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CD56^{bright} Human NK Cells Differentiate into CD56^{dim} Cells: Role of Contact with Peripheral Fibroblasts

Antoni Chan,[1]* Deng-Li Hong,† Ann Atzberger,† Simon Kollnberger,* Andrew D. Filer,‡ Christopher D. Buckley,‡ Andrew McMichael,* Tariq Enver,† and Paul Bowness*

Human NK cells are divided into CD56^{bright}CD16^{-} cells and CD56^{dim}CD16^{+} cells. We tested the hypothesis that CD56^{bright} NK cells can differentiate into CD56^{dim} cells by prospectively isolating and culturing each NK subset in vitro and in vivo. Our results show that CD56^{bright} cells can differentiate into CD56^{dim} both in vitro, in the presence of synovial fibroblasts, and in vivo, upon transfer into NOD-SCID mice. In vitro, this differentiation was inhibited by fibroblast growth factor receptor-1 Ab, demonstrating a role of the CD56 and fibroblast growth factor receptor-1 interaction in this process. Differentiated CD56^{dim} cells had reduced IFN-γ production but increased perforin expression and cytolysis of cell line K562 targets. Flow cytometric fluorescent in situ hybridization demonstrated that CD56^{bright} NK cells had longer telomere length compared with CD56^{dim} NK cells, implying the former are less mature. Our data support a linear differentiation model of human NK development in which immature CD56^{bright} NK cells can differentiate into CD56^{dim} cells. *The Journal of Immunology,* **2007, 179: 89–94.**

Human NK cells are classified into two populations according to the intensity of CD56 (neural cell adhesion molecule (NCAM)[2]) surface expression, as well as possession of CD16, the FcγRIII. CD56^{dim}CD16^{bright} make up ~90% of circulating NK cells, whereas CD56^{bright}CD16$^{-/dim}$ comprises the remaining 10% (1, 2). By contrast, CD56^{bright} NK cells predominate in lymph nodes and sites of inflammation. CD56^{bright} NK cells produce abundant cytokines (e.g., IFN-γ) (3) and have immunoregulatory function (4), whereas CD56^{dim} play a key role in natural and Ab-mediated cell cytotoxicity (2, 5). The phylogeny and ontogeny of these human NK subsets are poorly understood. The absence of a murine homolog of the human CD56 molecule prevents comparative studies. Models of human CD56 subset development have therefore been based on in vitro studies (6). Three possible models have been proposed. First, a common NK cell precursor could differentiate into either CD56^{bright} or CD56^{dim} (7). A second possibility is that these subsets may switch from one form to another depending on the microenvironment. Third, as originally proposed by Nagler et al. (8), CD56^{bright} may be precursors of CD56^{dim} (or vice versa). CD56 is a ligand for fibroblast growth factor receptor (FGFR)1 that is constitutively expressed on fibroblasts in both membrane-bound and secreted forms (9). We hypothesized that heterophilic adhesion of CD56^{bright} NK cells with peripheral tissue fibroblasts via a CD56-FGFR1 interaction may aid the differentiation into CD56^{dim} cells. In this study, we show a terminal differentiation step in NK subset development in which CD56^{bright} cells, in contact with fibroblasts, can differentiate into CD56^{dim} cells, supporting a sequential linear differential model of NK subset development.

Materials and Methods

Cell isolation and culture

PBMC were obtained by Ficoll-Hypaque density centrifugation of eight healthy adult buffy coat leukocyte concentrates (Blood Transfusion Service). CD56 NK subsets (CD56^{bright}CD16^{-} or CD56^{dim}CD16^{bright}) were isolated by FACS sorting (MoFlo; DakoCytomation) of NK cells enriched from PBMC using the MACS NK Negative Isolation kit (Miltenyi Biotec). Cell purity exceeded 99%. Fibroblast-like synoviocytes (FLS) and skin fibroblasts were obtained from four patients with spondyloarthritis and three patients with rheumatoid arthritis, respectively, after informed consent. This study had Central Oxfordshire Research Ethics Committee approval (C00.114) and the South Birmingham Local Research Ethics Committee (no. 5735). Fibroblasts were isolated from synovial or skin tissue as previously described (10). Briefly, tissue samples were enzymatically dissociated and cultured to confluence in RPMI 1640 (Invitrogen Life Technologies), supplemented with 20% heat-inactivated FCS, 1% penicillin/streptomycin, 1% L-glutamine, 1% sodium pyruvate, and 1% nonessential amino acid (Sigma-Aldrich). FLS were defined by morphology and expression of fibronectin and prolyl 4-hydroxylase, being negative for the expression of CD1, CD3, CD19, CD31, CD68, CD80, CD86, von Willebrand factor, and cytokeratin. FLS were used between passages 3 and 5. FLS were added to 96-well flat-bottom plates (BD Falcon) at 3000 cells/well. Experiments were performed with confluent FLS prepared 24 h before contact. Once confluence was reached, the fibroblasts were washed twice in PBS and cocultured with 1×10^5 NK cells in RPMI 1640 supplemented with 10% heat-inactivated FCS, 1% penicillin/streptomycin, and 1% L-glutamine. IL-2 (100 IU/ml), IL-15 (10 ng/ml), 5×10^4 gamma-irradiated LBL721.221 cells, or 10% synovial fluid were added to NK cells in some experiments. All experimental conditions were performed in duplicate.

