Human natural killer cells: a unique innate immunoregulatory role for the CD56^{bright} subset

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During the innate immune response to infection, monocyte-derived cytokines (monokines), stimulate natural killer (NK) cells to produce immunoregulatory cytokines that are important to the host's early defense. Human NK cell subsets can be distinguished by CD56 surface density expression (ie, CD56^{bright} and CD56^{dim}). In this report, it is shown that CD56^{bright} NK cells produce significantly greater levels of interferon-γ, tumor necrosis factor-β, granulocyte macrophage–colony-stimulating factor, IL-10, and IL-13 protein in response to monokine stimulation than do CD56^{dim} NK cells, which produce negligible amounts of these cytokines. Further, qualitative differences in CD56^{bright} NK-derived cytokines are shown to be dependent on the specific monokines present. For example, the monokine IL-15 appears to be required for type 2 cytokine production by CD56^{bright} NK cells. It is proposed that human CD56^{bright} NK cells have a unique functional role in the innate immune response as the primary source of NK cell–derived immunoregulatory cytokines, regulated in part by differential monokine production. (Blood. 2001;97:3146-3151)

Introduction

Natural killer (NK) cells are innate immune effectors that produce immunoregulatory cytokines, such as interferon (IFN)-γ and granulocyte macrophage–colony-stimulating factor GM-CSF, critical to early host defense against a variety of viral, bacterial, and parasitic pathogens.[1-4] Human NK cells comprise approximately 10% of all peripheral blood lymphocytes and are characterized phenotypically by the presence of CD56 and the lack of CD3.[1] There are 2 distinct subsets of human NK cells identified by cell surface density of CD56. The majority (approximately 90%) of human NK cells are CD56^{dim} and express high levels of FcγRIII (CD16), whereas a minority (approximately 10%) are CD56^{bright} and CD16$^{dim/neg}$.[5]

CD56^{bright} NK cells constitutively express the high- and intermediate-affinity IL-2 receptors and expand in vitro and in vivo in response to low (picomolar) doses of IL-2.[6-8] These NK cells also express the c-*kit* receptor tyrosine kinase whose ligand enhances IL-2–induced proliferation.[9,10] In contrast, resting CD56^{dim} NK cells express only the intermediate affinity IL-2 receptor, are c-*kit*^{neg}, and proliferate weakly in response to high doses of IL-2 (1 to 10 nM) in vitro, even after induction of the high-affinity IL-2 receptor.[6,7] Resting CD56^{dim} NK cells are more cytotoxic against NK-sensitive targets than CD56^{bright} NK cells.[11] However, after activation with IL-2 or IL-12, CD56^{bright} cells exhibit similar or enhanced cytotoxicity against NK targets compared to CD56^{dim} cells.[11-13]

NK cell subsets have differential natural killer receptor (NKR) repertoires. All resting CD56^{bright} NK cells have high expression of CD94/NKG2 C-type lectin receptors.[14] A small percentage (less than 10%) expresses killer cell immunoglobulin-like receptors (KIR),[15] while most (more than 85%) resting CD56^{dim} NK cells are KIR^{+} and have low expression of CD94/NKG2.

CD56^{bright} NK cells also express the adhesion molecule L-selectin (CD62L), which mediates initial interactions with vascular endothelium.[16] CD56^{dim} NK cells lack this receptor but have recently been found to express PEN5, an NK cell-restricted sulfated lactosamine epitope that partially mediates the binding of L-selectin,[15] thus suggesting the potential for differential trafficking of human NK cell subsets in vivo. Therefore, CD56^{bright} and CD56^{dim} NK cells differ in their proliferative response to IL-2, intrinsic cytotoxic capacity, NKR repertoire, and adhesion molecule expression.

NK cells constitutively express receptors for monocyte-derived cytokines (monokines) and produce critical cytokines, such as IFN-γ, in response to monokine stimulation.[17-20] In the current study we examine CD56^{bright} and CD56^{dim} NK cell production of multiple cytokines—including IFN-γ, tumor necrosis factor (TNF)-β, IL-10, IL-13, TNF-α, and GM-CSF—in response to stimulation with monokines. We show that CD56^{bright} NK cells are the primary population responsible for NK cell cytokine production in response to monokines. These data support a model whereby CD56^{bright} and CD56^{dim} NK cells represent functionally distinct subsets of mature human NK cells.

