Enhancement of Antibody-Dependent Cellular Cytotoxicity of Neonatal Cells by Interleukin-2 (IL-2) and IL-12

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Newborn infants are more susceptible to infections due in part to deficiencies in the cytotoxic functions of their lymphocytes. We investigated the ability of interleukin-2 (IL-2) and IL-12 to enhance the cytotoxicity of neonatal (cord blood) and adult mononuclear cells (MNCs) in both natural killer (NK) cell and antibody-dependent cellular cytotoxicity (ADCC) assays. The cytotoxic activity of cord blood MNCs was less than 50% that of adult MNCs in most assays prior to exposure to cytokines. Incubation with IL-2 (100 U/ml) or IL-12 (1 ng/ml) for 18 h increased the NK cell activity (using K562 target cells) of both cord blood and adult MNCs, and the combination of IL-2 and IL-12 increased cord blood cytotoxicity threefold, making the cytotoxicity of cord blood cells equivalent to that of adult cells treated with the same cytokines. In ADCC assays with chicken erythrocyte targets, the combination of IL-2 and IL-12 increased the cytotoxicities of both cord blood and adult MNCs, with greater enhancement again seen with cord blood cells. In assays with NK cell-resistant CEM cells coated with human immunodeficiency virus (HIV) gp120 antigen in the presence of hyperimmune anti-HIV immunoglobulin, ADCC of cord blood MNCs was about 50% that of adult MNCs; ADCC of cord blood MNCs increased two- to threefold with the addition of IL-2 and IL-12, whereas ADCC of adult MNCs did not increase. Incubation of cord blood cells, but not adult cells, with IL-2 or IL-12 for 1 week increased the percentage of CD16+/CD56+ cells two- to fivefold and enhanced ADCC activity. Thus, IL-2 and IL-12 greatly enhance both the NK cell and ADCC activities of neonatal MNCs and increase the number of NK cells in long-term culture.

Natural killer (NK) cells are large granular lymphocytes capable of lysing many types of tumor and virus-infected cells without prior sensitization. NK cells also have Fc receptors (CD16) on their surfaces. These receptors allow NK cells to kill antibody-coated target cells, thus providing another form of immune defense (12, 35). Several defects have been identified in NK cells of newborn infants, and these defects may make newborn infants particularly more susceptible to viral infections than adults (1, 11, 24, 27, 47, 52). NK cell cytolytic function, as measured by the level of killing of K562 cells, is decreased about 50% in newborns compared to that in adults (25), and neonatal NK cells have a decreased ability to kill cells infected with herpesvirus (18, 21). Newborn mononuclear cells (MNCs) have decreased antibody-dependent cellular cytotoxicity (ADCC) compared to the ADCC of MNCs from adults (11, 12), and newborns have decreased numbers of NK cells with both CD16 and CD56, a phenotype associated with greater cytotoxicity (32).

Previous studies have demonstrated that MNCs from newborn infants have a reduced capacity to produce cytokines, particularly interleukin-2 (IL-2) and gamma interferon (IFN-γ) (1, 6, 22, 27, 48), both of which are important in upregulating the cytotoxicity of NK cells (4, 5, 45). The cytotoxicity of neonatal NK cells against K562 targets (22, 38, 50) and virus-infected cells (17) is augmented by IL-2 but remains significantly lower than that of IL-2-activated adult MNCs. Mechanisms that may explain these deficits in cord lymphocytes include a decreased ability to translate IL-2 from mRNA (46) and reduced levels of expression of the γ chain of the IL-2 receptor on the cell membrane (30, 37, 43, 44, 52).

More recently, IL-12 has been found to play an important role in activating various lymphocyte functions. IL-12 is a 75-kDa disulfide heterodimeric protein that is secreted by monocytes, macrophages, neutrophils, and dendritic cells and that stimulates NK cells and T cells (29). IL-12 augments the cytotoxicity of NK cells in healthy adults and also in human immunodeficiency virus (HIV)-infected patients who produce decreased levels of IL-12 (19). IL-12 also enhances the killing of K562 and HIV-infected cells by neonatal NK cells (5, 10, 25). The level of expression of mRNA for IL-12 as well as the level of production of IL-12 was recently reported to be decreased in neonatal MNCs compared to that in adult MNCs (20).

