The Functional Characterization of Interleukin-10 Receptor Expression on Human Natural Killer Cells

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Human natural killer (NK) cells are large granular lymphocytes that constitutively express functional forms of the interleukin-2 receptor (IL-2R) and lyse tumor and virally infected cells without prior sensitization. NK cells with high density expression of CD56 (CD56bright) express the high affinity IL-2R and proliferate in response to low (picomolar) concentrations of IL-2. CD56dim NK cells express the intermediate affinity IL-2R and demonstrate enhanced cytotoxic activity without proliferation in response to high (nanomolar) concentrations of IL-2. In the present study, we characterized IL-10R expression on human NK cells and the functional consequences of IL-10 binding directly to highly purified subsets of CD56bright and CD56dim NK cells. Binding studies using 125I-IL-10 indicated that resting human NK cells constitutively express the IL-10 receptor protein at a surface density of approximately 90 receptor sites per cell, with a kD of ~1 nmol/L. Alone, IL-10 did not induce proliferation of CD56bright or CD56dim NK cell subsets. However, at low concentrations (0.5 to 5 ng/mL), IL-10 significantly augmented IL-2-induced proliferation of the CD56bright NK cell subset mediated via the high-affinity IL-2R. In the absence of IL-2, IL-10 was able to induce significant NK cytotoxic activity against NK-resistant tumor cell targets in both subsets of NK cells in a dose-dependent fashion. Furthermore, the combination of IL-10 and IL-2 had an additive effect on NK cytotoxic activity, whereas that of IL-10 and IL-12 did not. Production of interferon-γ, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor by IL-2–activated NK cells was also significantly enhanced by IL-10. Neither resting nor activated human NK cells appear to produce human IL-10 protein. In summary, NK cells constitutively express the IL-10R protein in low density, and the functional consequences of IL-10 binding directly to human NK cell subsets appear to be stimulatory and dose-dependent. In contrast to its direct effects on human T cells and monocytes/macrophages, IL-10 potentiates cytokine production by human NK cells.

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Materials and Methods

Cytokines. All cytokines used in this study were purified recombinant proteins of human origin. IL-10 was provided by Schering-Plough Research Institute (Kenilworth, NJ; specific activity, 11 × 10^6 IU/mg). IL-2 was obtained from Hofmann-LaRoche (Nutley, NJ; specific activity, 1.53 × 10^7 IU/mg). Tumor necrosis factor-α (TNF-α; specific activity, 2 × 10^7 IU/mg) was obtained from the Asahi Chemical Corp (Fugi City, Japan). IL-12 (specific activity, 4.5 × 10^6 IU/mg) was obtained from Genetics Institute (Cambridge, MA). All cytokines were reconstituted in RPMI-1640 supplemented media.


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with 0.1% human albumin (Armour Pharmaceutical Co, Kankakee, IL).

**MoAbs.** Anti-CD3 and CD4 sterile mouse ascites were a generous gift of Dr Stuart Schlossman (Dana Farber Cancer Institute, Boston, MA). 3F8B11 is an anti–HLA-DR murine MoAb that was used as an isotype control.59 Phycoerythrin (PE)-conjugated anti-CD56 MoAb (NK11-RDI) was purchased from Coulter Immunology (Hialeah, FL). Fluorescein isothiocyanate (FITC)-labeled MoAbs to CD2, CD11a (LFA-1), CD25 (IL-2Rα), CD54 (ICAM-1), and CD58 (LFA-3) were purchased from Becton Dickinson (San Jose, CA). CD122 (IL-2Rβ) was purchased from Endogen (Boston, MA). Anti-Tac MoAb (anti-CD25 or anti-IL-2Rα) sterile mouse ascites was a generous gift of Dr Kendall Smith (Cornell Medical School, New York, NY). Antibody was purified from ascites using the Affigel-gel protein A MoAb purification system (Bio-Rad Laboratories, Richmond, CA) in accordance with the manufacturer’s instructions.