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Materials and methods

Cell culture reagents and antibodies

Human NK cells and macrophages were cultured in RPMI-1640 with 10% human serum (C-6 Diagnostics, Mequon, WI) and antibiotics. Recombinant human IL-12 was provided by Genetics Institute (Cambridge, MA); rIL-15 was a gift from Immunex (Seattle, WA); rIL-18 was a gift from BASF Bioresearch (Worcester, MA); and rIL-1β was purchased from Peprotech (Rocky Hill, NJ). PMA and ionomycin (calcium salt) were obtained from Calbiochem (La Jolla, CA). Anti-CD56-phycoerythrin (PE) (NKH1), CD16-fluorescein isothiocyanate (FITC) monoclonal antibodies, and isotype controls were purchased from Coulter (Miami, FL).

Purification of human NK cell subsets and macrophages

Human NK cells were isolated from fresh normal donor leukopacs (American Red Cross, Columbus, OH) as previously described[9,18] or with RosetteSep NK cell cocktail (StemCell Technologies, Vancouver, BC) according to the manufacturer's directions. NK cells were stained with anti–CD56-PE or control PE, and subsets were purified based on CD56 cell-surface density by FACS (Elite Flow Cytometer; Coulter) as previously described.[6] Cells were routinely greater than 98% pure by post-FACS cytometric analysis of CD56 and CD16, as shown in Figure 1. Human macrophages were isolated by adherence and gentle scraping (more than 85% $CD14^+$ by flow cytometry).

NK cell monokine and PMA–ionomycin stimulation

Purified NK cells (5×10^4 cells/well) were stimulated with combinations of IL-12 (10 ng/mL), IL-15 (100 ng/mL), IL-18 (100 ng/mL), IL-1β (10 ng/mL), PMA (20 ng/mL), or ionomycin (5 μM), and cell-free culture supernatants were harvested at 72 hours. Cell culture supernatants were assayed for IFN-γ, IL-10, GM-CSF, TNF-α, IL-13, IL-5 (Endogen, Woburn, MA), and TNF-β (R&D Systems, Minneapolis, MN) protein in duplicate enzyme-linked immunosorbent assay (ELISA) wells. Results represent the mean ± SEM of 3 or more donors.

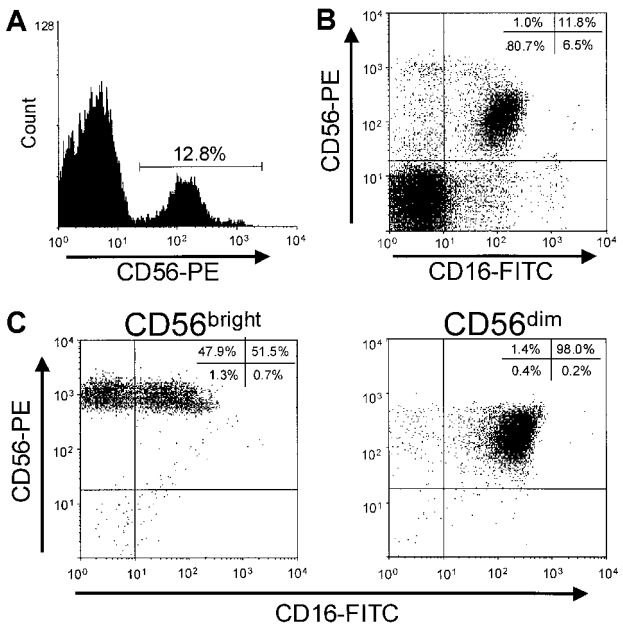

Figure 1. Purification of human NK cell subsets. $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets were FACS-purified from fresh peripheral blood lymphocytes. Flow cytometric analysis of CD56-PE (A) and CD56-PE/CD16-FITC expression before (B) and after (C) FACS purification of a representative donor is shown, and the percentages of cells are indicated in the upper right quadrant. (A) Gating on total peripheral blood lymphocytes; 12.8% of unpurified lymphocytes were $CD56^+$, and (B) 11.8% were positive for both CD56 and CD16. (C) Purified $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets were more than 98% pure, as shown by flow cytometry analysis of CD56 and CD16. Of $CD56^{bright}$ cells, 51.5% expressed CD16 at low levels (mean fluorescence intensity [MFI] 49.2), whereas 98% of $CD56^{dim}$ cells had high expression of CD16 (MFI 213.9). NK cell subsets were gated on total viable cells (more than 98% of all collected events).

Quantitation of cytokine transcripts by real-time RT-PCR

FACS-purified $CD56^{bright}$ and $CD56^{dim}$ NK cells (1×10^5) were either immediately lysed for RNA (Qiagen RNeasy lysis buffer; Qiagen, Valencia, CA) or cultured at 1×10^5 cells/well with recombinant monokines. Cells were harvested at 24 hours and lysed with 300 μL RNA lysis buffer. Total cellular RNA was isolated (Qiagen RNeasy Mini-kits; Qiagen) and cDNA was generated with random hexamer primers and MMLV-RT according to the manufacturer's recommendations (Gibco Life Technologies, Rockville, MD). cDNA was then used as a template for real-time polymerase chain reaction (PCR).