We sought to determine if IL-2 and IL-12 could enhance the ADCC activity of neonatal MNCs. MNCs from umbilical cord blood and from healthy adult controls were used as effector cells, and targets included K562 cells, chicken erythrocytes (CRBCs), and an NK cell-resistant CEM cell line coated with an HIV antigen. We found that both IL-2 and IL-12 enhance NK cell activity and, more significantly, the ADCC activity of MNCs from newborns. These cytokines also increase the number of NK cells in long-term culture, which may further contribute to their potential therapeutic benefit.

MATERIALS AND METHODS

Subjects. MNCs were obtained from healthy adult volunteers and from the umbilical cords of full-term newborns following normal vaginal deliveries according to guidelines established by the Human Subjects Protection Committee of the University of California at Los Angeles. Cord blood was collected in sterile tubes and was processed within 24 h of birth.

Effector cells. MNCs were separated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. The isolated cells were resuspended at 10^6 cells/ml in RPMI 1640 (Irvine Scientific, Santa Ana, Calif.) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, Utah).

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with 10% heat-inactivated fetal calf serum (FCS). MNCs were then placed in a 15-mL culture tube (Falcon 2095; Becton Dickinson, Oxnard, Calif.) or a 25-cm² tissue culture flask (Sarstedt Inc., Newton, N.C.) with no cytokines or with IL-2 (Cetus, Emeryville, Calif.) or IL-12 (R&D Systems, Minneapolis, Minn.), or both. The cells were incubated without cytokines or with various cytokine combinations and concentrations in short-term (18 h) or long-term (7 days) cultures at 37°C in 5% CO₂. Interleukin dosages for optimal in vitro NK cell and ADCC activity were used as described previously (5, 10, 22, 25, 36, 38, 50). Because of the short half-life of the cytokines, IL-2 or IL-12 or both, was added to the culture every other day to maintain optimal activity in the longer-term experiments (7 days). Following incubation, the MNCs were pelleted and resuspended at 2.5 × 10⁶ cells/mL for cytotoxicity assays.

In some experiments, MNCs were placed directly in 96-well U-bottom microtiter plates (Falcon 3077; Becton Dickinson, Lincoln Park, N.J.) and were then incubated for 18 h with or without cytokines. These cells were not washed prior to the addition of the target cells so that the cytokines were present throughout the cytotoxicity assay.

**Target cells.** Cytotoxicity was measured by determining the amount of ¹⁵⁶Cr released from target cells. The tumor cell line K562 and CRBCs were used as target cells and were prepared as described previously (42). CEM cells (an NK cell-resistant human T-lymphoblast tumor cell line) were maintained in long-term cultures in RPMI 1640 with 10% FCS (34). On the day of the assay, 2 × 10⁶ to 5 × 10⁶ CEM cells were pelleted in a 5-mL centrifuge tube, resuspended, and then incubated with 100 μCi of ¹⁵⁶Cr and 1 μg of HIV type 1 (HIV-1)-strain MN gp120 recombinant protein (McGowanGenSys, Menlo, Conn.) for 2.5 × 10⁵ targets in a 37°C water bath for 2 h with mixing every 15 to 20 min. After incubation, the chromated cells were washed three times with 5 mL of RPMI 1640, resuspended at 2 × 10⁶/mL, and added to the wells.

**NK cell and ADCC assays.** When K562 cells were used as targets, 10⁵ cells in 100 μL of RPMI containing 10% FCS was added to each well with 5 × 10⁶ MNCs to achieve a 10:1 effector cell:target cell ratio. To determine the total and spontaneous release, plates containing target cells and effector cells were centrifuged at 100 × g for 3 to 5 min and then were incubated at 37°C in a CO₂ incubator at various effector cell:target cell ratios as described previously (42). All assays included additional wells with effector cells and target cells in the absence of antibody to allow for determination of the spontaneous lysis of the target cells. Supernatants (40% of the total volume) were harvested and counted to assess the total release and spontaneous release of target cells.