**Cell lines.** The NK-resistant human colon adenocarcinoma cell line COLO 205 (ATCC, Rockville, MD) was used as a target for cytotoxic assays. Ba.I is the Ba/F3 murine cell line that has been transfected with the human IL-10R CDNA pSW8.1 construct and expresses IL-10R (pSW8-1 clone, kindly provided by Dr Kevin Moore, DNAX Research, Palo Alto, CA) with reference to the L7 hybridization signal as a standard. For the purposes of comparison, background was subtracted from the IL-10R and 7.7 hybridization signals were made on an Apple OneScanner (Apple Computer, Cupertino, CA) with reference to the L7 hybridization signal as a standard. For the purposes of comparison, background was subtracted from the IL-10R and L7 signals and the ratio of their intensities was calculated using Scan Analysis software (Biosoft, Ferguson, MO).

**Northern analysis for human IL-10R transcript.** RNA was isolated from highly purified (>94%) resting and IL-2-activated NK cells and control cell lines using RNAzol (Tel-Test Inc, Friendswood, TX), as per the manufacturer’s recommendations.18 IL-2 activation of NK cells was achieved by 72 hours of culture in medium containing 10% HAB and antibiotics and prehybridized following the manufacturer’s specifica-

**Proliferation assays.** Sorted CD56bright or CD56dim NK cells (2 × 10⁶) were resuspended in RPMI-1640 with 10% HAB and antibiotics and plated in the presence or absence of cytokines in 96-well U-bottom plates in a total volume of 200 µL per well. Plates were centrifuged at 37°C for 3 hours, after which supernatants were harvested, aliquoted, and frozen at −70°C. At a later date, culture supernatants were thawed at 37°C in a water bath and assayed by enzyme-linked immunosorbent assay (ELISA) for production of human TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF; Quantikine; R & D Systems, Minneapolis, MN), IFN-γ (GIBCO BRL, Gaithersburg, MD), and IL-10 (Biosource International, Camarillo, CA).

**Northern analysis for human IL-10R transcript.** RNA was isolated from highly purified (>94%) resting and IL-2-activated NK cells and control cell lines using RNAzol (Tel-Test Inc, Friendswood, TX), as per the manufacturer’s recommendations. IL-2 activation of NK cells was achieved by 72 hours of culture in medium containing 10% HAB and antibiotics and prehybridized following the manufacturer’s specifications. Blots were then hybridized with a 32P dCTP (Pharmacia, Piscataway, NJ) random-labeled full-length cDNA probe for human IL-10R (pSW8.1 clone, kindly provided by Dr Kevin Moore, DNAX Research, Palo Alto, CA). After two washes, hybridized membranes were exposed to Kodak X-OMAT AR film (Kodak, Rochester, NY) random-labeled full-length cDNA probe for human IL-10R (pSW8.1 clone, kindly provided by Dr Kevin Moore, DNAX Research, Palo Alto, CA). After two washes, hybridized membranes were exposed to Kodak X-OMAT AR film (Kodak, Rochester, NY) at −70°C. Quantitative measurements of IL-10R hybridization signals were made on an Apple OneScanner (Apple Computer, Cupertino, CA) with reference to the L7 hybridization signal as a standard. For the purposes of comparison, background was subtracted from the IL-10R and L7 signals and the ratio of their intensities was calculated using Scan Analysis software (Biosoft, Ferguson, MO).
buffer containing 10% glycerol. Layered cell preparations were pelleted by centrifugation (400g) at 4°C and frozen in liquid nitrogen. Cell pellets were then severed and counted in a Clinigamma 1272 counter (Pharmacia). Nonspecific binding was determined by performing the identical binding assay in the presence of a 1,000-fold molar excess of unlabeled IL-10.21 In some experiments, half of the purified NK cells were first incubated in 1 nmoI/L IL-2 for 72 hours and then assayed for $^3_{25}$-IL-10 binding specificity.

In saturation binding experiments, twofold serial dilutions of an 8 nmoI solution of $^{125}$I-IL-10 were used. Nonspecific binding was determined in a parallel series of experiments using identical dilutions of $^{125}$I-IL-10 in the presence of a 1,000-molar excess of unla- beled rIL-10. The dissociation constant (kd) and maximal concentration of ligand bound to cells ($B_{max}$) were calculated by Scatchard analysis of the saturation binding data using linear regression analysis with the EBDA program (Elsevier-Biosoft, Cambridge, UK). The results of Scatchard analysis were replotted using the Cricket Graph program (Computer Associates International, San Diego, CA).

