Decreased Interleukin-15 From Activated Cord Versus Adult Peripheral Blood Mononuclear Cells and the Effect of Interleukin-15 in Upregulating Antitumor Immune Activity and Cytokine Production in Cord Blood

By John X. Qian, Sun min Lee, Yu Suen, Eva Knoppel, Carmella van de Ven, and Mitchell S. Cairo

Interleukin-15 (IL-15) is an important lymphokine regulating natural killer (NK) activity, T-cell proliferation, and T-cell cytokotoxic activities. We hypothesized that the reduced expression and production of IL-15 from cord blood (CB) may contribute to the immaturity of CB immunity and potentially delay immune reconstitution after CB transplantation. We compared the expression and production of IL-15 from activated cord versus adult mononuclear cells (MNCs) and the regulatory mechanisms associated with IL-15 expression in CB MNCs. We have also studied the effect of exogenous IL-15 stimulation on CB and adult peripheral blood (APB) MNCs in terms of NK and lymphokine-activated killer (LAK) activities and cytokine induction. Lipopolysaccharide (LPS)-stimulated CB and APB MNCs were used to determine IL-15 expression and protein production by Northern analysis and Western immunoblot analysis. IL-15 mRNA expression and protein accumulation in CB MNC were 25% ± 2.0% (12 hours, n = 4, P < .05) and 30% ± 2.5% (12 hours, n = 3, P < .05), respectively, when compared with APB MNCs. Nuclear run-on assays showed no differences between CB and APB MNCs during basal levels of transcription and after transcriptional activation. However, the half-life of IL-15 mRNA was approximately twofold lower in activated CB MNCs than in activated APB MNCs (CB: 101 ± 5.8 minutes vs APB: 210 ± 8.2 minutes, n = 3, P < .05). Exogenous IL-15 significantly enhanced CB NK and LAK activities up to comparable levels of APB (P < .05). IL-15 also significantly induced interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) protein production (days 1, 3, and 6, P < .05, n = 3) in CB MNCs. IL-15-stimulated LAK cells induced a significant lytic response against two acute lymphoblastic cell lines and two pediatric neuroblastoma cell lines. Both NK and LAK activities were augmented by the combination of IL-12 and IL-15, and the low-dose combination of IL-12 and IL-15 achieved similar levels of in vitro NK and LAK cytotoxicity compared with higher doses of either lymphokine. The present study suggests that IL-15 mRNA and protein expression is decreased in activated CB, secondary, in part, to altered posttranscriptional regulation. The reduced production of IL-15 from CB MNCs in response to stimulation may contribute to the decrease in IFN-γ and TNF-α production and CB cellular immunity. However, exogenous IL-15 enhanced IFN-γ and TNF-α production and NK and LAK cytotoxicities in CB MNCs. The reduced production of IL-15 from activated CB may contribute to the immaturity of CB cellular immunity and delayed immune reconstitution after unrelated CB transplantation. Exogenous IL-15 administration may compensate for the immaturity of CB immunity. The synergistic in vitro effects of low-dose IL-12 and IL-15 also implies the possible use of low doses each of IL-12 and IL-15 for enhancing immune reconstitution and/or possibly as a form of antitumor immunotherapy after CB transplantation.

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Comparatively, IL-2 is predominantly expressed and produced by activated T cells. IL-15 binds to only the β and γ subunits of the IL-2 receptor complex without requiring the use of the α subunit to exert its biologic activities. IL-15 has been reported to enhance nonspecific NK and lymphokine-activating killer (LAK) cytotoxic activities. IL-15 also induces NK cell production of IFN-γ, TNF-α, and GM-CSF. In vitro and in vivo studies have shown IL-15 to induce a variety of antitumor effects, including induction of CTL and LAK activities.

We hypothesize that the reduced production of IL-15 from activated CB MNCs compared with APB MNCs may contribute, in part, to immaturity of CB cellular immunity and potentially the delay in immune reconstitution after unrelated CB transplantation. Furthermore, exogenous IL-15 administration may enhance CB cellular immunity and has the potential for enhancing immune reconstitution and for immunotherapy after unrelated CB transplantation. In this study, therefore, we investigated the expression and production of IL-15 and regulatory mechanisms associated with IL-15 expression in CB compared with APB MNCs. We sought to determine if IL-15 could enhance CB IFN-γ and TNF-α production and CB NK and LAK cytotoxicities compared with APB and also to determine if IL-15 would be additive or synergistic with IL-12 with regard to in vitro antitumor immunity.

