Interleukin (IL) 15 Is a Novel Cytokine That Activates Human Natural Killer Cells via Components of the IL-2 Receptor

By William E. Carson,* Judith G. Giri,‡ Matthew J. Lindemann,* Michael L. Linett,* Minoo Ahdieh,‡ Raymond Paxton,‡ Dirk Anderson,‡ June Eisenmann,‡ Kenneth Grabstein,‡ and Michael A. Caligiuri*

*From the *Departments of Medicine, Surgery, and Molecular Medicine, Roswell Park Cancer Institute, Buffalo, New York 14263; and ‡Immunex Research and Development Corporation, Seattle, Washington 98101*

Summary

Interleukin 15 (IL-15) is a novel cytokine that has recently been cloned and expressed. Whereas it has no sequence homology with IL-2, IL-15 interacts with components of the IL-2 receptor (IL-2R). In the present study we performed a functional analysis of recombinant IL-15 on phenotypically and functionally distinct populations of highly purified human natural killer (NK) cells. The $CD56^{bright}$ subset of human NK cells constitutively expresses the high affinity IL-2R and exhibits a brisk proliferative response after the binding of picomolar amounts of IL-2. Using a proliferation assay, IL-15 demonstrated a very steep dose–response curve that was distinct from the dose–response curve for IL-2. The proliferative effects of IL-15 could be abrogated by anti-IL-2Rβ (p75), but not by anti-IL-2Rα (p55). The proliferative effects of IL-2 on $CD56^{bright}$ NK cells could be inhibited by both antibodies. $CD56^{dim}$ NK cells express the intermediate affinity IL-2R in the absence of the high affinity IL-2R. Activation of $CD56^{dim}$ NK cells by IL-15 was similar to that of IL-2 as measured by enhanced NK cytotoxic activity, antibody-dependent cellular cytotoxicity, and NK cell production of interferon γ, tumor necrosis factor α, and granulocyte/macrophage colony-stimulating factor. The IL-15–enhanced NK cytotoxic activity could be completely blocked by anti-IL-2Rβ monoclonal antibody. The binding of radiolabeled IL-2 and IL-15 to $CD56^{dim}$ NK cells was inhibited in the presence of anti-IL-2Rβ. Scatchard analysis of radiolabeled IL-15 and IL-2 binding to NK-enriched human lymphocytes revealed the presence of high and intermediate affinity receptors for both ligands. IL-15 is a ligand that activates human NK cells through components of the IL-2R in a pattern that is similar but not identical to that of IL-2. Unlike IL-2, IL-15 is produced by activated monocytes/macrophages. The discovery of IL-15 may increase our understanding of how monocytes/macrophages participate in the regulation of NK cell function.

Human NK cells are LGL that do not rearrange TCR genes, and demonstrate cytotoxic activity against tumor and virally infected cell targets without prior sensitization or MHC restriction. As such, NK cells appear to have an important role in the early defense against viral infection and malignant transformation (for a review see reference 1).

NK cells can be identified phenotypically by the expression of CD56, an isoform of the human neural cell adhesion molecule, as well as CD16 or FcRγIII (1). Approximately 10% of NK cells have high density expression of CD56 ($CD56^{bright}$). The $CD56^{bright}$ NK cells constitutively express the high affinity heterotrimeric IL-2 receptor (IL-2R$\alpha\beta\gamma$), as well as the intermediate affinity heterodimeric IL-2R$\beta\gamma$ (2, 3). The more abundant $CD56^{dim}$ NK cells constitutively express the intermediate affinity heterodimeric IL-2R$\beta\gamma$ (4). IL-2 binding to the high affinity IL-2R expressed on $CD56^{bright}$ NK cells results in a strong proliferative response, whereas complete saturation of the intermediate affinity IL-2R on either $CD56^{bright}$ or $CD56^{dim}$ NK cells results in enhanced cytotoxic activity with little or no effect of proliferation (2). Thus, NK cells appear to have relatively distinct functional responses after the binding of IL-2 to the high or intermediate affinity IL-2R.

IL-15 is a novel cytokine that has recently been cloned from the simian kidney epithelial cell line, CV1/EBNA (5). Whereas the sequence of IL-15 shows no homology with IL-2, IL-15 can bind to a variety of cell lines, human mononuclear cells, and activated T cells. On activated T cells, IL-15 appears to

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use components of the IL-2R for binding and signal transduction (5, 6). In contrast to IL-2, IL-15 appears to be much more abundantly expressed in a wide variety of tissues, including placenta, skeletal muscle, kidney, and activated monocytes/macrophages (5). Thus, IL-15 may be very important for NK cell function as NK cells are unique among human lymphocytes in their constitutive expression of functional IL-2R. We therefore investigated the functional response of human NK cells to IL-15, and compared these responses to those of IL-2.

Materials and Methods

Cytokines. Purified, yeast-derived, recombinant human IL-15 was provided by Immunex Research and Development Corporation. Purified rIL-2 was obtained from Hoffmann LaRoche (Nutley, NJ; sp act, 1.53×10^7 U/mg). Purified rIL-12 (sp act, 4.5×10^6 U/mg) was obtained from Genetics Institute Inc. (Cambridge, MA). Recombinant human TNF-α (sp act, 2×10^5 U/μg) was obtained from the Asahi Chemical Corporation (Fuji City, Japan). All cytokines were constituted in RPMI-1640 with 0.1% human albumin (Armour Pharmaceutical Co., Kankakee, IL).

mAbs. The anti-p75 (anti-IL-2Rβ or anti-CD122) mAb (7) and the anti-p55 (anti-IL-2Rα or anti-CD25) mAb (8) were kindly provided as ascites by Dr. Kendall Smith (Cornell Medical School, New York). Antibody was purified from ascites using the Affi-gel protein A mAb purification system II kit (Bio-Rad Laboratories, Richmond, CA) in accordance with the manufacturer's instructions. 3F5B11 is an anti-HLA-DR murine mAb that was purified in an identical fashion and was used as an isotype control (9). Anti-CD3 and CD4 sterile mouse ascites were a generous gift of Dr. Stuart Schlossman (Dana Farber Cancer Institute, Boston, MA). PE-conjugated anti-CD56 mAb (NKH1-RD1) was purchased from Coulter Immunology (Hialeah, FL). Polyclonal rabbit anti-mouse lymphocyte serum was purchased from Accurate Chemical and Scientific Corp. (Westbury, NY).