RESULTS

Northern blot analysis for the IL-10R mRNA. Liu et al.14 have recently shown that both T cells and NK cells constitutively express the IL-10R mRNA and that T-cell activation leads to a downregulation of this transcript. We proceeded to analyze resting NK cells and CD3+ CD56+ IL-2-activated NK cells for IL-10R transcript. As shown in Fig 1A, we confirm that purified (>94%) populations of resting NK cells express abundant IL-10R transcript. However, after 72 hours of culture in 15 nmoI/mL IL-2 (1 nmoI/L or ~230 U/mL), the same CD3+ CD56+ NK cells showed an approximate fourfold decrease in IL-10R transcript by densitometric analysis (Fig 1A through C).

Binding of radiolabeled IL-10 to human NK cells. To determine if human NK cells were in fact expressing a protein receptor specific for human IL-10, highly purified (>94%) NK cells were incubated with $^{125}$I-IL-10 in the presence or absence of a 1,000-fold molar excess of unlabeled human IL-10. Both resting and IL-2-activated NK cells were tested in this assay. As shown in Fig 2A, radiola beled IL-10 binds to resting NK cells with a significant degree of specificity. This level of specific IL-10 binding is comparable to that seen in IL-10-responsive cell types that we have previously tested.21 However, despite the reduction in IL-10R transcript found when human NK cells are cultured for 72 hours in 1 nmoI/L IL-2 (Fig 1), there does not appear to be a significant decrease in IL-10R surface protein expression in IL-2-activated NK cells as measured by the specific binding of radiolabeled ligand (Fig 2A).

To further characterize IL-10R protein expression on resting human NK cells, we sought to determine the binding affinity and estimate the number of binding sites or receptors per cell. A typical saturation binding curve with freshly isolated highly purified resting human NK cells is shown in Fig 2B. Maximal binding occurs at approximately 2 to 4 nmoI/L of radiolabeled IL-10. Scatchard analysis of representative binding data provided a linear graph with a slope that yields a kd of approximately 1 nmoI/L and a $B_{max}$ of 3.4 x 10^12 mol/L (Fig 2C). If one assumes that each IL-10R unit binds one dimer of human IL-10 ligand,21 then these results provide an estimate of approximately 90 receptors/cell for human NK cells.

The effect of IL-10 on human NK cell proliferation. In contrast to B cells,22 IL-10 alone did not support the survival of CD56[high] NK cells, as shown by vital dye exclusion (data not shown). We next tested the proliferation of CD56[high] NK cells in response to varying concentrations of IL-10 in the presence or absence of 1.5 ng/mL IL-2 (100 pmol/L). As seen in Fig 3, IL-10 alone did not induce proliferation of CD56[high] NK cells at any concentration. CD56[high] NK cells constitutively express a high-affinity IL-2R that mediates a brisk proliferative response in the presence of picomolar concentrations of IL-2.23 The proliferation of CD56[high] NK cells in response to 1.5 ng/mL (100 pmol/L) IL-2 was potentiated by the addition of IL-10 beginning at IL-10 concentrations of 0.05 nmol/L, with a peak 60% increase over baseline occurring at 0.5 ng/mL. At higher concentrations of IL-10, a progressive diminution in CD56[high] proliferation was seen. At 50 ng/mL of IL-10, IL-2-induced [H]-thymidine uptake was no longer augmented by IL-10. When cell enumeration in vital dye was performed in parallel experiments, changes in NK cell number did correlate with the increases in [H]-thymidine incorporation seen under the various experimental conditions described above. However, the absolute number of CD56[high] NK cells did not increase drastically, likely because of the fact that a significant fraction of this population is not responsive to IL-2 and consequently undergoes cell death.24

As shown in Fig 4A, 0.5 ng/mL of IL-10 potentiated the IL-2-induced proliferation of CD56[high] NK cells over a limited range of IL-2 concentrations. IL-10 caused a shift in the IL-2 dose-response curve at IL-2 concentrations that partially saturate the high-affinity IL-2R, ranging from 0.015 to 1.5 ng/mL (1 to 100 pmol/L). However, at an IL-2 concentra- tion of 15 ng/mL (1 nmol/L), the high-affinity IL-2R remains completely saturated for the 96-hour incubation period, and partial saturation of the intermediate affinity IL-2R also occurs.25 At this point of maximal IL-2-induced proliferation, IL-10 is no longer able to potentiate the proliferative response to IL-2.