Real-time quantitative reverse transcription (RT)-PCR is a novel method to accurately measure amplified target copy number through the use of a dual-labeled fluorogenic probe.[21] Real-time PCR reactions for human IFN-γ, GM-CSF, and IL-10 transcripts were performed as previously described and as multiplex reactions with primer and probe sets specific for the cytokine transcript of interest and an internal control (rRNA, 18s; PE Applied Biosystems, Foster City, CA).[18] cDNA from PHA-activated human lymphocytes served as positive controls for cytokine transcripts, and murine cDNA (P815 cell line) was used as a negative control. Reactions were performed using an ABI prism 7700 sequence detector (Taqman; PE Applied Biosystems), and data were analyzed with the Sequence Detector version 1.6 software to establish the PCR cycle at which the fluorescence exceeded a set threshold, C_T, for each sample. Data were analyzed according to the comparative C_T method, as previously described, using internal control (18s) transcript levels to normalize differences in sample loading and preparation. Results are semiquantitative and represent the n-fold difference of transcript levels in a particular sample compared to calibrator cDNA (for these experiments, cDNA samples of resting NK cells from each donor). Results are expressed as the mean ± SEM of triplicate reaction wells.

NK cell and macrophage co-cultures

Purified $CD56^{bright}$ and $CD56^{dim}$ NK cells (1.0×10^5) were co-cultured with autologous macrophages (1.0×10^5) as previously described[22] and stimulated with 10 μg/mL lipopolysaccharide (LPS; serotype 0127 B8; Sigma, St Louis, MO) for 72 hours.

Statistical analysis

Statistical analysis was performed using the Student paired *t* test; $P < .05$ was considered significant.

Results

$CD56^{bright}$ NK cells produce abundant type 1 and type 2 cytokines compared to $CD56^{dim}$ NK cells

We stimulated sorted resting $CD56^{bright}$ and $CD56^{dim}$ NK cells (Figure 1) with the recombinant monokines IL-12, IL-15, IL-18, and IL-1β alone and in combination with IL-12 or IL-15. To examine the stimulation of NK cells independent of monokine receptor expression, NK cell subsets were also activated with phorbol esters (PMA) plus ionomycin. The $CD56^{bright}$ subset produced significantly more of the type 1 cytokines IFN-γ and TNF-β than $CD56^{dim}$ NK cells cultured under identical conditions after stimulation with monokines or PMA plus ionomycin (Figure 2). $CD56^{bright}$ NK cells co-stimulated with IL-18 plus IL-12

