggests that therapeutic approaches to enhance protective immune responses to HIV will have to be tailored to the host and to stage of disease. Augmenting cellular capacity for ADCC in the newborn at risk of HIV infection may be a viable strategy to interrupt vertical transmission.

REFERENCES

1. Rogers MF, Ou CY, Kilbourne B 1991 Advances and problems in the diagnosis of human immunodeficiency virus infection in infants. Pediatr Infect Dis J 10:523–531
2. Blanche S, Rouzioux C, Moscato MG, Veber F, Mayaux MJ, Jacomet C, Tricoire J, Deville A, Vial M, Firtion G, deCrepy A, Douard D, Robin M, Courpotin C, Ciraru-Vigneron N, le Diest F, Griscelli C 1989 A prospective study of infants born to women seropositive for HIV type 1. N Engl J Med 320:1643–1648
3. European Collaborative Study 1991 Children born to women with HIV infection: natural history and risk of transmission. Lancet 337:253–260
4. Shore SL, Cromeons TL, Romano TJ 1976 Immune destruction of virus-infected cells early in the infection cycle. Nature 262:695–696
5. Tanneau F, McChesney M, Lopez O, Sansonetti P, Montagnier L, Riviere Y 1990 Primary cytotoxicity against the envelope glycoprotein of human immunodeficiency virus-1: evidence for antibody-dependent cellular cytotoxicity *in vivo*. J Infect Dis 162:837–843
6. Tyler DS, Nastala CL, Stanley SD, Matthews TJ, Lyerly HK, Bolognesi DP, Weinhold KJ 1989 Gp120 specific cellular cytotoxicity in HIV-1 seropositive individuals. Evidence for circulating CD16+ effector cells armed *in vivo* with cytophilic antibody. J Immunol 142:1177–1182