MATERIALS AND METHODS

Isolation of MNCs from CB and APB. Peripheral blood was obtained by venipuncture from healthy adult volunteers in accordance with the principles of the Declaration of Helsinki. Blood samples were also obtained from the umbilical cords of the placentas of normal, full-term, nonstressed infants immediately after scheduled cesarean section. The samples were collected in heparinized syringes. CB and APB MNCs were isolated from whole blood by density gradient separation on Ficoll-Hypaque gradients (density = 1.077 g/mL; Sigma Chemical Co, St Louis, MO) for 30 minutes. The MNCs at the interface were collected, washed twice, and resuspended in RPMI-1640 (GIBCO, Grand Island, NY) culture medium supplemented with 10% heat-inactivated human AB serum (Sigma). MNCs isolated by this density gradient separation were purified to greater than 98% homogeneity, and cell viability as measured by trypan blue exclusion was more than 99%. There was no difference in the MNC differential between CB and APB: CB: 82% ± 8.0% lymphocytes and 8.8% ± 4.0% monocytes; APB: 86% ± 4.0% lymphocytes and 7.2% ± 3.0% monocytes). The cells were cultured at a density of 1 × 10^6 cells/mL in culture medium for the following assays.

RNA isolation and Northern blotting. To determine IL-15 mRNA expression, CB and APB MNCs (60–10^6 cells) were stimulated with lipopolysaccharide (LPS; from Escherichia coli 0127:B8 at 10 μg/mL; Sigma) for up to 48 hours. Total cellular RNA was extracted from stimulated and unstimulated cells by the method of Chomczynski and Sacchi. Polyadenylated (A+)-RNA from cytoplasmic total RNA was then purified with oligo(dT) celluloose column (mRNA purification kit from Pharmacia Biotech Inc, Piscataway, NJ) and electrofocused on 1% agarose and 5% formaldehyde gel. The samples were heated at 40°C formamide and 14% formaldehyde at 65°C for 15 minutes and then cooled before the addition of 1 μg/mL ethidium bromide. RNA was transferred to nitrocellulose and baked for 2 hours. Hybridization with an antisense probe made by transcription of a human cDNA (kindly provided by Dirk Ander-
was normalized to the amount of GAPDH mRNA in each sample
and then expressed as a percentage, setting the amount of mRNA
at time 0 equal to 100%. The data were plotted against the time
after addition of actinomycin D, and the half-life of each transcript
was calculated based on the resultant graphs.

**Induction of cytokine production and NK and LAK cytotoxicity.**
Recombinant simian IL-15 (specific activity on CTLL proliferation,
3.33 × 10^7 U/μg) was kindly provided by Dr T. Troutt (Immunex),
and the recombinant human IL-12 (specific activity on phytohemag-
glutinin blast proliferation, 5.26 × 10^7 U/μg) was kindly provided
by Dr S. Wolf (Genetics Institute, Cambridge, MA). MNCs at 5 ×
10^5 cells/mL were placed into a plastic petri dish for 1 hour of
incubation at 37°C to remove monocytes. Monocyte-depleted MNCs
(MD MNCs) were adjusted to 1 × 10^6 cells/mL, seeded in 24-well
flat-bottom plates containing varying concentrations of cytokines,
and incubated at 37°C in a 5% CO2 humidified incubator. NK activity
was measured against K562 target cells (NK-sensitive, a human
erythroleukemic cell line; ATCC) after 18 hours of stimulation, and
LAK activity was measured against Daupi target cells (LAK-sensi-
tive, a human Burkitt’s lymphoma; ATCC) after 72 hours of stimula-
tion. At the end of the incubation, the effector cells were harvested,
washed, and resuspended in appropriate concentrations based on the
ratios of effector to target cells (E:T ratio) for the cytotoxicity assays.