**ADCC assays.** CRBCs (2.5 × 10⁶/mL) were treated with HIV-1 gp120 protein in medium containing 5% newborn bovine serum albumin (FCS) alone, or medium containing 5% newborn bovine serum albumin and HIV-hyperimmune intravenous immunoglobulin (HIVIG; North American Biologicals, Miami, Fla.) was used at a dilution of 1:5,000 to assess ADCC. HIVIG is a 5% solution of 99% immunoglobulin G from pooled plasma from asymptomatic HIV-1-seropositive donors with CD4 counts of >500/mm³ and a high titer of antibody to HIV-2 (24). For assays with CRBCs as targets, rabbit anti-CRBC antiserum (Organon Teknika Corp., Durham, N.C.) was used at a dilution of 1:10,000 to assess ADCC. CRBCs (2.5 × 10⁶) in RPMI 1640 containing 10% FCS were added to each well, with the wells containing proportional increases in the numbers of effector cells to the addition of the target cells so that the cytokines were present throughout the incubation. The fluorescent stained cells were analyzed on a FACScan (Becton Dickinson, San Jose, Calif.) flow cytometer. Electronic gates were set to enable analysis of the fluorescence of the lymphocytes or monocytes in each preparation. The fluorescence of the lymphocytes or monocytes in each preparation was analyzed by using MNCs from healthy adult controls as NK cell activity against K562 cells and effects of IL-2 and IL-12. The effects of IL-2 and IL-12 on NK cell killing of K562 cells determined by using MNCs from healthy adult controls as effector cells are presented in Fig. 1. The MNCs were tested after 18 h of incubation with the cytokines. Concentrations of IL-12 greater than 1 ng/mL did not result in higher cytotoxicity when used alone (data not shown). An IL-2 concentration of 100 U/mL was selected for further testing because the augmentation by IL-12 was not evident at higher concentrations of IL-2. The concentrations selected for most assays in this study were not maximal so that the effects of combining the cytokines could be examined and so that the levels which were pharmacologically tolerable in vivo could be approximated (16).

The abilities of adult and cord blood MNCs to lyse K562 cells after 18 h of incubation are presented in Fig. 2A. The activity of cord blood MNCs in medium alone was only about half that of the adult cells at either effector cell:target cell ratio. However, the addition of IL-2 (100 U/mL), IL-12 (1 ng/mL), or the combination significantly enhanced the ability of MNCs from both adults and cord blood to lyse K562 cells (P < 0.0001). At the 25:1 effector cell:target cell ratio (Fig. 2A), the NK cell response of adult MNCs increased from 44% ± 4% (mean ± standard error of the mean [SEM]) lysis in medium alone to 56% ± 4% lysis with the addition of IL-2 to 58% ± 4% lysis with the addition of IL-12, and to 71% ± 4% lysis with the addition of the combination. The cytotoxins caused an even greater relative increase in cytotoxicity when cord blood MNCs were used. Compared to the cytotoxicity in medium alone (23% ± 2%), there was an approximate twofold increase in the cord blood NK cell activity of cord blood MNCs after incubating the MNCs with IL-2 (45% ± 4%) or IL-12 (53% ± 4%) and a threefold increase after incubating the MNCs with the combination (67% ± 5%). Similar increases in both adult and...
cord blood NK cell activities were seen at the 10:1 ratio (data not shown). Thus, the combination of cytokines can increase the cytotoxicity of cord blood cells to the levels for similarly activated adult cells.

The effects of incubating adult and cord MNCs in IL-2 and IL-12 for 1 week are presented in Fig. 2B. In the absence of cytokines, the lytic activity of cord blood cells (23% ± 4%) was less than half that of adult cells (56% ± 8%). When the cord blood cells were incubated in IL-2, an approximately threefold increase (76% ± 2%) in cytotoxicity occurred. In contrast, the cytotoxicity of adult MNCs did not increase after incubation with IL-2 (52% ± 8%). When the cells were incubated with IL-12, there was also an increase in the cytotoxicity of cord blood cells (63% ± 4%) but not that of adult cells (58% ± 5%). Incubation with the combination of IL-2 and IL-12 increased the cytotoxicity of cord blood MNCs (51% ± 6%), although it was lower than that after incubation with either cytokine alone, unlike the results seen in the 18-h incubation (Fig. 2A). Similar results for cord blood cells were seen at the 10:1 effector cell:target cell ratio (data not shown).