Cell Lines. Target cells for LAK activity and antibody-dependent cellular cytotoxicity (ADCC)[1], assays were COLO 205, a NK-resistant human colon adenocarcinoma cell line, and P815, a NK-resistant murine mastocytoma cell line, respectively. Cells were grown in RPMI-1640 supplemented with 10% heat-inactivated FCS (Sigma Chemical Co., St. Louis, MO), anti-PPLO agent, and antibiotics (GIBCO BRL, Gaithersburg, MD).

Isolation of Human NK Cells. Mononuclear cells were obtained from fresh leukopacs (American Red Cross, Buffalo, NY) using Ficoll-Hypaque density gradient centrifugation (Sigma Chemical Co.). Cells were then washed twice in RPMI-1640 supplemented with 10% human AB serum (HAB), and adhered to plastic for 2 h. T cells, B cells, and remaining monocytes were depleted using goat anti-mouse immunomagnetic beads (Advanced Magnetics, Inc., Cambridge, MA), and a combination of murine mAb reactive against CD3, CD4, and HLA-DR, as described (9). Non-depleted cells were then stained with CD56-PE, washed in 10% HAB, and sorted for $CD56^{bright}$ and $CD56^{dim}$ NK cells on a FACStar Plus ® (Becton Dickinson & Co., Mountain View, CA) (2). Sorted $CD56^+$ NK cells were >97% pure by FACStar® analysis.

Proliferation Assays. 2×10^4 $CD56^{bright}$ or $CD56^{dim}$ NK cells were plated in U-bottom plates in the presence or absence of IL-15 or IL-2 and incubated at 37°C for 84 h. In blocking experiments, cells were preincubated with saturating concentrations of either anti-p55, anti-p75, or isotype nonreactive control mAb for 1 h before the addition of cytokine. Proliferation was measured by methyl-[^{3}H]thymidine incorporation during the last 12 h of incubation. Results represent the mean of triplicate wells ± SE and are expressed as counts per minute of [^{3}H]thymidine incorporation.

^{51}Chromium-release Cytotoxicity Assay. Sorted $CD56^{dim}$ NK cells were plated in 96-well V-bottom plates (Costar Corp., Cambridge, MA) in 200 μl of RPMI-1640 media supplemented with 10% HAB with or without cytokine. After an 18-h incubation at 37°C, 4×10^3 ^{51}Cr-labeled COLO 205 tumor target cells were added to each well. Plates were then centrifuged for 3 min at 800 rpm and incubated for an additional 4 h at 37°C. Plates were then centrifuged and the supernatant from each well was harvested using a filter harvesting system (Skatron Inc., Sterling, VA). Minimum and maximum release were determined in 10% HAB and 1% NP-40 detergent, respectively. Specific lysis was determined as previously described (10). ADCC assays were performed against P815 cells preincubated with either medium alone or a 1:100 dilution of polyclonal rabbit anti-mouse lymphocyte serum as previously described (11).

NK Cell Cytokine Production. 10^5 sorted $CD56^{dim}$ NK cells were plated in the presence or absence of cytokines in 96-well U-bottom plates in a total volume of 200 μl of per well. Medium consisted of RPMI-1640 plus 10% HAB. Cells were then incubated at 37°C for 72 h, after which supernatants were harvested and assayed by ELISA for production of human TNF-α, GM-CSF (Quantikine; R & D Systems, Minneapolis, MN), and IFN-γ (GIBCO BRL). Results represent the mean of duplicate wells ± SE.

Scatchard Analysis of IL-15 Binding to Resting NK Cells. Recombinant simian IL-15 expressed in yeast and purified as described by Grabstein et al. (5) was radiolabeled using immobilized lactoperoxidase (Enzymeobead reagent, Bio-Rad Laboratories) to sp act, 1.8×10^{15} cpm/mmol. Initial protein concentration of IL-15 was determined by amino acid analysis and the bioactivity of the radiolabeled protein was measured in an IL-15–dependent CTLL-2 proliferation assay as described in further detail by Giri et al. (6).

Binding experiments were carried out at 4°C for 1 h, with conditions empirically determined to result in saturable binding and reduced rate of internalization of ^{125}I-IL-15 bound to cell surface receptors. Binding experiments were performed using the phthalate oil separation method and data analysis as described by Dower et al. (12). In each experiment, between 5×10^7 and 1×10^8 resting human NK cells were used. Resting human NK cells were purified by depleting nonadherent PBMC of T cells and B cells with immunomagnetic purging as described above. Cells were between 75 and 90% $CD3^-CD56^+$ by FACS® analysis (Becton Dickinson & Co.). In some experiments, depleted cells were again labeled with anti-CD3 mAb and subsequently passed over a magnetic-activated cell sorter (MACS) column (Milentyi Biotec, Sunnyvale, CA), which brought the purity of $CD3^-CD56^+$ cells up to 90% by FACS® analysis. Freshly depleted resting NK cells were sent by overnight courier from Buffalo, NY to Seattle, WA on wet ice. Upon arrival, IL-2 and IL-15 binding studies were carried out as described above. To confirm that overnight shipment had not led to artifactual findings, reagents for isolation of NK cells were sent to Seattle on one occasion and binding studies were performed on freshly isolated NK cells with identical results.