The CD56[dim] NK cell population expresses only the intermediate-affinity IL-2R that provides a relatively weak proliferative signal when activated with nanomolar concentrations of IL-2.2 By itself, 0.5 ng/mL IL-10 did not induce CD56[dim] NK cell proliferation (data not shown). IL-2-induced prolif-eration mediated via the intermediate-affinity IL-2R was only minimally enhanced by IL-10 (0.5 ng/mL), and the overall response remained weak relative to CD56[high] NK cells (Fig 4B).

To determine if IL-10 was potentiating IL-2-induced proliferation mediated via the high-affinity IL-2R, we stimulated CD56[high] NK cells with both IL-2 (0.3 ng/mL or 20 pmol/ L) and IL-10 (0.5 ng/mL) in the presence or absence of anti-Tac (anti-CD25) MoAb. Anti-Tac MoAb abrogates the CD56[high] NK cell proliferative response mediated via the high-affinity IL-2R by blocking the binding of IL-2 to the IL-2Rα (p55) protein subunit of the heterotrimeric receptor complex.23 As can be seen in Fig 5, 0.3 ng/mL IL-2 in the presence of an isotype control MoAb stimulates a brisk proliferative response upon binding to the high-affinity IL-2R. The presence of 0.5 ng/mL IL-10 potentiates this re-
Response by approximately 60%. In contrast, anti-Tac MoAb abrogates the IL-2–induced proliferative response, which also eliminates the synergistic effect of IL-10. Thus, the potentiating effect of IL-10 in this NK cell population appears to require the binding of IL-2 to the high-affinity IL-2 receptor.

**Effect of IL-10 on NK cell cytotoxicity.** Hsu et al. have shown that the cytotoxic activity of IL-2–activated peripheral blood mononuclear cells (PBMC) is not inhibited by IL-10, but the direct effects of IL-10 on human NK cell cytotoxicity have not been investigated. Using highly purified, sorted populations of CD3⁻CD56⁺ NK cell subsets, we performed additional studies to determine the direct effect of human IL-10 on both NK and cytotoxic activity. As shown in Fig 6A, both CD56bright and CD56dim resting NK cells showed significant cytotoxic activity against NK-resistant tumor cell targets when incubated overnight in medium containing 50 ng/mL of IL-10 compared with incubation in medium alone. This effect of IL-10 alone seemed to be more pronounced in CD56bright NK cells. A dose-response curve measuring NK cytotoxic activity against NK-resistant tumor cell targets in a sorted population of CD56dim NK cells illustrates that the enhanced cytotoxic effect is most pronounced at high concentrations (5 to 50 ng/mL) of IL-10 (Fig 6B). Culture of CD56dim NK cells in 50 ng/mL IL-10 resulted in 22% killing against COLO 205 versus 4% when incubated in media alone (P < .05).

Greater enhancement of CD56dim NK cell cytotoxicity could be achieved by the incubation of effectors in medium plus 15 ng/mL (1 nmol/L) of IL-2, which resulted in 40% cytotoxicity against COLO 205. The combination of IL-2 and IL-10 had an additive effect on NK cytotoxic activity.
such that incubation of CD56\textsuperscript{dim} NK effectors in 15 ng/mL IL-2 plus 50 ng/mL IL-10 resulted in 75% lysis of COLO 205 (Fig 6C). A similar additive effect was not apparent with the CD56\textsuperscript{bright} NK cells, possibly because of the dramatic increase in cytotoxic activity seen with IL-10 alone (see above). In similar cytotoxicity experiments using CD56\textsuperscript{dim} NK effectors and IL-12 as an NK cell stimulatory cytokine, IL-10 had no effect on IL-12-induced NK cell cytotoxicity. Antibody-dependent cell cytotoxicity was not potentiated by IL-10 either alone or in combination with IL-2 or IL-12 (data not shown).