ADCC of CRBC targets. Cytokines increased the ADCC activities of both adult and cord blood MNCs against CRBC targets after an 18-h incubation (Fig. 3). In the absence of anti-CRBC antiserum, minimal cytolysis occurred with or without cytokines, although incubation with the combination of IL-2 and IL-12 slightly increased the NK cell response of cord blood cells, but the increased response was not statistically significant. The ADCC of cord blood MNCs in the absence of cytokines was 44% ± 4% at the 5:1 ratio (Fig. 3A), which is approximately 30% lower than that of adult cells (66% ± 3%). The adult cell ADCC was significantly \( P < 0.005 \) enhanced by incubation with IL-2 alone (75% ± 4%) and the combination of IL-2 and IL-12 (90% ± 5%) compared to that for incubation with medium alone. The activity of cord blood cells was significantly augmented by incubation with IL-12 alone (54% ± 5%; \( P < 0.05 \)), and incubation with the combination of IL-2 and IL-12 gave the highest response (71% ± 5%; \( P < 0.005 \)). At the 1:1 ratio (Fig. 3B) the greatest increase in ADCC activity again occurred after incubation with the combination of IL-2 and IL-12, with cord blood ADCC increasing almost 100%. Thus, incubation with the combination of IL-2 and IL-12 increases the ADCC activities of both adult and cord blood MNCs, with the most dramatic increases seen with the cord blood cells.

Enhancement of ADCC against gp120-coated CEM cells. Adult and cord blood cytotoxicities were next tested by culturing CEM cells coated with HIV protein gp120 in the absence
(NK cell activity) and the presence (ADCC) of antibody for 18 h (Fig. 4). The NK cell activities of both adult and cord blood cells were much lower against this NK cell-resistant target than against K562 cells (Fig. 2). NK cell lysis could be augmented by the addition of cytokines, with the greatest increase occurring with the combination of IL-2 (100 U/ml) and IL-12 (1 ng/ml). MNCs were tested at effector cell:target cell ratios of 25:1 (A) and 10:1 (B). The minimum number of determinations for each point was 13 adults and 26 umbilical cords; data are expressed as means ± SEMs. The P values compare the response for samples not treated with cytokines with the response for samples treated with cytokines.

![Cytokines Added](A)

![Cytokines Added](B)

**FIG. 4.** Short-term effects of cytokines on MNC-mediated ADCC of CEM cells coated with the HIV gp120 antigen. Cytolysis was tested in the absence of a specific antibody (NK cell activity) and in the presence of HIVIG (NK cell activity plus ADCC). MNCs from healthy adult controls and umbilical cord blood were incubated for 18 h alone or in the presence of IL-2 (100 U/ml), IL-12 (1 ng/ml), and IL-2 plus IL-12. MNCs were tested at effector cell:target cell ratios of 25:1 (A) and 10:1 (B). The minimum number of determinations for each point was 13 adults and 26 umbilical cords; data are expressed as means ± SEMs. The P values compare the response for samples not treated with cytokines with the response for samples treated with cytokines.

![Cytokines Added](A)

![Cytokines Added](B)

**FIG. 5.** Long-term effects of cytokines on MNC-mediated ADCC of CEM cells coated with the HIV gp120 antigen by using MNCs from healthy adult controls and umbilical cord blood as effectors. MNCs were incubated for 1 week alone or in the presence of IL-2 (100 U/ml), IL-12 (1 ng/ml), and IL-2 plus IL-12 and were tested at effector cell:target cell ratios of 25:1 (A) and 10:1 (B). The minimum number of determinations for each point was 9 adults and 13 cords; data are expressed as means ± SEMs. The P values compare the response for samples not treated with cytokines with the response for samples treated with cytokines.