Results

Effects of IL-15 on Human NK Cell Proliferation. Initial studies with IL-15 had shown that it could stimulate lym-

[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; HAB, human AB serum.

phocytes via components of the IL-2R (5, 6). We therefore proceeded to compare the proliferative effects of IL-15 and IL-2 on highly purified NK cells that constitutively express the IL-2R. CD56^{bright} NK cells constitutively express a high affinity IL-2R and exhibit a proliferative response to concentrations of IL-2 that partially or fully saturate this receptor (2, 3). As shown in Fig. 1 *A*, significant proliferation was achieved with IL-2 concentrations that partially saturate the

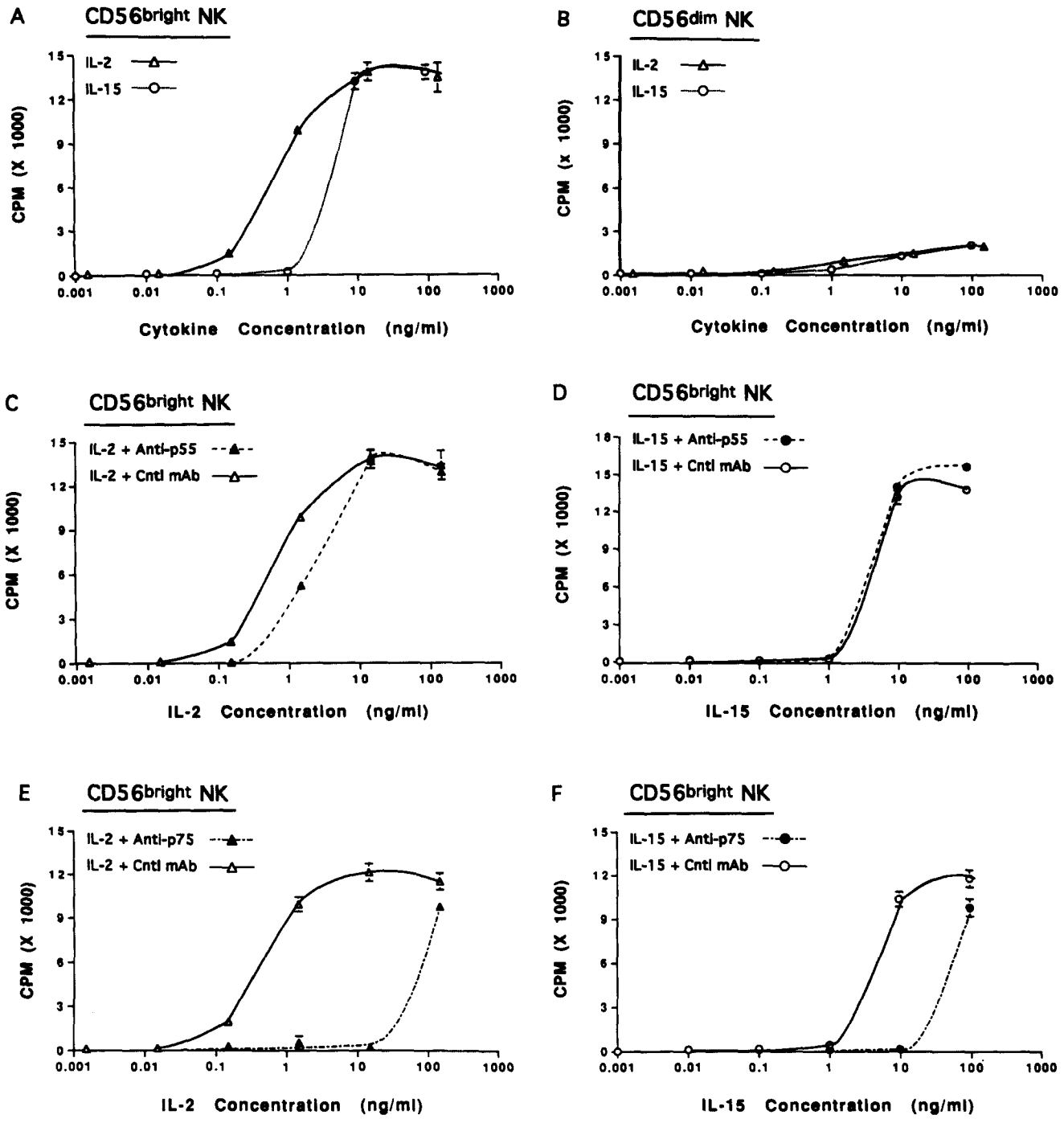

Figure 1. Proliferation of NK cell subsets in the presence of rIL-2 or rIL-15. (*A*) CD56^{bright} NK cells were sorted from fresh PBL and cultured in medium plus varying concentrations of IL-2 or IL-15 for 84 h and then assayed for methyl-[^{3}H]thymidine incorporation during the final 12 h of incubation. Results represent the mean ± SE of triplicate wells of a representative experiment. (*B*) Identical assay on sorted CD56^{dim} NK cells. (*C*) Proliferation of CD56^{bright} NK cells in the presence of varying concentrations of IL-2 plus anti-IL-2Rα (*anti-p55* mAb) or isotype control mAb. (*D*) Proliferation of CD56^{bright} NK cells in the presence of varying concentrations of IL-15 plus anti-IL-2Rα or isotype control mAb. (*E*) Proliferation of CD56^{bright} NK cells in the presence of varying concentrations of IL-2 plus anti-IL-2Rβ (*anti-p75* mAb) or isotype control mAb. (*F*) Proliferation of CD56^{bright} NK cells in the presence of varying concentrations of IL-15 plus anti-IL-2Rβ or isotype control mAb.

high affinity IL-2R (i.e., 0.10 and 1.0 ng/ml). 10 ng/ml of IL-2 afforded full saturation of the high affinity IL-2R and partial saturation of the intermediate affinity IL-2R, and resulted in a maximal proliferative response. Further increases in the concentration of IL-2 to 100 ng/ml fully saturated the intermediate affinity IL-2R expressed on CD56^{bright} NK cells, but did not increase proliferation (2).