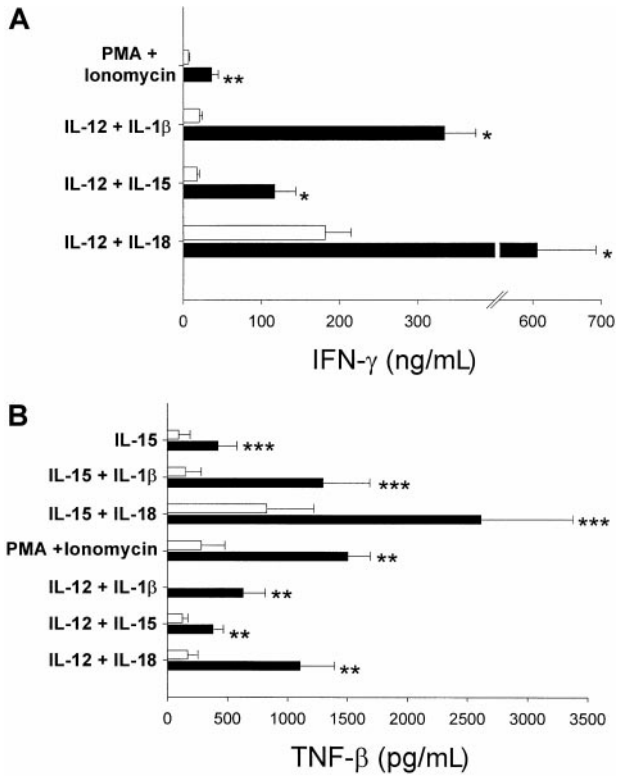

Figure 2. NK cell production of type 1 cytokines IFN-γ and TNF-β. (A) IFN-γ protein was measured by ELISA in cell culture supernatants of freshly isolated CD56^{bright} and CD56^{dim} NK cells after culture with monokines or PMA plus ionomycin for 72 hours. CD56^{bright} NK cells produced significantly more IFN-γ in response to all positive stimuli; data represent the mean ± SEM of 5 to 10 donors (*$P < .004$; **$P < .03$). IL-12 or IL-15 alone induced minimal IFN-γ (less than 2 ng/mL) whereas no IFN-γ was detected in cultures with IL-18, IL-1β, PMA, or ionomycin alone (data not shown; sensitivity < 25 pg/mL). (B) CD56^{bright} NK cells also produced significantly more TNF-β protein after activation with monokines or PMA plus ionomycin (n = 3-5 donors per condition; **$P < .03$; ***$P < .05$). There was no detectable TNF-β protein with IL-12, IL-1β, IL-18, PMA, or ionomycin alone (data not shown; sensitivity less than 20 pg/mL). □ indicates CD56^{dim}; ■, CD56^{bright}.

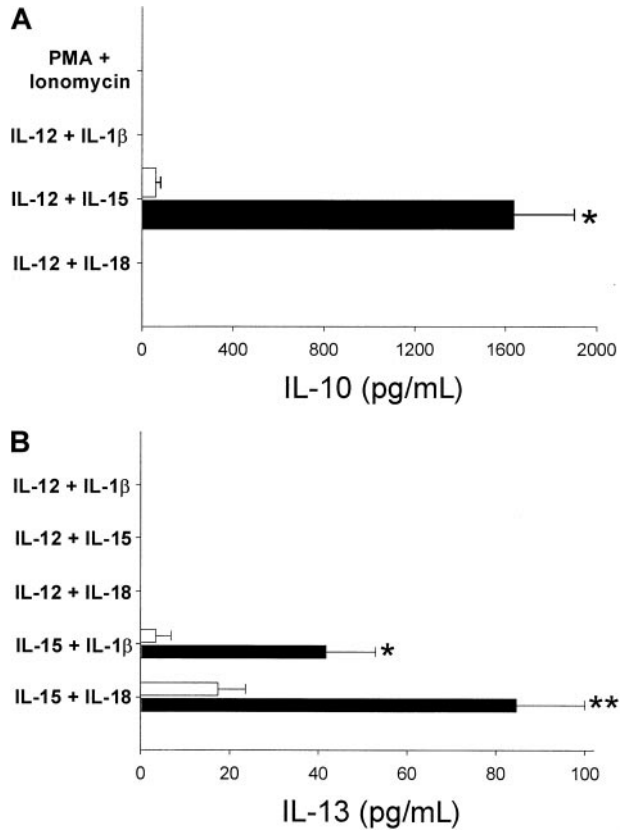

Figure 3. CD56^{bright} NK cells produce the type 2 cytokines IL-10 and IL-13. (A) IL-12 plus IL-15 was the only monokine combination that induced IL-10 production in human NK cell subsets. Significantly more protein was detected in CD56^{bright} NK cells (1635.5 ± 266.3 vs 61.2 ± 21.3 pg/mL CD56^{bright} vs CD56^{dim}; *$P < .0012$; n = 8). (B) The CD56^{bright} subset produced significantly more IL-13 protein than CD56^{dim} NK cells after stimulation with IL-15 plus IL-1β or IL-18 (*$P < .0018$; **$P < .014$; n = 7). No IL-13 was detected in cultures with IL-12 plus IL-1β, IL-15, or IL-18 (n = 3) or with PMA plus ionomycin (n = 2). □ indicates CD56^{dim}; ■, CD56^{bright}.

produced the most IFN-γ protein (Figure 2A), whereas stimulation with IL-18 plus IL-15 or IL-1β plus IL-15 induced the highest levels of TNF-β protein production.

NK cell production of IL-10, a type 2 cytokine, was only detected after co-stimulation with IL-12 plus IL-15 (Figure 3A), with CD56^{bright} NK cells producing in excess of 25-fold more IL-10 protein than CD56^{dim} cells. Interestingly, other monokine combinations, including IL-12 plus IL-18, failed to elicit any production of IL-10 from either subset. Modest amounts of IL-13, another cytokine produced by committed Th2 cells, were detected in cultures of CD56^{bright} NK cells stimulated with IL-15 plus IL-18 or IL-1β, and this production was always significantly greater than in CD56^{dim} NK cells (Figure 3B). We did not detect any NK cell production of IL-5 protein in response to monokine or PMA plus ionomycin stimulation (data not shown; sensitivity less than 2 pg/mL).