A 24-well Transwell system (Corning; Costar) was used in some coculture experiments. The system consists of two compartments separated by a porous matrix (0.4 μm), which allowed exchange of soluble factors while preventing direct contact. FLS were grown to confluence in the bottom well, and NK cells were either added to the same well (allowing contact) or in the top well (avoiding contact). Blocking Abs were added at the following concentrations: anti-human FGFR1 mAb (R&D Systems) at 5 μg/ml, anti-human FGFR2 mAb (R&D Systems) at 10 μg/ml, and mouse isotype control IgG1 (BD Pharmingen) at 5 μg/ml.

Abs, FACS analysis, and immunostaining

NK cells were stained with directly conjugated CD3, CD16, CD56, CD34, CD62 ligand (CD62L), CD94, CD45, anti-human mAbs (Serotec), killer

*Medical Research Council Human Immunology Unit, and †Medical Research Council Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom; and ‡Division of Immunity and Infection, Medical Research Council, Centre for Immune Regulation, University of Birmingham, Birmingham, United Kingdom

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[1] Address correspondence and reprint requests to Dr. Antoni Chan, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, U.K. E-mail address: achan@hammer.imm.ox.ac.uk

[2] Abbreviations used in this paper: NCAM, neural cell adhesion molecule; FGFR, fibroblast growth factor receptor; PNA, peptide nucleic acid; KIR, killer Ig-related receptor; FLS, fibroblast-like synoviocyte; CD62L, CD62 ligand.

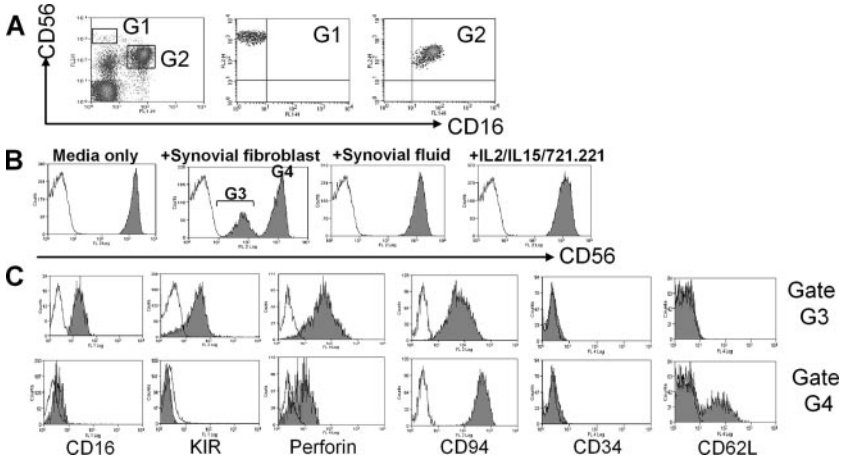

FIGURE 1. Synovial fibroblasts promote the differentiation of CD56^{bright} to fully functional CD56^{dim} cells. *A*, CD56 and CD16 expression by FACS of PBMC used for cell sorting (*left*) are shown, and cells were first gated on lymphocyte and CD3$^-$. FACS analysis of CD56^{bright} (*middle*) and CD56^{dim} (*right*) NK subsets following FACSort. *B*, CD56 expression of CD56^{bright} NK cells cultured for 7 days in medium alone or with the addition of synovial fibroblasts, synovial fluid, or IL-2/IL-15/LBL721.221 mix. The percentage of cells in gate G3 was 30% and in gate G4 was 70%. CD56 staining (gray-filled histogram) and isotype control Ab staining (open histogram) are shown. *C*, CD16, KIR, perforin, CD94, CD34, and CD62L expression of CD56^{dim} cells generated from culture with synovial fibroblasts (gate G3, *top*) and of undifferentiated CD56^{bright} NK cells (gate G4, *bottom*). Staining of NK cell markers (gray-filled histogram) and isotype control Ab staining (open histogram) are shown. Experiments were performed on buffy coats from eight different individuals in three independent experiments with similar results.