In contrast to IL-2, IL-15 did not induce proliferation of CD56^{bright} NK cells at concentrations ranging from 0.01 to 1.0 ng/ml in over 20 separate experiments. However, at 10 ng/ml, IL-15 produced a maximal proliferative response in CD56^{bright} NK cells. Thus IL-15 had a much steeper dose–response curve than IL-2 (Fig. 1 *A*). Like IL-2, additional increases in the concentration of IL-15 did not further enhance the proliferative response of CD56^{bright} NK cells. In data not shown, the median effective concentration (EC_{50}) for IL-15 in these CD56^{bright} NK proliferation experiments ranged between 3.0 and 7.0 ng/ml (i.e., ~0.2 and 0.5 nM), whereas the EC_{50} in identical experiments with IL-2 ranged between 0.45 and 0.7 ng/ml (i.e., ~30 and 50 pM).

The vast majority of CD56^{dim} NK cells constitutively express the intermediate affinity IL-2R (4), and exhibit minimal proliferation in the presence of saturating concentrations of IL-2 (2). As shown in Fig. 1 *B*, incubation of CD56^{dim} NK cells in increasing concentrations of either IL-2 or IL-15 produced very little proliferation relative to the CD56^{bright} NK cells (Fig. 1 *A*).

We next examined the effects of anti-IL-2Rα and anti-IL-2Rβ mAbs on IL-2– and IL-15–induced proliferation in CD56^{bright} NK cells. Preincubation of CD56^{bright} NK cells with anti-IL-2Rα resulted in significant inhibition of IL-2–induced proliferation at concentrations of IL-2 that selectively saturate the high affinity IL-2 receptor (Fig. 1 *C*) (2, 3). In contrast, anti-IL-2Rα had no effect on IL-15–induced proliferation of CD56^{bright} NK cells (Fig. 1 *D*). Anti-IL-2Rβ mAb inhibited IL-2–induced proliferation of CD56^{bright} NK cells at low concentrations of IL-2 (0.1–10 ng/ml), but could be overcome at higher concentrations of IL-2 (Fig. 1 *E*). Anti-IL-2Rβ mAb could also inhibit IL-15–induced proliferation of CD56^{bright} NK cells at 10 ng/ml, but this could be overcome at 10-fold higher concentrations of IL-15 (Fig. 1 *F*). Thus, whereas IL-2 interacts with both the IL-2Rα and IL-2Rβ components of the IL-2R to induce a proliferative response in CD56^{bright} NK cells, IL-15 appears to bind to IL-2Rβ but not IL-2Rα in producing a similar functional response, as shown in other systems (5, 6).

As shown in Fig. 1 *A*, an increase in IL-15 concentration from 1 to 10 ng/ml results in CD56^{bright} NK cells going from no proliferative response to a maximum proliferative response, respectively. At concentrations of IL-15 between 1 and 10 ng/ml (i.e., ~2 ng/ml), a submaximal proliferative response is noted (Fig. 2). It is important to note that the same proliferative response is produced with IL-2 at approximately 10-fold lower concentrations (i.e., ~0.15 ng/ml of IL-2). Submaximal concentrations of IL-15 can combine with low concentrations of IL-2 to produce an additive proliferative response to CD56^{bright} NK cells, but only before full

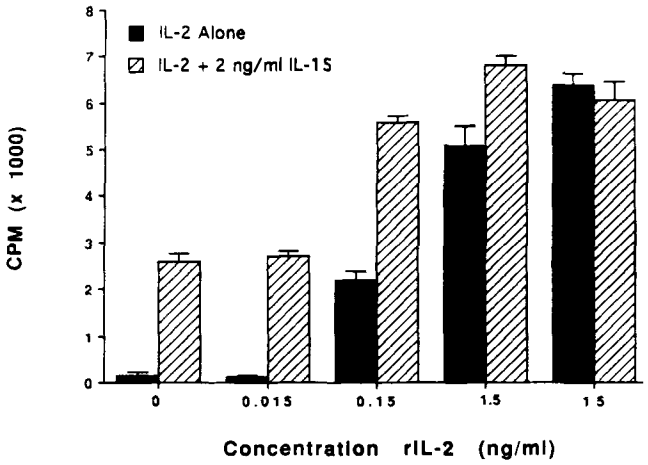

Figure 2. A suboptimal concentration of IL-15 potentiates proliferation of CD56^{bright} NK cells cultured in suboptimal concentrations of IL-2. Sorted CD56^{bright} NK cells were cultured in medium plus increasing concentrations IL-2 in the presence or absence of 2 ng/ml of IL-15 for 84 h and then assayed for methyl-[^{3}H]thymidine incorporation. Once IL-2 fully saturates the high affinity IL-2R (15 ng/ml), the potentiating effect of IL-15 is lost. Results represent the mean ± SE of triplicate wells.

saturation of the high affinity IL-2R with IL-2, which occurs at 15 ng/ml of IL-2. At that point, the two cytokines are no longer additive (Fig. 2). Furthermore, when IL-15 induced a maximal proliferative response of the CD56^{bright} NK cells at 10 ng/ml, combinations with lower concentrations of IL-2 which only saturate the high affinity IL-2R, did not further augment proliferation (data not shown).

Activation of NK Cytotoxic Activity with IL-15. NK cells display LAK activity against NK-resistant target cells after incubation in concentrations of IL-2 that saturate the intermediate affinity IL-2 receptor (2). This activity can be abrogated in the presence of anti-IL-2Rβ mAb (13). Incubation of CD56^{dim} NK cells in the presence of IL-15 resulted in LAK activity against the NK-resistant cell line COLO 205 in a dose-dependent fashion (Fig. 3 *A*). The IL-2 dose–response curve was identical to that of IL-15 (data not shown). Like IL-2, IL-15–induced LAK activity was completely abrogated in the presence of anti-IL-2Rβ mAb (Fig. 3 *B*). Thus IL-15 binds to IL-2Rβ to enhance cytotoxic activity of human NK cells. A similar dose–response curve was obtained when NK cells were incubated with increasing concentrations of IL-15 and assayed for ADCC against the P815 murine mastocytoma cell line in the presence of a rabbit anti–mouse antiserum (Fig. 3 *C*).