Thus, CD56^{bright} NK cells produce high levels of 2 principal type 1 cytokines, IFN-γ and TNF-β, after stimulation with monokines or phorbol esters plus ionomycin. However, only specific combinations of monokines that included IL-15 as a co-stimulus induced IL-10 or IL-13 protein in cultures of CD56^{bright} NK cells, suggesting that production of these type 2 cytokines by human NK cells requires specific monokine (eg, IL-15)-induced signaling.

CD56^{bright} NK cell production of other pro-inflammatory cytokines, GM-CSF, and TNF-α

CD56^{bright} NK cells produced significantly more of the macrophage-activating GM-CSF than CD56^{dim} NK cells in response to all

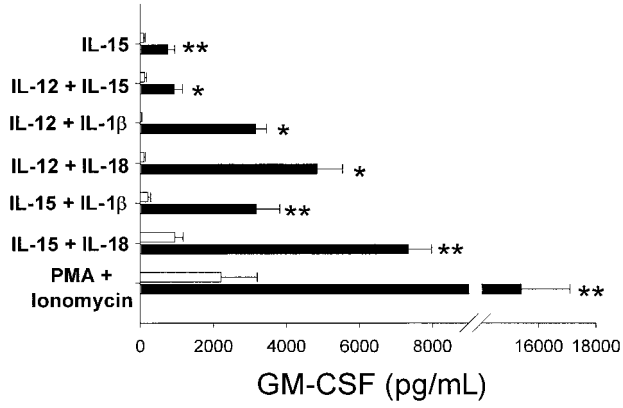

Figure 4. GM-CSF production by human NK cell subsets. Stimulation with monokines or PMA plus ionomycin induced significantly more GM-CSF protein in cultures of CD56^{bright} NK cells than in CD56^{dim} cells (*$P < .007$; **$P < .04$; n = 3-7 donors per condition). IL-15 alone induced moderate levels of GM-CSF (751.7 ± 190.4 pg/mL, CD56^{bright} NK cells), whereas there was no detectable GM-CSF in cultures with IL-12, IL-1β, IL-18, PMA, or ionomycin alone (sensitivity less than 15 pg/mL). □ indicates CD56^{dim}; ■, CD56^{bright}.

positive stimuli (Figure 4). IL-15 was the only monokine that induced GM-CSF protein without a co-stimulus (sensitivity less than 15 pg/mL). The combination of IL-15 plus IL-18 was the most potent monokine stimulus for GM-CSF production, similar to TNF-β. Unlike other NK-derived cytokines, stimulation with PMA plus ionomycin induced the highest levels of GM-CSF protein from CD56^{bright} NK cells. CD56^{bright} NK cell production of the pro-inflammatory cytokine TNF-α, after culture with monokines or PMA plus ionomycin, was modest (less than 300 pg/mL) and somewhat variable, but it was consistently greater than in the CD56^{dim} NK cell subset (data not shown).

Cytokine transcript levels in NK cell subsets

To determine whether NK cell subsets have differential baseline expression of cytokines not detectable by ELISA that might account for observed differences in protein production, we measured transcript levels of 3 primary NK-derived cytokines in resting and monokine-activated NK cell subsets by real-time quantitative RT-PCR. Resting subsets lacked any detectable expression of IL-10 (data not shown), but both CD56^{bright} and CD56^{dim} NK cell subsets expressed equal amounts of IFN-γ and GM-CSF transcript (data not shown; n = 3). Therefore, there is no detectable difference in the baseline production of these cytokine transcripts by resting NK cell subsets. After 24 hours of monokine stimulation, the CD56^{bright} NK subset produced higher levels of IFN-γ and GM-CSF transcript than the CD56^{dim} NK subset, consistent with their production of the respective proteins (Figure 5A,B).

CD56^{bright} NK cells produce significantly more IFN-γ than CD56^{dim} NK cells when cultured with LPS-activated macrophages

Gram-negative bacteria-derived LPS stimulate macrophages to secrete a number of monokines, including IL-1, IL-12, and IL-15.[22-24] We have previously shown that the production of monokines by LPS-activated macrophages induces IFN-γ production by human NK cells in vitro.[22] To determine the subset of NK cells responsible for IFN-γ production after monokine activation by LPS, purified CD56^{bright} and CD56^{dim} NK cells were co-cultured with LPS-stimulated autologous macrophages (Figure 6). Similar to results obtained with recombinant monokines, CD56^{bright} NK cells produced 8-fold more IFN-γ protein than CD56^{dim} cells (n = 7). Thus, CD56^{bright} NK cells are the primary producers of IFN-γ in response to both recombinant and endogenous monokines.