**Cytotoxicity assay.** A standard 3-hour 51Cr-release assay was
performed to measure the cytotoxicity. Briefly, the target cells were
labeled with 100 μCi of Na2CrO4, washed twice, and resuspended
in a concentration of 5 × 10^3 cells/mL. One hundred microliters of
each target and effector cell suspension with E:T ratios (20:1, 10:1,
and 5:1) was added to a V-bottom 96-well culture plate. The mixture
was centrifuged briefly and incubated at 37°C for 3 hours. At the
end of the incubation, 150 μL of cell-free supernatant was collected
each well. The radioactivity was measured in a Beckman LS 1800
Liquid Scintillation Counter (Beckman, Fullerton, CA). All of
the samples were run in triplicate. The percentage of lysis was
calculated at each E:T ratio using the formula [(1 – Spontaneous –
Spontaneous Release/Spontaneous Release)] × 100% and then converted
to lytic units (LU; 30% target cell killing in 10^7 effector cells) using a computer-assisted program. To determine
the tumoricidal spectrum of CB non-specific cytokotoxicity, two acute
lymphoblastic leukemia cell lines, CCRF-CEM (T cells; ATCC) and
CCRF-SB (B cells; ATCC), and two neuroblastoma cell lines, NB-
19 and SK-N-MC (ATCC), were used as target cells.

**The study of effects of IL-15 and IL-12 on combination on CB NK
and LAK activities.** A method previously described by DeBlaker-
Hohe et al was used to determine if IL-15 and IL-12 in combination
induces a synergistic or additive NK and LAK activity from CB
MNCs. To avoid a deviation of using controls twice while comparing
the cytolytic response from the combination of two cytokines with
the sum of that from each single cytokine, the method was modified
by subtracting the control from each single data before being applied
to the calculation. Each cytotoxicity result of 17 combinations from
5 doses of IL-15 (0.1, 0.5, 1.0, 10, and 100 ng/mL) and 4 doses of
IL-12 (0.1, 0.5, 1.0, and 10 U/mL) was compared with the sum of
that from the same dose of each single cytokine using the following
formula: [(Cytotoxicity in Combination – Sum of Each Cytokine
Alone)/(Sum of Each Cytokine Alone)] × 100%. Based on the calcu-
lated results, the synergistic effect was arbitrarily defined as the
cytolytic response from the combination of two cytokines exceeding
the sum of that of each single cytokine by more than 10%, additive
as 0% to 10% and nonadditive as less than 0%.

**TNF-α and IFN-γ enzyme-linked immunosorbent assay (ELISA).**
CB and APB MNCs at a concentration of 1 × 10^6 cells/mL were
stimulated by IL-15 (50 ng/mL) for 24, 72, and 144 hours. The
supernatant was collected and the protein level was measured by
ELISA (Biosource) following the manufacturer’s protocol. All of
the samples were run in duplicate and data were presented as the
mean ± SEM. The sensitivity of the assay was 7.8 pg/mL.

**Statistical analysis.** Results from cytotoxicity studies and
ELISA were presented as the mean ± SEM of three or more samples.
Student’s t-test was used for determining significant differences be-
tween two groups, and Kruskal-Wallis nonparametric ANOVA test
was used for comparing multiple groups with Bartlett’s test as the
posttest for determining specific significant subgroups (Instat Graph
Pad Software, San Diego, CA). A P value <.05 was considered
significant.

**RESULTS**

**Reduced IL-15 mRNA expression in stimulated cord versus
adult MNCs.** Northern blot analyses of CB and APB MNCs were performed simultaneously under identical conditions
to compare the IL-15 mRNA expression before and after LPS stimulation. Unstimulated MNCs from both CB
and APB had an undetectable expression of IL-15 mRNA.
In a time course study of IL-15 mRNA expression after
LPS (10 μg/mL) stimulation, IL-15 mRNA expression was
induced within 6 hours upon LPS stimulation and reached
a peak level at 12 hours in both CB and APB MNCs, re-
turned to basal level after 24 hours. However, IL-15 mRNA
expression in CB MNCs was only 25% that from the same dose of each single cytokine using the following
formula: 

\[
\text{Synergistic effect} = \frac{\text{Cytotoxicity in Combination} - \sum \text{Cytotoxicity alone}}{\sum \text{Cytotoxicity alone}} \times 100%
\]