IL-12 is known to induce LAK activity in human NK cells, and coincubation of NK cells with both IL-12 and IL-2 can have an additive effect on NK cytotoxic activity (14, 15). As can be seen in Fig. 3 *D*, combinations of IL-12 and IL-15 resulted in additive LAK activity that was nearly identical to that seen with IL-12 and IL-2. However, there was no synergistic or even additive effect on LAK activity when NK cells were incubated with equivalent amounts of IL-2 and

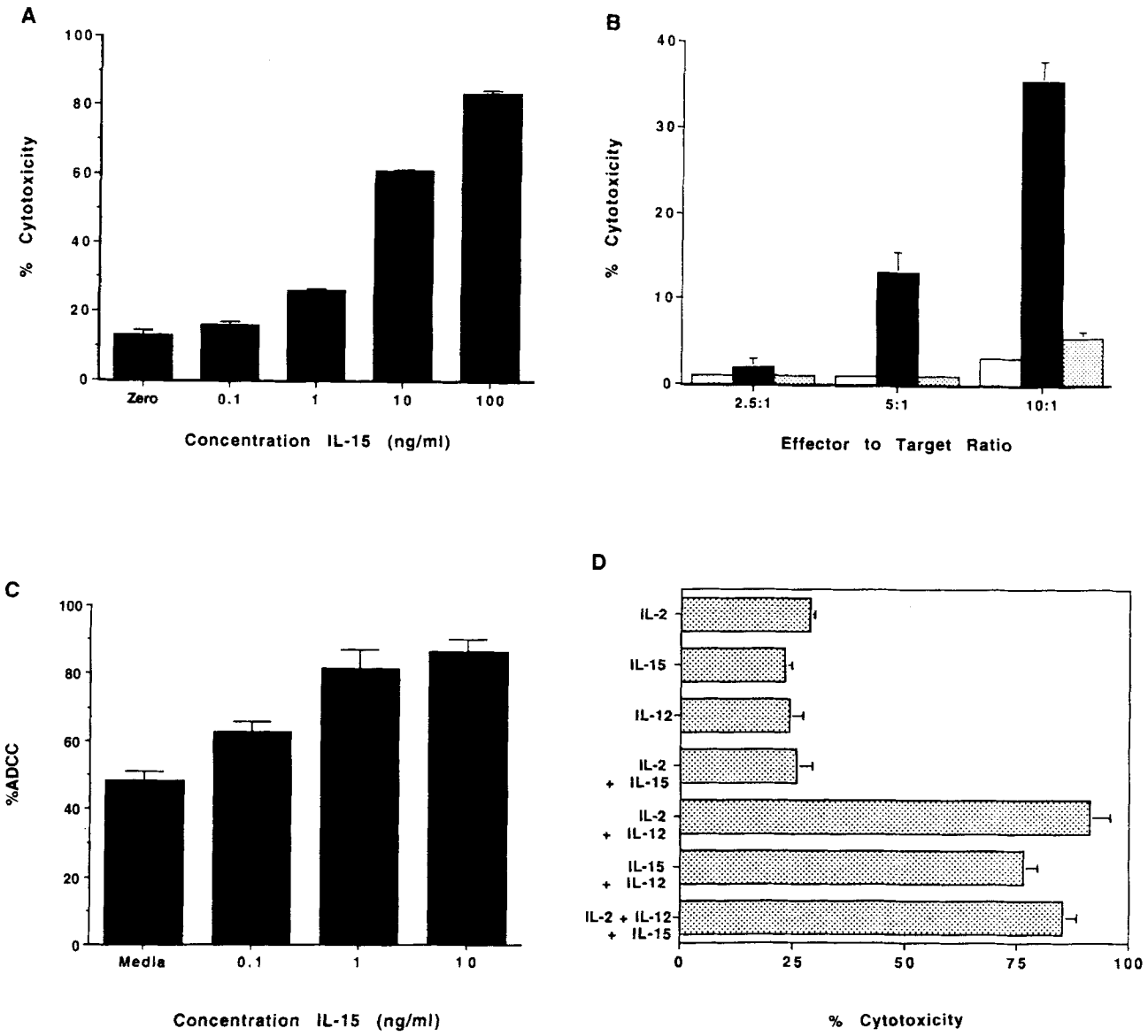

Figure 3. IL-15 induces LAK activity in CD56^{dim} NK cells. (*A*) Purified CD56^{dim} NK cells were incubated at increasing concentrations of IL-15 for 18 h and then tested against COLO 205 target cells in a standard 4-hour Cr-release assay. E/T ratio was 5:1. Results represent the mean ± SE of triplicate wells. (*B*) IL-15–induced LAK activity of CD56^{dim} NK cells is abrogated by an anti-IL-2Rβ mAb. CD56^{dim} NK cells were cultured in medium alone (□), medium supplemented with isotype control mAb and 1 ng/ml IL-15 (■), or medium supplemented with anti-IL-2Rβ mAb and 1 ng/ml IL-15 (▨). E:T ratios of 2.5:1, 5:1, and 10:1 were used against COLO 205 target cells. (*C*) IL-15 potentiates NK cell ADCC. Purified CD56^{dim} NK cells were incubated at increasing concentrations of IL-15 and then assayed for ADCC against the P815 cell line in the presence or absence of goat anti-mouse lymphocyte polysera. Specific ADCC was calculated by subtracting the percent lysis of noncoated targets from that of targets coated with polysera. Results represent the mean ± SE of triplicate wells. (*D*) IL-15 or IL-2 synergize with IL-12 to enhance the cytotoxic activity of CD56^{dim} NK cells. CD56^{dim} NK cells were incubated in 1 ng/ml IL-2, 1 ng/ml IL-15, 10 U/ml IL-12, alone or in combination, for 24 h and then tested for cytotoxicity against COLO 205 target cells at an E/T ratio of 5:1. Results represent the mean ± SE of triplicate wells.