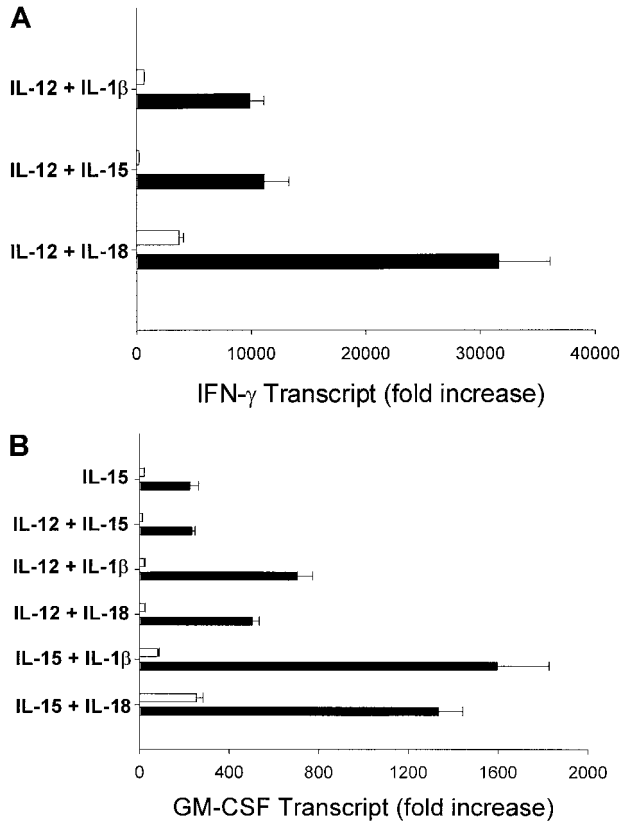

Figure 5. CD56^{bright} cells produce abundant IFN-γ and GM-CSF transcript. NK cell subsets were activated for 24 hours with the indicated monokines, and RNA was harvested. Resultant cDNA was analyzed for IFN-γ and GM-CSF transcripts by real-time PCR. Results represent fold-increase in cytokine transcript compared to resting NK cell subsets ± SEM and are representative of 3 experiments. (A) CD56^{bright} NK produced high levels of IFN-γ transcript in response to IL-12 plus IL-1β, IL-15, and IL-18 compared to the CD56^{dim} subset. (B) CD56^{bright} NK cells also produced more GM-CSF transcript in response to monokine stimulation, including activation with IL-15 alone. All results were calibrated to an internal control (18s ribosomal RNA) to normalize for differences in total cDNA. □ indicates CD56^{dim}; ■, CD56^{bright}.

Discussion

Collectively, our results reveal that CD56^{bright} human NK cells are the primary source of NK-derived immunoregulatory cytokines, including IFN-γ, TNF-β, IL-10, IL-13, and GM-CSF, whereas the CD56^{dim} NK cell subset consistently produces significantly less of these cytokines in vitro. The results confirm our earlier observations of CD56^{bright} NK IFN-γ production[18] but also provide evidence for a more generalized property that can be attributed to this distinct human NK cell subset. Although the possibility exists that differences in cytokine production may be attributed to differential monokine receptor expression, density, or both, the activation of NK cell subsets with phorbol esters plus ionomycin, which is not dependent on monokine receptor activation, resulted in significantly greater production of IFN-γ, TNF-β, and GM-CSF by CD56^{bright} NK cells. Furthermore, LPS, a bacterial component recognized by host innate immune effector cells, indirectly induced CD56^{bright} NK cells to produce much greater IFN-γ than CD56^{dim} NK cells when co-cultured with macrophages. Therefore, the CD56^{bright} NK subset has a significantly higher capacity for cytokine production than the CD56^{dim} subset.

Peritt et al[25] recently reported the differentiation of human NK cells into NK1 and NK2 subsets by generating NK cell clones after 8-day in vitro culture under type 1– or type 2–inducing conditions. After stimulation with PMA plus ionomycin, NK1 cells produced IFN-γ and TNF-β but also produced IL-10, a type 2 cytokine, and NK2 cells produced IL-5 and IL-13. However, because of the clear role of NK cells as efficient producers of cytokines early in the innate immune response to infection long before clonal responses are mounted, it is unlikely that prolonged peripheral NK cell differentiation is necessary in vivo. In this report we show that freshly isolated CD56^{bright} NK cells produce abundant type 1 and type 2 cytokines immediately after monokine stimulation, consistent with their known in vivo role. Hence, though we do not exclude the possibility for subsets of human NK cells that produce type 1 or type 2 cytokines, our data suggest that these subsets would be found within the resting CD56^{bright} NK cell population.