The expression level reached a maximum at 12 hours in both CB and APB MNCs, but the expression level in CB
MNCs was only 25% that from the same dose of each single cytokine using the following formula:

\[
\text{Synergistic effect} = \frac{\text{Cytotoxicity in Combination} - \sum \text{Cytotoxicity alone}}{\sum \text{Cytotoxicity alone}} \times 100%
\]

The expression level reached a maximum at 12 hours in both CB and APB MNCs, but the expression level in CB
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\[
\text{Synergistic effect} = \frac{\text{Cytotoxicity in Combination} - \sum \text{Cytotoxicity alone}}{\sum \text{Cytotoxicity alone}} \times 100%
\]
mine whether the differential regulation was occurring at the posttranscriptional level. CB and APB MNCs were stimulated with LPS (10 \( \mu \)g/mL) for 12 hours before actinomycin D (10 \( \mu \)g/mL) was added for various time periods (0 to 360 minutes.). Northern blots of poly (A)\(^+\) RNA were hybridized with an antisense riboprobe made by transcription of human cDNA. The levels of IL-15 mRNA progressively decreased during actinomycin D exposure in both CB and APB MNCs. Transcripts were quantitated by densitometric scanning of the autoradiographs. As shown in Fig 4, the measured mRNA half-life of IL-15 from stimulated CB MNCs was approximately twofold lower than that from stimulated APB MNCs (\( t_{1/2} \): 101 ± 5.8 minutes v 210 ± 8.2 minutes, CB v APB, mean ± SEM, \( n = 3 \), \( P < .05 \)).

Enhanced CB and APB NK and LAK cytotoxic activities after IL-15 stimulation. The baseline NK and LAK cytotoxicities were determined after 18 and 72 hours of incubation in the absence of cytokines. The NK activity of CB against K562 was significantly lower than that of APB MD MNCs (CB v APB: 60 ± 9 v 115 ± 18 LU, \( P < .05 \), \( n = 10 \); Fig 5). However, the LAK activity of CB was similar to APB (CB v APB: LAK, 43 ± 10 v 44 ± 19 LU, \( P = \) NS, \( n = 10 \); Fig 6). After incubation of MD MNCs with IL-15 (10 ng/mL), both CB and APB NK activities (18 hours) were significantly increased over control (control v IL-15 at 10 ng/mL: CB, 45 ± 9 v 533 ± 72 LU, \( P < .01 \), \( n = 10 \); APB, 115 ± 18 v 537 ± 68 LU, \( P < .05 \), \( n = 6 \)). CB NK activity reached a comparable level of APB (IL-15 at 10 ng/mL: CB v APB, 533 ± 72 v 539 ± 68 LU, \( P = \) NS). IL-15 (10 ng/mL) also induced a significant increase of both CB and APB LAK activities against Daudi target cells (control v IL-15 at 10 ng/mL: CB, 43 ± 10 v 420 ± 107 LU, \( P < .01 \), \( n = 6 \); Fig 6). However, CB MD MNCs was more responsive to IL-15 stimulation than APB for induction of LAK activity (CB v APB: IL-15 at 1.0 ng/mL, 262 ± 62 v 144 ± 91, \( P < .05 \); IL-15 at 10 ng/mL, 843 ± 64 v 420 ± 107 LU, \( P < .01 \); Fig 6).