7. Vivier E, Morin P, O'Brien C, Druker B, Schlossman SF, Anderson P 1991 Tyrosine phosphorylation of the Fc gamma RIII (CD16):zeta complex in human natural killer cells. J Immunol 146:206–210
8. Komiyama A, Kawai H, Yabuhara A, Yanagisawa Y, Miyagawa Y, Ota M, Hasekura H, Akabane T 1990 Natural killer cell immunodeficiency in siblings: defective killing in the absence of natural killer cytotoxic factor activity in natural killer and lymphokine-activated killer cytotoxicities. Pediatrics 85:323–330
9. Jewett A, Giorgi JV, Bonavida B 1990 Antibody-dependent cellular cytotoxicity against HIV-coated target cells by peripheral blood monocytes from HIV seropositive asymptomatic patients. J Immunol 145:4065–4071
10. Szelc CM, Mitcheltree C, Roberts RL, Stiehm ER 1992 Deficient polymorphonuclear cell and mononuclear cell antibody-dependent cellular cytotoxicity in pediatric and adult human immunodeficiency virus infection. J Infect Dis 166:486–493
11. Blumberg RS, Paradis T, Hartshorn KL, Vogt M, Ho DD, Hirsch MS, Leban J, Sato VL, Schooley RT 1987 Antibody-dependent cell-mediated cytotoxicity against cells infected with the human immunodeficiency virus. J Infect Dis 156:878–884
12. Ljunggren K, Fenyo E, Biberfeld G, Jondal M 1987 Detection of antibodies which mediate human immunodeficiency virus-specific cellular cytotoxicity (ADCC) *in vitro*. J Immunol Methods 104:7–14
13. Nishanian PG, Ojo-Amaize E, Uittenbogaart CH, Giorgi JV 1988 A simple and rapid method for preparation of HIV-expressing targets for functional assays. J Immunol Methods 107:261–271
14. Evans LA, Thompson-Honnebier G, Steimer K, Paoletti E, Perkus ME, Hollander H, Levy JA 1989 Antibody-dependent cellular cytotoxicity is directed against both the gp120 and gp41 envelope proteins of HIV. AIDS 3:273–276
15. Norley SG, Mikschy U, Werner A, Staszewski S, Helm EB, Kurth R 1990 Demonstration of cross-reactive antibodies able to elicit lysis of both HIV-1-and HIV-2-infected cells. J Immunol 145:1700–1705
16. Yabuhara A, Kawai H, Komiyama A 1990 Development of natural killer cytotoxicity during childhood: marked increases in number of natural killer cells with adequate cytotoxic abilities during infancy to early childhood. Pediatr Res 28:316–322
17. Kohl S, Loo LS, Gonik B 1984 Analysis of defective human neonatal antibody-dependent cellular cytotoxicity and natural killer cytotoxicity to herpes simplex virus-infected cells. J Infect Dis 150:14–19
18. Sancho L, de la Hera A, Casas J, Vaquer S, Martinez C, Alvarez-Mon M 1991 Two different maturational stages of stages in natural killer lymphocytes in human newborn infants. J Pediatr 119:446–454
19. Montagna D, Maccario R, Ugazio AG, Mingrat G, Burgio GR 1988 Natural cytotoxicity in the neonate: high levels of lymphokine activated killer (LAK) activity. J Immunol 71:177–181
20. Shaw GM, Hahn BH, Arya SK, Groopman JE, Gallo RC, Wong-Staal F 1984 Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. Science 226:1165–1171
21. Blumberg RS, Sandstrom EG, Paradis TJ, Neumeyer DN, Sarngadharan MG, Hartshorn KL, Byington RE, Hirsch MS, Schooley RT 1986 Detection of human T-cell lymphotropic virus type III-related antigens and anti-human T-cell lymphotropic virus type III antibodies by anticomplementary immunofluorescence. J Clin Microbiol 23:1072–1077
22. Kohl S, Starr SE, Oleske JM, Shore SL, Ashman RB, Nahmias AJ 1977 Human monocyte-macrophage mediated antibody-dependent cytotoxicity to herpes simplex virus-infected cells. J Immunol 118:729–735
23. Kunitomi T, Takigawa H, Sugita M, Nuono S, Suemune M, Inue M, Kodani N, Oda M, Ikeda M 1990 Antibody-dependent cellular cytotoxicity and natural killer activity against HTLV-1 infected cells. Acta Pediatr Japonica 32:16–19
24. Sinclair AL, Habeshaw JA, Muir L, Chandler P, Forster S, Cruikshank K, Dalgleish AG 1988 Antibody-dependent cell-mediated cytotoxicity: comparison between HTLV-I and HIV-1 assays. AIDS 2:465–472
25. Zheng ZY, Zucker-Frankilin D 1992 Apparent ineffectiveness of natural kiler cells vis-a-vis retrovirus-infected targets. J Immunol 148:3679–3685
26. Rappocciolo G, Toso JF, Torpey DJ, Gupta P, Rinaldo C 1989 Association of alpha interferon production with natural killer cell lysis of U937 cells infected with human immunodeficiency virus. J Clin Microbiol 27:41–48
27. Vivier E, Morin PM, O'Brien C, Schlossman SR, Anderson P 1991 CD2 is functionally linked to zeta-natural killer receptor complex. Eur J Immunol 21:1077–1080
28. Kay HD, Bonnard GD, West WH, Herberman RB 1977 A functional comparison of human Fc-receptor-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. J Immunol 118:2058–2066
29. Bonavida B, Katz J, Gottlieb M 1986 Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome and AIDS related complex, I. Defective trigger on NK cells for NKCF production by target cells, and partial restoration with IL-2. J Immunol 137:1157–1163
30. Katz JD, Mitsuyatsu R, Gottlieb MS, Lebow LT, Bonavida B 1987 Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome and AIDS related complex, II. Normal antibody dependent cellular cytotoxicity mediated by effector cells defective in natural killer cytotoxicity. J Immunol 139:55–60
31. Ojo-Amaize E, Nishanian PG, Heitjan DF, Rezai A, Esmail I, Korns E, Detels R, Fahey J, Giorgi JV 1989 Serum and effector cell antibody dependent cellular cytotoxicity activity remains high during human immunodeficiency virus disease progression. J Clin Immunol 9:454–461
32. Ziegner U, Frank I, Rutstein R, Campbell D, MacGregor R, Starr S 1991 Mononuclear cells of children with perinatal HIV infection fail to mediate ADCC against HIV-coated target cells. VII International Conference on AIDS, Florence, June 16–21, 1991(abstr W.A.1146)
33. Wong VK, Roth M, Karpas A, Mitcheltree C, Miles S, Gillespie S, Chen I, Stiehm ER 1991 Immune plasma treatment of an HIV infected child. Clin Res 39:57A(abstr)
34. Schwartz H, Ihle JN, Wecker E, Fischinger PJ, Thiel HJ, Bolognesi DP, Schafer W 1981 Properties of mouse leukemia viruses. XVII. Factors required for successful treatment of spontaneous AKR leukemia by antibodies against gp71. Virology 111:568–578
35. De Noronha F, Schafer W, Essex M, Bolognesi DP 1978 Influence of antisera to oncornavirus glycoprotein (gp71) on infections of cats with feline leukemia virus. Virology 85:617–621
36. Ljunggren K, Moschese V, Broliden PA, Giaquinto G, Quinti I, Fenyo E, Wahren B, Jondal M 1990 Antibodies mediating cellular cytotoxicity and neutralization correlate with a better clinical stage in children born to HIV-infected mothers. J Infect Dis 161:198–202
37. Kohl S 1991 The role of antibody dependent cellular cytotoxicity as a defense against herpes simplex virus infection. Rev Infect Dis 13:108–114
38. Ullberg M, Jondal M 1981 Recycling and target binding capacity of human natural killer cells. J Exp Med 153:615–628