Additional data presented here indicate that the qualitative and quantitative production of monokines after host infection are likely

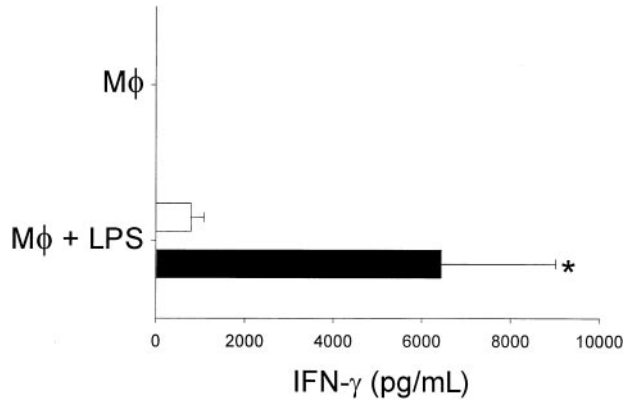

Figure 6. CD56^{bright} NK cell co-cultured with LPS-activated macrophages produce abundant IFN-γ. Resting CD56^{bright} and CD56^{dim} NK cells (1×10^5/well) were co-cultured with autologous macrophages (1×10^5/well) and control (phosphate-buffered saline) or LPS for 72 hours, and cell culture supernatants were assayed for IFN-γ protein. CD56^{bright} NK cells co-cultured with LPS-activated macrophages (Mϕ + LPS) produced significantly more IFN-γ protein than CD56^{dim} NK cells (6439.41 ± 2579.16 vs 791.5 ± 289.2 pg/mL; $P < .05$; n = 7). No IFN-γ protein was detected in co-cultures without LPS (Mϕ) or in cultures of LPS-activated NK cells or macrophages (data not shown). □ indicates CD56^{dim}; ■, CD56^{bright}.

important in distinguishing the induction of type 1 and type 2 cytokines by CD56^{bright} NK cells. IL-15 co-stimulation was requisite for CD56^{bright} NK cell production of type 2 cytokines (eg, IL-10 and IL-13), whereas IL-12 co-stimulation was required for optimal production of the type 1 cytokine IFN-γ. Although each of these monokines had the capacity to stimulate both type 1 and type 2 responses (eg, IL-12 co-stimulation of IL-10 and IL-15 co-stimulation of optimal TNF-β production), the relative quantities of each and the presence of other monokines (eg, IL-1 or IL-18) could influence the predominant CD56^{bright} NK cell cytokine response. Thus, the monokine milieu induced by infection may dictate CD56^{bright} NK cell production of type 1 or type 2 cytokines, which could then, in part, influence the consequent development of a T-helper response.[26,27]

It has been hypothesized that CD56^{bright} and CD56^{dim} NK cells represent different stages of NK cell maturation, with CD56^{dim} NK cells being the more differentiated cell type.[11] This paradigm is based on observations that resting CD56^{dim} NK cells are more cytotoxic than CD56^{bright} NK cells, express high surface density expression of both KIR[15] and CD16 (which mediate natural NK cytotoxicity[28] and antibody-dependent cellular cytotoxicity,[29] respectively), and do not readily proliferate in response to IL-2. In the current study we provide new evidence to suggest that CD56^{bright} NK cells are the major cytokine-producing subset of human NK cells, an attribute that is associated with essential host function. Based on these data, we propose that CD56^{bright} and CD56^{dim} NK cells represent functionally and phenotypically distinct subsets of NK cells with unique immunoregulatory roles in vivo (Figure 7).

Numerous studies from our laboratory and those of others have definitively identified IL-15 as the critical factor for the development of human and murine NK cells.[30-32] Culture of CD34$^+$Lin^{neg} human hematopoietic cells with IL-15 results in the differentiation of mature, functional CD56$^+$ NK cells. Culturing progenitor cells with flt3 ligand (FL) or c-*kit* ligand (KL) can increase the NK cell precursor frequency through up-regulation of the IL-15R complex.[30,33] However, in vitro-generated NK cells are consistently CD56^{bright}CD16$^{dim/neg}$KIR$^{low/neg}$, making them more phenotypically similar to the minor (approximately 10%) population of CD56^{bright} peripheral blood NK cells.[33] Further, these cell also produce immunoregulatory cytokines. Until recently, evidence for the differentiation of distinct CD56^{bright} and CD56^{dim} NK cell populations has been lacking because there were no reports of in vitro–generated CD56^{dim} NK cells, lending support to the theory that CD56^{bright} NK cells may be the more immature cell type. The discovery of a novel cytokine, IL-21, by Foster et al[34] may shed some light on the developmental differences between CD56^{bright} and CD56^{dim} human NK cell subsets. IL-21 is a unique cytokine most closely related to IL-2 and IL-15, with effects on B- and T-cell proliferation and NK cell differentiation, proliferation, and cytotoxicity. The authors cultured CD34$^+$Lin^{neg} hematopoietic progenitors with FL ± IL-21 and FL + IL-15 ± IL-21. IL-21, in combination with IL-15 and FL, induced the differentiation of CD56$^+$CD16$^+$ NK cells, which, by flow cytometric analysis, appear to be CD56^{dim}CD16$^+$. FL plus IL-21 alone did not induce NK cell differentiation, whereas stimulation with FL plus IL-15 resulted in the expected population of CD56^{bright}CD16^{neg} NK cells.[34] The discovery of this cytokine, which can, in combination with IL-15, induce differentiation of CD56^{dim}CD16$^+$ NK cells, supports a hypothesis whereby human CD56^{bright} and CD56^{dim} NK cells are terminally differentiated cell types that develop within the bone marrow under the influence of differential growth factors, such as IL-15 and IL-21. Further studies are required to investigate definitively the developmental relation between CD56^{bright} and CD56^{dim} NK cells. It will be interesting to determine the role of