Cellular adhesion molecules (CAM) are critical to the formation of conjugates between NK effectors and their targets and are responsible at least in part for the development of IL-2-induced cytotoxic activity.\textsuperscript{18} Having determined that IL-10 alone could induce significant NK cell cytolytic activity and potentiate IL-2-induced cytotoxicity against

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**Fig 2.** Binding of radiolabeled IL-10 to human natural killer cells. (A) Resting CD56\textsuperscript{dim} human NK cells (\textgeq 94\% pure) were isolated as described in Materials and Methods. One half of these purified NK cells were incubated in 1 nmol/L IL-2 for 72 hours before performing binding experiments. The bars represent the binding of \( ^{125}\text{I}-\text{IL-10} \) to resting or IL-2-activated NK cells in either the presence or absence of 1,000-fold molar excess unlabeled human IL-10. These results represent the mean ± SE for triplicate samples using approximately 5 x 10\(^6\) cells per sample. (B) Saturation binding of radiolabeled IL-10 to resting NK cells. Purified human NK cells (5 x 10\(^6\)) were incubated with increasing concentrations of \( ^{125}\text{I}-\text{IL-10} \). Specific binding was determined by subtracting nonspecific binding from total binding. Results represent the mean of triplicate samples. (C) Scatchard analysis of equilibrium binding data for resting human NK cells. The dissociation constant (kd) and maximal concentration of ligand bound to cells (\( B_{max} \)) were calculated by Scatchard analysis of the saturation binding data using linear regression analysis.
Concentration IL-2 (ng/ml)

Fig 4. Effect of IL-10 on the IL-2-induced proliferation of NK cell subsets. (A) CD56\textsuperscript{bright} NK cells were sorted from fresh PBL and cultured in medium plus varying concentrations of IL-2 in either the absence (○) or presence (●) of 0.5 ng/mL IL-10 for 96 hours. Cultures were then assayed for methyl-[3H]thymidine incorporation during the final 12 hours of incubation. Results represent the mean ± SE of triplicate wells. (B) Identical assay on sorted CD56\textsuperscript{dim} NK cells.

NK-resistant targets, the effects of IL-10 on NK cell CAM expression were examined. Sorted CD56\textsuperscript{+} NK cells were cultured for up to 72 hours in media alone (negative control), 15 ng/mL IL-2 alone (positive control), 50 ng/mL IL-10, or 15 ng/mL IL-2 in combination with 50 ng/mL IL-10 and then analyzed for cell surface expression of CD2, CD11a, CD54, CD56, and CD58 via flow cytometry. In contrast to IL-2 alone, IL-10 had no significant effect on NK cell CAM expression, either alone or in combination with IL-2. Additional studies showed that incubation of human NK cells in the presence of 50 ng/mL IL-10 or in combination with IL-2 did not significantly alter the cell surface expression of IL-2R\textalpha{} or IL-2R\beta{} (data not shown).

Effect of IL-10 on NK cell cytokine production. NK cells have been shown to produce GM-CSF, TNF-α, and IFN-γ after stimulation with monocyte-derived factors such as TNF-α and IL-12, both alone or in combination with IL-2.\textsuperscript{24-27} Indeed, the ability of IL-10 to suppress NK cell IFN-γ production occurs indirectly via suppression of monocyte/macrophage cytokine production.\textsuperscript{10,11} In the present study, we assessed the direct effects of IL-10 on NK cell cytokine production. Highly purified populations of CD56\textsuperscript{+} NK cells were sorted from fresh blood and cultured for 72 hours with IL-2 and/or IL-12, either alone or in combination with IL-10. At the end of the incubation period, NK cell culture supernatants were assayed for the production of IFN-γ, TNF-α, or GM-CSF. Two observations were made (Fig 7A through C). First, IL-10 alone did not have any stimulatory effect on NK cell cytokine production. Secondly, IL-10 consistently enhanced the NK cell production of IFN-γ, TNF-α, and GM-CSF in the presence of IL-2 but not of IL-12. The augmentation of cytokine production after 72 hours of incubation in IL-10 plus IL-2 appeared to result from an increase in the endogenous production of cytokine, because there was no relative or absolute increase in NK cell numbers cultured under these conditions when compared with cells cultured in IL-2 alone.

In similar experiments we tested sorted CD56\textsuperscript{dim} NK cells for their ability to produce IL-10. Using an ELISA with a sensitivity of 40 pg/mL, we were unable to detect human IL-10 production from resting NK cells, nor could this activity be induced after 72 hours of culture of NK cells in IL-2, IL-12, or TNF-α either alone or in combination (data not shown).