IL-15. Consistent with this, incubation with IL-2, IL-15, and IL-12 did not enhance NK cytotoxic activity any more than the combination of IL-2 with IL-12 or IL-15 with IL-12.

NK Cell Cytokine Production in Response to IL-15. A number of studies have shown that activation of human NK cells with IL-2, IL-12, or TNF-α can result in cytokine production by human NK cells (16–18). We assayed for the production of IFN-γ, TNF-α, and GM-CSF, by NK cells after activation by IL-15. Highly purified populations of NK cells were sorted from fresh blood and cultured for 72 h with IL-2 and IL-15 alone or in combination with TNF-α or IL-12. As shown in Fig. 4 *A*, IFN-γ production by NK cells was negligible in the presence of TNF-α, IL-12, IL-15, or IL-2. The combination of IL-2 plus IL-15 did not result in significant

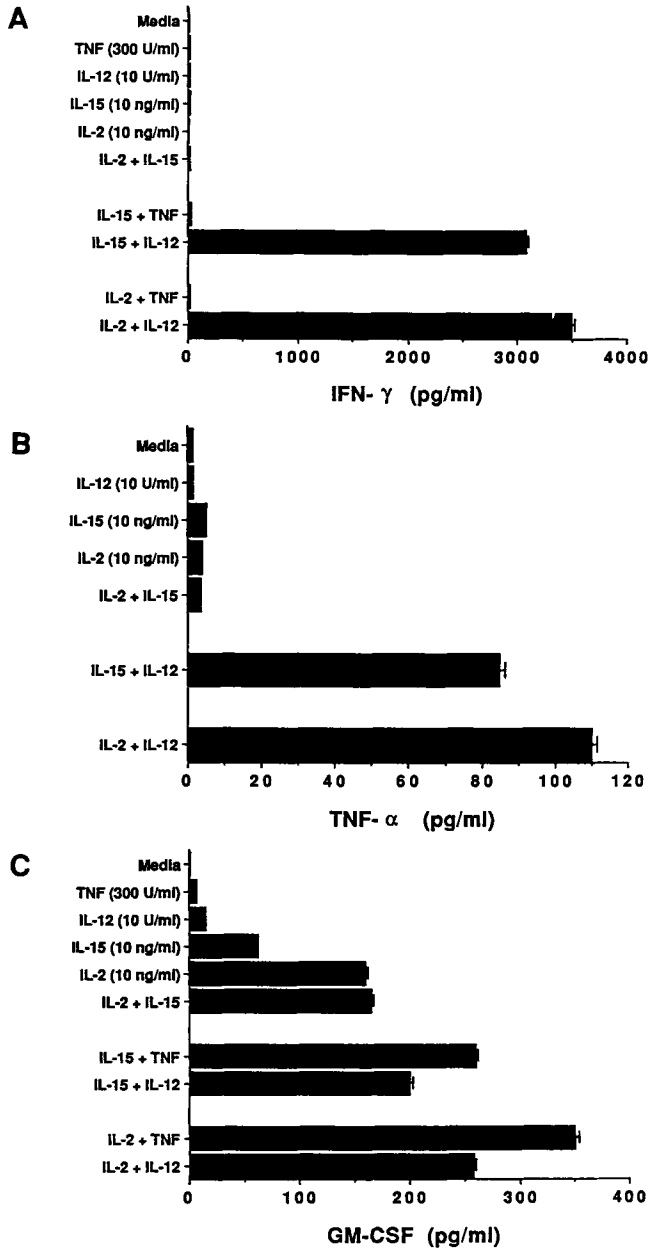

Figure 4. IL-15 and IL-2 stimulate CD56^{dim} NK cell cytokine production in a similar fashion. 10^5 sorted CD56^{dim} NK cells (>97% pure) were plated in U-bottom wells in 200 μl of medium supplemented with one or more of the following cytokines: TNF-α (300 U/ml), IL-12 (10 U/ml), IL-2 (10 ng/ml), or IL-15 (10 ng/ml). Cells were incubated for 72 h at which time the culture supernatants were harvested and assayed for the presence of either IFN-γ (*A*), TNF-α (*B*), or GM-CSF (*C*), using an ELISA. Cytokine production is measured in pg per ml.

IFN-γ production. When IL-12 was combined with either IL-2 or IL-15, significant and nearly equivalent amounts of INF-γ were produced. The same was true of TNF-α production by NK cells (Fig. 4 *B*). However, in repeated experiments, the production of GM-CSF by IL-2–stimulated NK cells was nearly twice that of IL-15–stimulated NK cells. It is important to note that there was no additive production