The effects of incubating MNCs with IL-2 and IL-12 in long-term culture (1 week) on lysis of the gp120-coated CEM cell targets are presented in Fig. 5. When no cytokines were present, the NK cell activity of the adult MNCs increased, whereas the NK cell activity of cord blood cells remained low (compare to Figure 4). At the 25:1 effector cell:target cell ratio, IL-2 significantly increased the total cytotoxicity of both adult (55% ± 4%) and cord blood (62% ± 4%) cells compared to the cytotoxicity when no cytokines were added to adult cells (36% ± 5%) or cord blood cells (16% ± 3%) (Fig. 5A). The addition of IL-12 also increased the total cytotoxicity of cord blood cells (52% ± 4%) and adult cells (39% ± 4%). In contrast to the 18-h incubation (Fig. 4), incubation with the combination of IL-2 and IL-12 did not significantly alter the cytotoxicity of adult or cord blood MNCs in the presence or absence of antibody. The 10:1 effector cell:target cell ratio gave similar results (Fig. 5B).
In some experiments, the cells were added directly to the microtiter plates along with the cytokines for an 18-h incubation (Table 1). These cells were not washed prior to the cytotoxicity assays and thus remained in the continuous presence of cytokines throughout the experiment. Cytotoxicity against both K562 and CEM targets was about 50% higher by this microtiter plate method compared to that after incubation of the MNCs in tubes or flasks overnight with cytokines and then washing out the cytokines prior to the assay (Fig. 2, 4, and 5). However, the pattern of response to IL-2 and IL-12 was similar, with the highest cytotoxicity occurring when both IL-2 and IL-12 were present. Thus, the cytotoxicity levels obtained when cells were washed prior to the assay were less than those found when the MNCs were continually exposed to cytokines without washing. In addition, the cytotoxicity levels of the cord blood and adult cells were more similar when the MNCs were continually exposed to cytokines compared to the levels found when cells were washed prior to the assay (Fig. 2A), which resulted in higher cytotoxicity when adult cells were used.

Effect of IL-2 and IL-12 on CD16+CD56+ expression. The occurrence of NK cells expressing both CD16 and CD56 was noted (22, 33, 38, 50), but enhancement of ADCC has not been reported. In this study, we found that IL-2 and IL-12 significantly enhance both the NK cell and the ADCC activities of adult and cord blood MNCs (Table 2). Freshly isolated cord blood MNCs had 7% CD16+CD56+ cells among the cord blood cells increased to 18% ± 3% with the addition of IL-2 (100 U/ml) and to 7% ± 2% with the addition of IL-12 (1 ng/ml) (compared to 3% ± 1% without unstimulated cells). By contrast, the percentage of CD16+CD56+ cells among adult MNCs was not increased after 1 week of incubation with IL-2 or IL-12. The combination of IL-2 and IL-12 did not significantly enhance the percentage of CD16+CD56+ cells among either adult or cord blood MNCs.

### DISCUSSION

Newborns have increased susceptibility to many pathogens including bacteria and viruses as a result of a developmentally deficient host defense system. This increased susceptibility has been attributed to a number of immunological defects such as defective neutrophil function, decreased levels of antibody production, and reduced levels of IL-2 and IFN-γ production by T cells (14, 21, 22, 25, 48). NK cell activity and ADCC mediated by NK cells are also decreased in newborns. Enhancement of NK cell activity by IL-2 and IL-12 has been noted (22, 33, 38, 50), but enhancement of ADCC has not been reported. In this study, we found that IL-2 and IL-12 significantly enhance both the NK cell and the ADCC activities of cells from newborns, with the combination of the two cytokines producing the greatest increase.