IL-15 induced nonspecific tumoricidal NK and LAK activities. Four cell lines, CCRF-CEM, CCRF-SB, NB-100, and SK-N-MC, were used to examine in vitro antitumor activity of CB NK and LAK cells after IL-15 stimulation. IL-15 (10 ng/mL) stimulation resulted in enhanced CB cytotoxicity against all of these tumor cell lines. As shown in Fig 7A, these cell lines, CCRF-CEM, SK-N-MC, and NB-100, be-
came more sensitive to CB NK cytotoxicity induced by IL-15 (control v IL-15 stimulated: CCRF-CEM, 50 ± 34 v 229 ± 34 LU, P < .05; SK-N-MC, 18 ± 17 v 269 ± 91 LU, P < .01; NB-100, 22 ± 4 v 208 ± 25 LU, P < .01, n = 4). IL-15–induced CB LAK cytotoxicity produced an enhanced lytic response against all four tumor cell lines over controls (control v IL-15 stimulated: CCRF-CEM, 55 ± 39 v 318 ± 3 LU, P < .01; CCRF-SB, 18 ± 12 v 436 ± 114 LU, P < .05; SK-N-MC, 50 ± 25 v 358 ± 13 LU, P < .01; NB-100, 33 ± 31 v 330 ± 40 LU, P < .01, n = 3; Fig 7B).

Additive and synergistic effects of low doses of IL-15 and IL-12 on CB NK and LAK cytotoxicity. The effects of the combination of IL-15 and IL-12 on NK and LAK cytotoxicity was examined by comparing the cytolytic response from two cytokines in combination with the sum of that from each single cytokine. The results in Table 1 show that using lower doses of IL-15 (0.1, 0.5, and 1.0 ng/mL) and IL-12 (0.1, 0.5, and 1.0 U/mL) concomitantly generated either synergistic or additive effects on CB NK cytotoxicity against K562 targets. The synergy from these lower dose combinations induced a comparable NK cytotoxicity compared with single higher doses of each cytokine individually. Specifically, IL-15 (1.0 ng/mL) and IL-12 (0.5 U/mL) induced a lytic response comparable to that induced by 10 ng/mL of IL-15 alone or 10 U/mL of IL-12 alone (532 ± 64 v 473 ± 70 LU, P = NS, and v 410 ± 48 LU, P = NS). Conversely, higher dose combinations of IL-12 and IL-15 induced only nonadditive effects on CB NK cytotoxicity. Table 2 summarizes the combined ef-

Fig 4. The half-life of IL-15 mRNA in cord (CB) (A) versus adult (APB) (B) MNCs. Actinomycin D (10 μg/mL) was added for the indicated times to cells from cord (A) and adult (B), stimulated with LPS (10 μg/mL) for 12 hours. Poly (A)+ RNA was analyzed by Northern blotting for the presence of IL-15 transcript (1.5 kb). The data were plotted against the time after the addition of actinomycin D. Results shown are representative of three different experiments (t1/2: 101 ± 5.8 minutes v 210 ± 8.2 minutes, CB v APB, P < .05, n = 3).
fecteds of IL-15 and IL-12 on CB LAK cytotoxicity against Daudi targets. The synergistic effect of IL-12 and IL-15 is seen at two combinations of lower doses of IL-15 and IL-12 (0.1 or 0.5 ng/mL of IL-15 + 0.1 U/mL of IL-12) and the lytic response from the latter combination is comparable to that of a single higher dose of IL-12 (10 U/mL), but much lower than that of IL-15 (10 ng/mL) (IL-15 + IL-12 v IL-12 alone v IL-15 alone: 512 ± 48 LU v 501 ± 54 LU, P = NS; and v 823 ± 68 LU, P < .01). The higher dose combinations of IL-15 and IL-12 induced a suppressive effect on LAK cytotoxicity when compared with the single higher dose of IL-12 and IL-15 (10 ng/mL IL-15 + 10 U/mL IL-12 v 10 ng/mL IL-15 alone v 10 U/mL IL-12 alone, 259 ± 77 v 823 ± 68 LU, P < .01; 259 ± 77 v 501 ± 54, P < .05, n = 10).