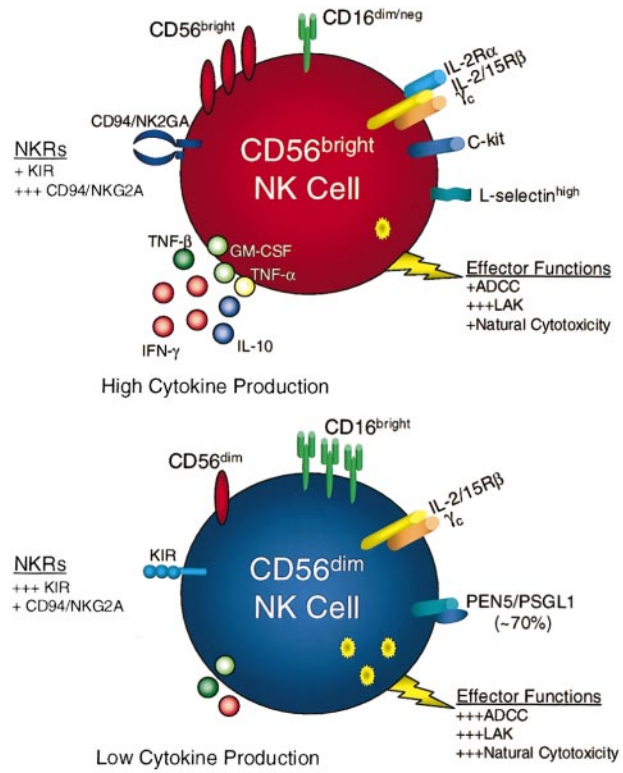

Figure 7. CD56^{bright} and CD56^{dim} NK cell subsets exhibit differential receptor profiles and innate immune functions. Many of the receptors and functions of CD56^{bright} (red cell) and CD56^{dim} (blue cell) NK cells are schematized in this figure. CD56^{bright}CD16$^{dim/neg}$ NK cells produce abundant immunoregulatory cytokines (some of which are depicted here) and exhibit potent LAK activity but are less effective mediators of ADCC and natural cytotoxicity. By contrast, CD56^{dim}CD16^{bright} NK cells produce low levels of NK-derived cytokines and are potent mediators of ADCC, LAK activity, and natural cytotoxicity.[11,12] CD56^{bright} NK cells have a less granular morphology when compared with CD56^{dim} NK cells.[5] CD56^{dim} NK cell exhibit much higher levels of KIR, whereas resting CD56^{bright} NK cells have high expression of CD94/NKG2A.[14,15] L-selectin is highly expressed on CD56^{bright} NK cells, whereas CD56^{dim} NK cells express PEN5/PSGL1 which appears to mediate interactions with L-selectin.[15,16] ADCC, antibody-dependent cellular cytotoxicity; KIR, killer-immunoglobulin-like receptor; LAK, lymphokine-activated killer; NKRs, natural killer receptors.

IL-21/IL-21R in the development of human NK cell subsets and NK receptors (eg, CD16 and KIR).

As we continue to develop immunotherapeutic strategies that target human NK cells, such as the selective expansion of CD56bright NK cells with low-dose IL-2 treatment of malignancies[8,35] and human immunodeficiency virus,[36] it will be important to understand the functional differences between these human NK cell subsets. Knowledge of the distinct functional attributes of CD56bright (eg, immunoregulatory cytokine production) and CD56dim (eg, cytotoxicity and antibody-dependent cellular cytotoxicity) human NK cell subsets and the factors involved in their development and expansion may enable us to design strategies that preferentially activate that subset with the greatest therapeutic potential for a particular disease.

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