In agreement with Gaddy et al. (10), we showed that over-

### TABLE 1. Effect of continuous exposure to IL-2 and IL-12 on NK cell and ADCC activities of adult and cord blood MNCs

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Adult MNCs</th>
<th>Cord blood MNCs</th>
<th>Adult MNCs</th>
<th>Cord blood MNCs</th>
<th>Adult MNCs</th>
<th>Cord blood MNCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium alone</td>
<td>54 ± 5 (12)</td>
<td>8 ± 2 (10)</td>
<td>43 ± 5 (10)</td>
<td>39 ± 6 (10)</td>
<td>6 ± 1 (19)</td>
<td>33 ± 3 (19)</td>
</tr>
<tr>
<td>IL-2 (100 U/ml)</td>
<td>73 ± 4 (12)</td>
<td>21 ± 4 (10)</td>
<td>54 ± 5 (10)</td>
<td>70 ± 5 (10)</td>
<td>22 ± 2 (19)</td>
<td>56 ± 4 (19)</td>
</tr>
<tr>
<td>IL-12 (1 ng/ml)</td>
<td>68 ± 4 (10)</td>
<td>18 ± 4 (9)</td>
<td>53 ± 5 (9)</td>
<td>66 ± 7 (9)</td>
<td>22 ± 3 (14)</td>
<td>55 ± 3 (14)</td>
</tr>
<tr>
<td>IL-2 + IL-12</td>
<td>78 ± 3 (10)</td>
<td>30 ± 5 (9)</td>
<td>60 ± 6 (9)</td>
<td>80 ± 5 (7)</td>
<td>36 ± 4 (10)</td>
<td>67 ± 3 (10)</td>
</tr>
</tbody>
</table>

* MNCs were incubated with cytokines for 18 h in microtiter plates without washing of cells. The effector cell:target cell ratio was 25:1. Values are means ± standard errors.

### TABLE 2. Effects of IL-2 and IL-12 on expression of CD16+CD56+ by adult and cord MNCs

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Fresh</th>
<th>18 h</th>
<th>1 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult MNCs</td>
<td>Cord blood MNCs</td>
<td>Adult MNCs</td>
</tr>
<tr>
<td>Medium alone</td>
<td>11 ± 3 (5)</td>
<td>7 ± 2 (11)</td>
<td>6 ± 3 (4)</td>
</tr>
<tr>
<td>IL-2 (100 U/ml)</td>
<td>8 ± 3 (4)</td>
<td>3 ± 0.3 (5)</td>
<td>9 ± 0.1 (4)</td>
</tr>
<tr>
<td>IL-12 (1 ng/ml)</td>
<td>8 ± 3 (3)</td>
<td>2 ± 1 (4)</td>
<td>4 ± 1 (4)</td>
</tr>
<tr>
<td>IL-2 + IL-12</td>
<td>8 ± 3 (3)</td>
<td>2 ± 1 (3)</td>
<td>5 ± 2 (4)</td>
</tr>
</tbody>
</table>

* MNCs were tested fresh after isolation (0 h), at 18 h, and at 1 week. Values are means ± standard errors.

b 0.01 when compared with no cytokines.

b P < 0.0001 when compared with no cytokines.

b P < 0.01 when compared with no cytokines.

b P < 0.05 compared with no cytokines.
night incubation with either IL-2 or IL-12 greatly augments cord blood cytosis of K562 cell targets. We additionally demonstrated that the levels of cytotoxicity induced by IL-2 and IL-12 in combination are higher than those induced by any one cytokine alone (Fig. 2). IL-2 has previously been shown to act synergistically with IL-12 to enhance lymphokine-activated cytotoxicity, induce the production of IFN-γ by NK cells (51), and promote the expression of genes for perforin and granzyme in fresh human NK cells (9); these factors may explain the greater enhancement seen with the combination of IL-2 and IL-12. Experiments with shorter exposure times (4 h) performed with cytokines present during the assays did not show significant effects (data not shown). In addition, the cytotoxicity levels of cord blood cells incubated with cytokines for 2 and 3 days were higher than those of cells incubated for only 18 h (data not shown).

We also examined the ability of cord blood MNCs to kill CEM tumor cells coated with gp120 HIV antigen. Although the activities of NK cells from both adults and infants were increased with cytokines, the antibody-dependent component was augmented in cord blood MNCs only, as calculated by subtracting the NK cell toxicity from the total cytotoxicity (Fig. 4 and 5). By using CRBC targets (Fig. 3), in which NK cell activity is negligible, the ADCC activities of both adult and cord blood MNCs were enhanced by the combination of IL-2 and IL-12, confirming the ADCC-enhancing properties of these cytokines.