**IL-15 enhancement of IFN-γ and TNF-α production from CB MNCs.** IL-15 (50 ng/mL) stimulation induced a significant increase of IFN-γ protein production in CB MNCs (IL-15 stimulated v control: day 1, 112 ± 13 v 3.3 ± 1.2 pg/mL, P < .01; day 3, 480 ± 75 v 8 ± 4 pg/mL, P < .01; day 6, 670 ± 20 v 135 ± 52 pg/mL, P < .01, n = 3). However, the IFN-γ levels in IL-15–stimulated CB were still significantly lower than stimulated APB (IL-15–stimulated CB v IL-15–stimulated APB: day 1, 112 ± 13 v 567 ± 44 pg/mL, P < .01; day 3, 480 ± 75 v 866 ± 30 pg/mL, P < .05; day 6, 670 ± 20 v 830 ± 20 pg/mL, P < .05, n = 3; Fig 8A). Similarly, IL-15 (50 ng/mL) stimulation also significantly increased TNF-α protein production in CB MNCs (IL-15 stimulated v control: day 1, 308 ± 81 v 50 ± 26 pg/mL, P < .01; day 3, 423 ± 76 v 70 ± 30 pg/mL, P < .01; day 6, 358 ± 44 v 128 ± 51 pg/mL, P < .05, n = 3). The TNF-α production in CB MNCs was still significantly lower than APB (IL-15–stimulated CB v stimulated APB: day 1, 308 ± 46 v 676 ± 88 pg/mL, P < .01; day 3, 423 ± 76 v 983 ± 179 pg/mL, P < .05; day 6, 358 ± 44 v 1,125 ± 108, P < .01, n = 3; Fig 8B).

**DISCUSSION**

Numerous studies have demonstrated the success of hematopoietic growth factors to enhance hematologic reconstitution after stem cell transplantation.44-51 Vowels et al52 re-
ported that the use of GM-CSF post-CB transplantation resulted in rapid engraftment and mild graft-versus-host disease. However, the delay in immune reconstitution post-CB transplantation may be due to the defects in CB cellular immunity and cytokine production.2-4 We have recently reported that the reduced expression and production of IL-12 from activated CB may contribute to the immaturity in CB cellular immunity.18 The number and functionality of donor-derived lymphocytes in patients after CB transplantation remains to be determined. The use of exogenous cytokines post-CB transplantation that enhance CB immune function may compensate for the immaturity of CB cellular immunity and enhance immune reconstitution and CB tumor immunity post-CB transplantation.

Although there are many similar biologic activities between IL-2 and IL-15, the regulation of IL-15 expression differs markedly from that of IL-2. IL-15 mRNA is expressed in a variety of tissues, including placenta, skeletal muscle, kidney, and activated monocytes/macrophages but not normal T cells.22 Enhancement of IL-15 protein production from monocytes occurs in response to a wide variety of agonists including LPS, IFN-γ, Bacillus Calmette-Guerin (BCG), Mycobacterium tuberculosis, Toxoplasma gondii, or Salmonella choleraesuis.53-55 During states of increased de-

**Table 1. The Synergistic and Additive Effects of IL-15 and IL-12 on Cord Blood NK Cytotoxicity Against K562**

<table>
<thead>
<tr>
<th>IL-12 (U/mL)</th>
<th>0</th>
<th>0.1</th>
<th>0.5</th>
<th>1.0</th>
<th>5.0</th>
<th>10</th>
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<tr>
<td>IL-15 (ng/mL)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>54 ± 10*</td>
<td>133 ± 19</td>
<td>179 ± 29</td>
<td>383 ± 61</td>
<td>473 ± 70</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>156 ± 23</td>
<td>227 ± 28 (+8%)†</td>
<td>383 ± 63 (+33%)</td>
<td>465 ± 59 (+21%)</td>
<td>534 ± 92 (–1%)</td>
<td>307 ± 71 (–51%)</td>
</tr>
<tr>
<td>0.5</td>
<td>283 ± 32</td>
<td>368 ± 63 (+9%)</td>
<td>486 ± 65 (+17%)</td>
<td>532 ± 64 (+15%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1.0</td>
<td>352 ± 54</td>
<td>449 ± 64 (+10%)</td>
<td>554 ± 65 (+14%)</td>
<td>489 ± 71 (–8%)</td>
<td>514 ± 107 (–30%)</td>
<td>495 ± 97 (–40%)</td>
</tr>
<tr>
<td>10</td>
<td>410 ± 48</td>
<td>509 ± 67 (+9%)</td>
<td>ND</td>
<td>559 ± 65 (–5%)</td>
<td>457 ± 92 (–42%)</td>
<td>631 ± 91 (–29%)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