DISCUSSION

In the present study, we have characterized IL-10R expression and the functional consequences of IL-10 binding directly to human NK cells. Using highly purified populations of human NK cells, we have shown that human IL-10 can directly induce NK cytotoxic activity against tumor-resistant targets as well as augment IL-2–induced proliferation, cytotoxicity, and cytokine production. Antigen-presenting cells...
and other lymphocytes were eliminated from our cultures by a three-step purification process that included adherence to plastic, immunomagnetic bead depletion of nonadherent cells to eliminate residual monocytes, B cells, and T cells, and a second distinct negative depletion process or by positive selection for CD56+ NK cells by fluorescence-activated cell sorting.

Liu et al\textsuperscript{14} showed that resting purified human NK cells express abundant IL-10R transcript, with similar levels of expression detected in resting B cells, T cells, and monocytes. Interestingly, activation of human T-cell clones with PMA or anti-CD3 resulted in a downregulation of human IL-10R mRNA levels.\textsuperscript{13} Our work confirms the presence of human IL-10R message in resting human NK cells and further shows that activation of NK cells with IL-2 leads to a significant downregulation of this mRNA species.

In the absence of an anti-IL-10R MoAb, additional characterization of IL-10R expression at the surface protein level was performed using \textsuperscript{125}I-IL-10. Assessment of \textsuperscript{125}I-IL-10 binding in the presence and absence of excess unlabeled IL-10 showed a modest amount of specific binding on purified NK cell populations. IL-10 does not appear to be produced endogenously by human NK cells, so it is unlikely to be competing with \textsuperscript{125}I-IL-10 binding in these assays. We have previously shown that the IL-10-responsive JY human B-cell line binds radiolabeled IL-10 with specificity comparable to human NK cells in the present study.\textsuperscript{21} These data and additional competitive binding experiments performed with soluble human IL-10R (J. Tan, unpublished observations) suggested that relatively few IL-10 binding sites on NK cells are required for functional responsiveness to IL-10.

The binding experiments performed with serial dilutions of \textsuperscript{125}I-IL-10 on resting human NK cells in the presence and absence of excess unlabeled IL-10 confirmed the estimate of a low number (~90) of IL-10R sites/cell and also provided information about the \textit{k}_d (~1 nmol/L) of this receptor. Furthermore, the repeat binding studies performed on human NK cells that had been activated in the presence of IL-2 for 72 hours suggested that, unlike the IL-10R transcript, the number of surface IL-10R protein sites does not change significantly under conditions of IL-2 activation. This finding is consistent with our observation that both IL-10 and IL-2 appear to have similar and at times additive stimulatory effects on human NK cells.

In some respects, the functional consequences of human IL-10 binding to CD56\textsuperscript{bright} NK cells are similar to those that we recently reported for the ligand to the tyrosine kinase receptor c-kit.\textsuperscript{16} Alone, c-kit ligand (KL) had no effect on proliferation of CD56\textsuperscript{bright} NK cells. However, KL was able to significantly augment the proliferative effect of IL-2, causing a marked shift in the dose-response curve at IL-2 concentrations that selectively saturate the high-affinity IL-2R.\textsuperscript{16} Similarly, IL-10 by itself had no effect on CD56\textsuperscript{bright} NK cell proliferation but was able to potentiate IL-2-induced proliferation mediated by the binding of IL-2 to the high-