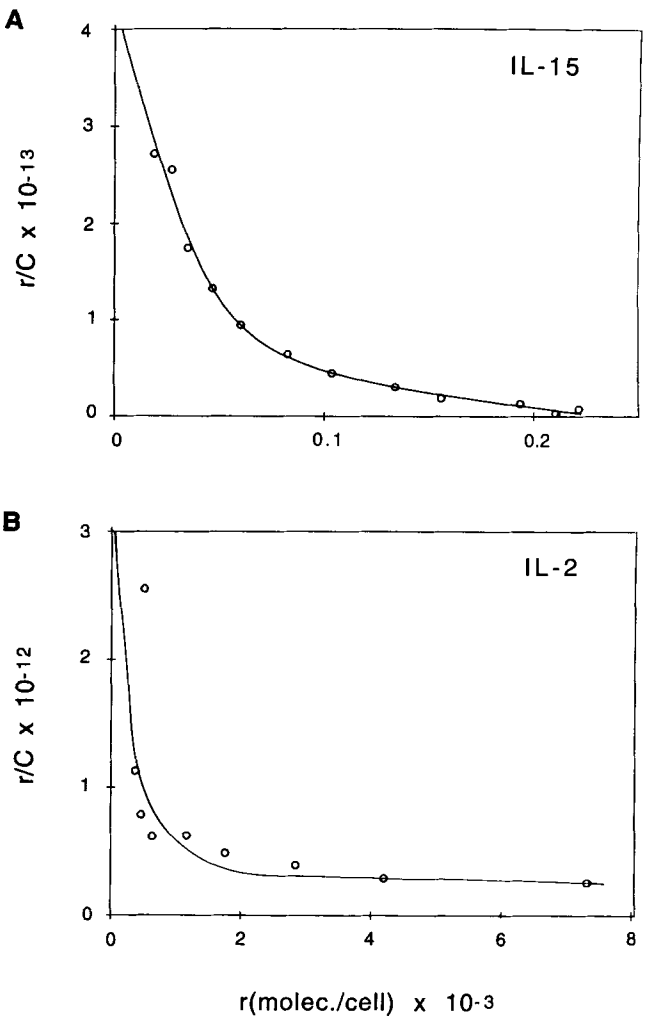

Figure 5. IL-15 binds to receptors on human NK cells. Scatchard analysis of ^{125}I-IL-15 (*A*) and ^{125}I-IL-2 (*B*) binding to NK-enriched resting PBL. Equilibrium binding conditions were as described by Giri et al. (6).

of GM-CSF when NK cells were incubated with both IL-2 and IL-15 (Fig. 4 *C*), again emphasizing the redundant nature of these two ligands. Both IL-15 and IL-2 synergized with TNF-α or IL-12 in inducing NK production of GM-CSF, but there appeared to be slightly greater production of GM-CSF when IL-2 was used in these combinations, as compared to IL-15.

Binding of Radiolabeled IL-2 and IL-15 to Human NK Cells. The results from the proliferation, cytotoxicity, and cytokine production assays strongly suggested that IL-2 and IL-15 were sharing components of the IL-2R on NK cells for binding and signal transduction. To investigate this further, radiolabeled IL-2 and IL-15 were used to perform binding inhibition assays on human CD56^{dim} NK cells that express the intermediate affinity IL-2Rβγ. In repeated experiments, incubation of highly purified (>97%) CD56^{dim} NK cells with ^{125}I-IL-2 and a 100-fold excess of unlabeled IL-15 resulted in 85–95% inhibition of ^{125}I-IL-2 binding compared to control (100-fold excess of unlabeled IL-2). Likewise, in-

cubation with ^{125}I-IL-15 and 100-fold excess of unlabeled IL-2 resulted in 85–95% inhibition of ^{125}I-IL-15 binding compared to control. Incubation with anti-IL-2Rβ resulted in a similar (>90%) inhibition of binding of both radiolabeled IL-2 and IL-15 (data not shown). Together, these results support the notion that IL-2 and IL-15 share common binding sites on CD56^{dim} NK cells. Sufficient numbers of cells could not be obtained to perform this assay on CD56^{bright} NK cells.

To better quantitate the affinity of the NK cell for both IL-15 and IL-2, we performed a series of Scatchard analyses of ^{125}I-IL-15 binding to NK-enriched (75–90% by FACS® analysis) resting PBL, and of ^{125}I-IL-2 binding to the same population. The analysis of one experiment for IL-15 is shown in Fig. 5 *A* and indicates that IL-15 binding sites include a low number of high affinity receptors (K_{D1} = 11.7 pM, 45 R_1 sites), and approximately 189 lower affinity R_2 sites (K_{D2} = 500 pM). Analysis of IL-2 binding in the same experiment showed the presence of high afffinity IL-2R (K_{D1} = 55 pM, 100 R_1 sites) and a larger number of intermediate affinity IL-2R (K_{D2} = 1.8 nM, 1110 R_2 sites, Fig. 5 *B*). Consistent with this, FACS® analysis showed the majority of NK cells expressing IL-2Rβ and ~10% of the NK cells expressing IL-2Rα, a component of the high affinity but not the intermediate affinity IL-2R (19). Approximately 10% of the cells were CD56^{bright}. In another experiment in which more than 90% of the cells were CD3$^-$CD56$^+$ by FACS® analysis, a similar binding pattern for iodinated IL-15 was detected (data not shown).

Discussion

The recent discovery and characterization of IL-15 has resulted in the identification of a novel ligand that uses components of the IL-2R (5, 6). Using cell lines transfected with the human IL-2Rβ and IL-2Rγ protein subunits, Giri et al. (6) have demonstrated that both subunits are required for IL-15 binding and signal transduction. The α subunit of the IL-2R is not involved in IL-15 binding (5, 6). The vast majority of human NK cells found in unstimulated peripheral blood constitutively express functional forms of the IL-2R, yet neither resting or activated NK cells produce IL-2 (1). As IL-15 appears to be abundantly and constitutively expressed in a variety of tissues, including activated monocytes/macrophages (5), this cytokine may have an important role in the regulation of NK cell function. We have therefore examined the effects of IL-15 on functionally distinct subsets of human NK cells.