* NK cytotoxicity (lytic units) in Table 1 is presented as the mean ± SEM (n = 8).
† (%) indicates the effects (synergistic > 10%, additive 0% to 10%, not additive < 0%) of IL-15 and IL-12 in combination on NK activity compared with the sum of each cytokine alone which is obtained by the formula as below. All the data are normalized by subtracting the control prior to being applied to calculation:

\[
\text{Cytotoxicity in Combination} - \frac{(\text{Sum of Each Cytokine Alone})}{\times 100%}.
\]
Table 2. The Synergistic and Additive Effects of IL-15 and IL-12 on CB LAK Cytotoxicity Against Daudi

<table>
<thead>
<tr>
<th>IL-12 (U/mL)</th>
<th>0</th>
<th>0.1</th>
<th>0.5</th>
<th>1.0</th>
<th>10</th>
<th>100</th>
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<tbody>
<tr>
<td>IL-15 (ng/mL)</td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>86 ± 24*</td>
<td>234 ± 62</td>
<td>348 ± 58</td>
<td>823 ± 68</td>
<td>930 ± 57</td>
<td></td>
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<tr>
<td>0.1</td>
<td>313 ± 54 (+19%)</td>
<td>512 ± 48 (+19%)</td>
<td>542 ± 44 (−1%)</td>
<td>576 ± 51 (−44%)</td>
<td>601 ± 76 (−47%)</td>
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</tr>
<tr>
<td>0.5</td>
<td>460 ± 51 (−3%)</td>
<td>519 ± 53 (−17%)</td>
<td>574 ± 68 (−22%)</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>1.0</td>
<td>487 ± 56 (−7%)</td>
<td>516 ± 63 (−23%)</td>
<td>573 ± 75 (−27%)</td>
<td>403 ± 60 (−68%)</td>
<td>372 ± 75 (−76%)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>375 ± 97 (−36%)</td>
<td>ND</td>
<td>354 ± 92 (−57%)</td>
<td>259 ± 77 (−80%)</td>
<td>381 ± 60 (−78%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

* LAK cytotoxicity (lytic units) in Table 2 is presented as mean ± SEM (n = 10).

† (%) indicates the effects (synergistic > 10%, not additive < 0%) of IL-15 and IL-12 in combination on LAK activity compared with the sum of each cytokine alone. All the data are normalized and calculated in the same way as in Table 1.
ing activity and with the levels of AU-rich binding factor. It seems likely that various protein factors interacting with specific mRNA sequences exist in vivo and are involved in the regulation of AU-rich mRNA decay. Any alteration in the expression and/or biologic activities of these various protein factors in stimulated CB MNCs could contribute to the reduction of IL-15 mRNA expression. Further studies are required to test these possibilities.

The immaturity of CB immunity, which is associated with decreased production of IL-2, IL-12, IL-15, IFN-γ, and TNF-α, may contribute to diminished CB NK, LAK, and CTL cytotoxicities. Seki et al. and others have reported that NK cytotoxicity is decreased in CB compared with APB. IL-2 can enhance the NK cytotoxicity of CB MNCs to the level of APB MNC activity. We have recently reported that IL-12 can enhance CB NK cytotoxicity up to levels of APB MNC activity. IL-15 not only enhances T-cell function, but also enhances cytolytic function of both CD56dim NK and CD8+ T cells. Carson et al. reported that activation of CD56dim NK cells by IL-15 was similar to that of IL-2. However, the IL-15–enhanced NK cytotoxic activity is completely IL-2 independent. Our present studies suggested that IL-15 also enhanced CB NK and LAK activities up to the adult level. CB LAK activity appeared to be more sensitive to exogenous IL-15 compared with APB (Fig 5). Tumoricidal studies showed that IL-15 induced significant CB NK and LAK activities against a broad range of neuroblastoma, leukemia, and lymphoma cell lines (Fig 7).