Fig 6. IL-10 enhances NK cytolytic activity in CD56\textsuperscript{bright} and CD56\textsuperscript{dim} NK cells and augments IL-2-induced cytolytic activity. (A) Purified CD56\textsuperscript{bright} or CD56\textsuperscript{dim} NK cells were incubated in medium alone (■) or medium plus 50 ng/mL IL-10 (●) for 18 hours and then tested against COLO 205 target cells in a standard 4-hour \textsuperscript{51}Cr-release assay. The E:T ratio was 10:1. Results represent the mean ± SE of triplicate wells. (B) Similar cytotoxicity assay performed using CD56\textsuperscript{bright} NK cells cultured in varying concentrations of IL-2. (C) Similar cytotoxicity assay performed using CD56\textsuperscript{dim} NK cells cultured in medium plus 15 ng/mL (~1 nmol/L) of IL-2 and varying concentrations of IL-10.
IL-10 augments IL-2-induced NK cell cytokine production. CD56\textsuperscript{dim} NK cells (1 x 10\textsuperscript{5}) were plated in U-bottom wells in 200 \muL of medium alone or medium supplemented with IL-12 (10 U/ml), IL-2 (15 ng/ml), or a combination of IL-12 plus IL-2. IL-10 (5 ng/ml) was added to parallel experiments performed under identical culture conditions. Cells were incubated for 72 hours, at which time the culture supernatants were harvested and assayed for the presence of either IFN-\gamma, TNF-\alpha, or GM-CSF by ELISA (Fig 6A, B, and C, respectively). Cytokine production is measured in picograms per milliliter. Results represent the mean of duplicate wells ± SE.

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affinity IL-2R. However, in contrast to KL, higher concentrations of IL-10 failed to augment CD56\textsuperscript{dim} NK cell proliferation, and IL-10 alone clearly augmented CD56\textsuperscript{dim} NK cell cytotoxic activity whereas KL did not. Furthermore, KL alone can prevent programmed cell death of CD56\textsuperscript{dim} NK cells in the absence of serum or additional growth factors, whereas IL-10 cannot. Thus, whereas the functional consequences of IL-10 binding to CD56\textsuperscript{dim} NK cells do overlap with those of KL, its full spectrum of activity on this population of human lymphocytes appears to be distinct. This finding is consistent with the fact that the IL-10R belongs to the class II subgroup of cytokine receptors, which includes the IFN receptor family. The structural motifs of this class of receptors bear little resemblance to either the tyrosine kinase or hematopoietin family of receptors. Indeed, the profile of IFN effects on human NK cells mirrors that of IL-10.

The mechanism by which IL-10 is able to induce NK cell cytolytic activation against resistant tumor cell targets appears to be at least partially distinct from that of IL-2. IL-10 did not upregulate NK cell CAMs. Unlike IL-2, IL-10 could not provide a costimulatory signal to potentiate IL-12–induced NK cytotoxic activity. The incubation of NK cells in the presence of both IL-10 and IL-2 had an additive effect on NK cytotoxic activity, even at maximum concentrations of IL-10, suggesting that these two cytokines are not mutually redundant in their effects on NK cells.

In contrast to its induction of cytotoxic activity, IL-10 alone was unable to induce NK cytokine production. However, IL-10 consistently potentiated IL-2–induced production of IFN-\gamma, GM-CSF, and TNF-\alpha in human NK cells in an even greater than additive fashion and yet failed to potentiate the IL-12–induced production of NK cell cytokines. The ability of IL-10 to costimulate with IL-2 to induce low but significant IFN-\gamma production in NK cells differs from the results reported by Hsu et al, who showed no NK cell production of IFN-\gamma by the combination of IL-2 plus IL-10. It is quite possible that this subtle difference is technical in nature because the length of NK cell incubation differed, and the amount of human IL-10 contained in the COS-human IL-10 supernatants was not specified in that report.

In summary, the direct effects of IL-10 on human NK cells are rather distinct from those on both T cells and monocyte/macrophages. Furthermore, they are distinct from the direct effects of another Th2 cytokine, IL-4, on human NK cells. Overall, IL-10 directly stimulates or potentiates NK cell function. At lower concentrations, IL-10 can augment IL-2–induced NK cell proliferation in the less abundant CD56\textsuperscript{dim} NK subset, whereas at higher concentrations IL-10 alone can directly enhance cytotoxic activity in both NK subsets. IL-10 can potentiate IL-2–induced NK cytotoxic activity as well as IL-2–induced NK cell cytokine production, suggesting that the signal transduction pathways used by their respective receptors may share some intracellular intermediates. It is of interest that, in certain animal models, NK cells have been shown to be critical in the protection against pathogens that secrete large amounts of IL-10.

The effects of IL-10 on NK cell function in vivo will be best studied in such animal models and will hopefully lend additional insights into the role this cytokine may play during the human innate immune response.
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The functional characterization of interleukin-10 receptor expression on human natural killer cells

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