The vast majority of CD56^{dim} NK cells express only the intermediate affinity heterodimeric IL-2R$\beta\gamma$, and display a weak proliferative but strong cytotoxic response to IL-2 (2, 4). Both the proliferative and cytotoxic responses of CD56^{dim} NK cells to IL-15 were indistinguishable from those of IL-2 at equivalent concentrations. Likewise, IL-15–induced NK cell cytokine production was strikingly similar to that seen with IL-2, with the possible exception of GM-CSF production. Functional responses of the CD56^{dim} NK cells to IL-15 could be abrogated in the presence of antibodies directed against IL-2Rβ, indicating that IL-15 was binding to this component of the intermediate affinity IL-2R. Studies performed with radiolabeled IL-2 and IL-15 and increasing concentrations of unlabeled IL-2 or IL-15 suggested that these two cytokines share common binding sites on CD56^{dim} NK cells (Carson, W.E., and M.A. Caligiuri, unpublished observations). Work by Giri et al. (6) suggests that IL-15, like IL-2 (20), also uses the γ subunit of IL-2R for binding and signaling. Studies to definitively address this in human NK cells must await the development of a mAb directed against the IL-2Rγ subunit.

It is important to note that whereas combinations of IL-15 or IL-2 could synergize equally well with IL-12 on CD56^{dim} NK cells, illustrating the NK cell's potential to markedly increase cytotoxicity and cytokine production, the combination of IL-15 with IL-2 never produced an additive or synergistic response, but exhibited the same stimulation as either cytokine acting alone. This would imply that for these functions on human CD56^{dim} NK cells, IL-15 and IL-2 are mutually redundant (21). However, the relative abundance of these two cytokines may lend some insight into which combination with IL-12 is likely to be more relevant in vivo. T cells transiently produce IL-2 after antigen activation, and require this cytokine in order to mount a successful immune response (22). As no other effector cell of the immune system is known to produce IL-2, its availability for NK cell activation via the intermediate affinity IL-2R is likely to be quite limited. In contrast, IL-12 production by activated monocytes/macrophages has been shown to be a critical component of host defense against invading pathogens via its stimulation of NK cell IFN-γ production (for a review see reference 23). As IL-15 is also produced by activated monocytes/macrophages (5), and can synergize with IL-12 in stimulating NK cell IFN-γ production, IL-15 may be the more important ligand for the intermediate affinity IL-2R expressed on human NK cells. The definitive experiments to test this hypothesis await the in vivo administration of neutralizing antibodies directed against IL-15 in animal models. Likewise, it remains to be determined if NK cells themselves can produce IL-15.

The CD56^{bright} NK cells constitutively express both a high affinity and intermediate affinity IL-2R, and in contrast to CD56^{dim} NK cells, the proliferative responses to IL-2 and IL-15 were distinct. At concentrations of 0.10 and 1.0 ng/ml of IL-2, a progressive increase in DNA synthesis was noted among the CD56^{bright} NK cells, whereas IL-15 showed no evidence of activity at identical concentrations. Previous work (2, 24) has shown that these concentrations of IL-2 result in partial to complete saturation of the heterotrimeric high affinity IL-2R$\alpha\beta\gamma$, supported in the present study by the partial inhibition seen in the presence of anti-IL-2Rα mAb. A 10-fold higher concentration of IL-2 (10 ng/ml) partially saturates the intermediate affinity IL-2R but also provides continual complete saturation of the high affinity IL-2R for the entire period of culture. The latter effect is likely to be responsible for the maximum proliferation noted at this point, as further saturation of the intermediate affinity IL-2R with

even higher concentrations of IL-2 does not enhance proliferation (2).

In distinct contrast to IL-2, the first CD56^{bright} NK proliferative response to IL-15 did not occur until log increases in concentration reached 10 ng/ml. Anti-IL-2Rα mAb did not inhibit this response, whereas anti-IL-2Rβ did. The explanation for the 10-fold difference between the IL-2 and IL-15 dose–response curves for CD56^{bright} NK cell proliferation is not clear at this time, but similar differences have been noted for PHA-activated human T cells (5). Scatchard analysis of radiolabeled IL-2 and IL-15 binding to unstimulated human NK cells showed that both cytokines identified high and intermediate affinity receptor sites. The high affinity IL-2R sites were few in number and are likely to represent those expressed by the CD56^{bright} NK subset (1% of PBL, 10% of NK cells), which is the only cell known to constitutively express high affinity IL-2R in unstimulated PBL (2, 3). The functional significance of NK cell receptors that bind IL-15 with apparent high affinity (K_{D1} of 11.7 pM) and lower affinity (K_{D2} of 500 pM) as shown in Fig. 5 cannot be determined at this time, as radiolabeled ligand binding assays were performed at 4°C, whereas biologic responses were assayed at 37°C. It is possible that IL-15 uses other receptor components not required for IL-2. Indeed, Grabstein et al. (5) and Giri et al. (6) have identified murine cell lines that proliferate vigorously in response to IL-2, but poorly or not at all to IL-15.

In summary, we have characterized the functional responses of human NK cell subsets to IL-15. IL-15 appears to use components of the IL-2R to induce proliferation, cytotoxic activity, and cytokine production in this subset of lymphocytes. Whereas IL-15 was found to share many biologic properties of IL-2 in this regard, certain properties of IL-15 were distinct from IL-2 in CD56^{bright} NK cells. In animal models, monocyte/macrophage–derived cytokines have been shown to be important in defense against invading pathogens via their regulation of NK cell IFN-γ production (20). Whereas we and others (25) have shown that IL-2 can potentiate these functions in vitro, the limited production of IL-2 by activated T cell subsets might suggest that this mechanism is not operative in vivo. In distinct contrast to IL-2, IL-15 appears to be abundantly expressed in a variety of tissues and cell types, including activated monocytes/macrophages (5). The constitutive expression of functional IL-2 receptors on human NK cells, and our in vitro demonstration that IL-15 can potentiate NK cell cytokine production and cytotoxic activity in combination with other monocyte/macrophage–derived cytokines suggests that this synergy may also be important in vivo. Additional studies with IL-15 should provide further insight into the role of this molecule in normal host immunity.

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Address correspondence to Dr. M. A. Caligiuri, Roswell Park Cancer Institute, Buffalo, NY 14263.

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