Although IL-2 has been shown to have therapeutic benefits for some cancer patients, the substantial toxicities associated with high doses of IL-2 have limited its use clinically. IL-12 has also experienced dose-limiting toxicities. Recently, IL-15 has been shown to mimic the antitumor activities of IL-2 with potentially less toxicity in an in vivo animal model. Further studies are required to evaluate this aspect. Combinations of lower doses of IL-15 and IL-12 might have the potential of augmenting in vivo antitumor immune function and minimizing toxicity. DeBlaker-Hoke et al. showed a synergistic effect on inducing APB NK and LAK activities by the combination of lower doses of IL-2 and IL-12. Carson et al. suggested that the combination of IL-12 and IL-15 had a synergistic effect on augmenting APB NK cytolytic activity and IFN-γ production. In the present study, we demonstrated that low-dose combinations of IL-12 (0.1 U/mL) and IL-15 (0.1 to 1.0 ng/mL) induced a synergistic or additive effect on CB NK cytotoxicity, except for the combination of IL-12 (1.0 U/mL) and IL-15 (1.0 ng/mL). The synergistic NK activity reached the same levels as a single high dose (IL-12, 10 U/mL; IL-15, 10 ng/mL) of either individual cytokine. Although no synergistic or additive effects from high-dose combinations of IL-12 (5 to 10 U/mL) and IL-15 (5 to 10 ng/mL) on CB NK activity seen, the cytotoxicity levels were still higher than that induced by the single dose of individual cytokine (Table 1). Similarly, low-dose combinations of IL-12 (0.1 U/mL) and IL-15 (0.1 to 0.5 ng/mL) had a synergistic effect on CB LAK activity that is comparable to the level as a single high dose of IL-12 (10 U/mL), but lower than a single high dose of IL-15 (10 to 100 ng/mL). However, the high-dose combination of IL-12 (1.0 to 10 U/mL) and IL-15 (10 to 100 ng/mL) had a suppressive effect on CB LAK activity (Table 2). This suppression may be due to NK cell apoptosis in which decreased numbers of killer cells resulted in a low level of LAK activity. This observation is consistent with the report by Ross and Caligiuri in which costimulation of IL-12 and IL-15 or IL-12 and IL-2 induced NK cell proliferation and IFN-γ production initially, followed by NK cell apoptosis and a decline in IFN-γ production.

The IFN-γ and TNF-α production in IL-15–stimulated CB MNCs was significantly induced and increased up to the unstimulated APB level, but far lower than the IL-15 stimulated APB level (Fig 8). This result is consistent with our earlier observation on IFN-γ production in IL-12–stimulated CB and APB MNCs. This partial compensation after IL-15 stimulation suggests that the decreased production of IFN-γ and TNF-α by CB MNCs may be due to at least two factors: defective CB IFN-γ and TNF-α production and defective CB IFN-γ and TNF-α–inducing cytokines, such as IL-15, IL-12, and IL-2. Interestingly, the effect of IL-15 on CB NK activity and IFN-γ production showed that IL-15 alone is capable of inducing CB NK activity up to the APB level; however, IL-15 alone cannot compensate for IFN-γ production up to the stimulated APB level (Figs 5 and 8). This disparity indicates the important role of intrinsically deficient cytokine production such as IL-12, IL-15, and IFN-γ in the pathogenesis of the immaturity of CB cellular immunity. Further studies are required to verify these observations.

In summary, the present study showed that IL-15 mRNA and protein production is decreased in activated CB compared with APB MNCs. This discrepancy in IL-15 production is secondary, at least in part, to altered posttranscriptional regulation. The reduced production of IL-15 from activated CB MNCs might contribute to the immaturity in CB cellular immunity. However, exogenous IL-15 stimulation may compensate for the immaturity in CB immunity by enhancing NK and LAK activities and by inducing IFN-γ and TNF-α production. The additional synergistic effects of lower doses of IL-15 in combination with IL-12 suggests the potential benefit of the combination of each cytokine to increase CB antitumor immunity and potentially decrease toxicity compared with higher doses of either of the cytokines alone. Further studies are needed to define the clinical implications of these findings and the potential use of IL-15 to enhance CB cellular immunity and/or accelerate immune reconstitution after CB transplantation.

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Decreased Interleukin-15 From Activated Cord Versus Adult Peripheral Blood Mononuclear Cells and the Effect of Interleukin-15 in Upregulating Antitumor Immune Activity and Cytokine Production in Cord Blood

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