Concurrent studies in our laboratories have found that IL-2 and IL-12 also enhance gp120-specific antibody-dependent cytotoxicity as well as NK cell activity when MNCs from HIV-infected patients are used (23). Using the same assay used in our study, Baum et al. (3) reported that HIV-positive individuals with high titers of antibodies mediating ADCC against gp120 targets had a better prognosis than those with lower titers. Infusions of IL-2 are now being given to AIDS patients to improve their immune status, but high doses are quite toxic (35). Prolonged infusions of low doses of IL-2 could minimize the side effects and may preferentially stimulate the in vivo expansion of NK cells (13, 39, 40).

IL-12 has been reported to increase the NK cell activity of cord blood MNCs against HIV-infected H9 cells (19), but the augmentation of ADCC activity was not demonstrated (25). However, we found that IL-2 or IL-12, or both, enhanced both NK cell activity and ADCC against gp120-coated CEM cells. A notable difference between our study and the previous report is that the baseline NK cell activity of the cord blood cells from term infants against the H9 cell targets was high (over 30%) (19). By contrast, the NK cell activity of cord blood MNCs against CEM targets was much lower (5% ± 1%). The high NK cell activity of cord blood cells against H9 cells may have masked the enhancement of ADCC due to cytokines. In addition, we demonstrated that after 18 h the combination of IL-2 and IL-12 increases ADCC activity against CRBC targets when MNCs from both adults and umbilical cords are used (Fig. 3). Sahin et al. (36) recently demonstrated that IL-12 would increase the cytotoxicity of NK cells for human tumors using a bispecific monoclonal antibody directed both to a tumor antigen and to CD16 on the NK cell. Thus, antibody-dependent cytotoxicity, as mediated by the CD16 receptor, is enhanced by IL-12.

High doses of IL-2 have been shown to enhance NK cell activity for up to 6 days in cultures of adult cells (26). We confirmed these findings for both adult and cord blood cells in culture for 7 days using IL-2 and also found that the antibody-dependent cytotoxicity of cord blood cells was enhanced. The increase in CD16+ cells after 1 week of incubation with IL-2 or IL-12 may explain why the antibody-dependent cytotoxicity of cord blood cells exceeds that of adult cells in these longer cultures. After 1 week of culture with the combination of IL-2 and IL-12, there was less cytotoxic activity and a decrease in the numbers of CD16+ cells compared with the cytotoxic activity and numbers of CD16+ cells after culture with either cytokine alone (Fig. 2 and 5; Table 2). This is in contrast to the data observed for 18 h of incubation, in which cells stimulated with the combination of cytokines elicited the greatest increase in cytotoxicity (Fig. 2). A possible mechanism for the reduced activity or generation of NK cells in long-term culture with IL-12 might be due to a negative-feedback effect such as an increased susceptibility to apoptosis or programmed cell death (2). IL-2, particularly in combination with IL-12, also promotes the production of IFN-γ, tumor necrosis factor alpha, and other cytokines which may have antiproliferative or inhibitory effects (7, 15). Kohl et al. (16) recently showed that in vitro incubation with IL-12 increased the NK cell toxicity of cells from HIV-positive patients, whereas in vivo administration decreased their ADCC and NK cell activities as well as their peripheral lymphocyte counts. One explanation for this paradoxical finding is that activated NK cells may have trafficked from the peripheral blood pool, although the production of increased levels of inhibitory cytokines such as IL-4 was also postulated.

IL-12 and IL-2 will augment the cytolytic activities of peripheral blood lymphocytes from patients with hematologic and solid malignancies, and both are being studied in clinical trials (31, 41). The study of the function of NK cells in cord blood is also relevant because cord blood cells may be used as an alternative to bone marrow for transplants in children with immunological or hematological disorders. NK cells may have significant graft-versus-leukemia effects (28) in leukemia patients receiving transplants and may provide protection against viral infections. Our studies show that both the NK cell and the ADCC activities of cord blood cells can be significantly enhanced after incubation with cytokines, and this might have beneficial effects in speeding the recovery of the transplant recipient. These experiments may also serve as a foundation for using cytokine therapy in conjunction with HIVIG infusions in patients with advanced HIV infection.

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REFERENCES

2. Armenti, M., G. Delespesse, and M. Sfatati. 1995. IL-2 and IL-7 but not IL-12 protect natural killer cells from death by apoptosis and up-regulate bcl2 expression. Immunology 85:331–337.