Ig-like receptors (KIR; pool of anti-CD158a, CD158b, KIR3DL1/2), perforin, and IFN-γ (BD Biosciences). Cells were analyzed by FACSCalibur (BD Biosciences) using CellQuest software.

^{51}Cr release assay

NK cytotoxicity was assessed by incubating CD56 NK subsets (effector cells) with ^{51}Cr-labeled erythroleukemia cell line K562 (target cells) as previously described (11). The levels of radioactivity released from target cells into supernatants were assessed by gamma scintillation after 4 h of incubation. All experiments were performed in triplicate in a 96-well microtiter plate.

CFSE labeling and analysis

NK cells were labeled with 5 μM CFSE (Molecular Probes) at 37°C as described (12, 13).

Flow cytometric measurement of telomere length using fluorescence in situ hybridization

Telomere-specific $(C_3TA_2)_3$ fluorescently labeled peptide nucleic acid (PNA) probes (Applied Biosystems) were used as previously described (14). Briefly, NK cells were mixed with control 1301 cells. Hybridization buffer (70% v/v deionized formamide, 1% w/v BSA, 10 mM Tris Cl (pH 7.2)) with or without fluorescently labeled PNA probe was added to the cell mixture. Cells were incubated for 10 min at 80°C in a shaking water bath and further incubated overnight at 20°C, protected from light. Following this process, 0.5 ml of 0.3-μg/ml HO334 nucleic acid stain in PBS was added to all tubes. Samples were run on Cyan flow cytometer (DakoCytomation) and analyzed with Summit software (DakoCytomation).

In vivo experimental procedures

NOD/LtSz-scid/scid (NOD-SCID) mice (15) (The Jackson Laboratory) were maintained under defined flora conditions. CFSE-labeled human CD56^{dim} or CD56^{bright} NK cells (>99% purity) were injected into the tail veins of 6-wk-old ^{137}Cs source 350 cGy irradiated mice. Two mice per condition were injected with 1×10^6 human NK cells together with 0.5×10^6 irradiated autologous non-NK PBMC carrier cells in 300 μl of PBS/1% BSA. Experiments were repeated three times, and mice were killed between days 7 and 10 after transfer.

Results

CD56^{bright} cells can differentiate into CD56^{dim} NK cells on contact with synovial fibroblasts

To test the hypothesis that human CD56^{bright} NK cells could give rise to CD56^{dim}, we prospectively isolated NK populations from human peripheral blood by FACS sorting (Fig. 1*A*). CD56^{bright} CD16$^-$ NK cells were cultured with medium alone or together with synovial fibroblasts, 10% synovial fluid, or IL-2/IL-15/gamma-irradiated LBL721.221 cell mix. Fig. 1*B* shows that a population (30%) of CD56^{dim} NK cells was generated from CD56^{bright} cells after culture with synovial fibroblasts. These cells had fully adopted a CD56^{dim} phenotype, now expressing CD16, KIRs, and perforin, and were also CD94^{low}, CD62L$^-$, and CD34$^-$ (Fig. 1*C*, *top row*). Before culture, these cells were all CD16$^-$ (Fig. 1*A*) and KIR$^-$ (data not shown). By contrast, undifferentiated CD56^{bright} cells were CD16$^-$, KIR$^-$, perforin$^{-/low}$, CD94^{high}, CD34$^-$, and CD62L$^{-/+}$ (Fig. 1*C*, *bottom row*).

The CD56^{dim} cells that had differentiated from CD56^{bright} NK cells did not produce IFN-γ upon stimulation with PMA/ionomycin (Fig. 2*A*, i). The lack of IFN-γ production was similar to that seen for sorted CD56^{dim} NK cells cultured in medium alone for 7 days before stimulation with PMA/ionomycin (Fig. 2*A*, iii). By contrast, undifferentiated CD56^{bright} NK cells cultured with synovial fibroblast and sorted CD56^{bright} cells cultured in medium alone responded to PMA/ionomycin stimulation with IFN-γ release (Fig. 2*A*, ii and iv, respectively). CD56^{bright} differentiation in the presence of synovial fibroblasts was not observed if a Transwell was present (data not shown). The CD56^{dim} KIR$^+$ CD16$^+$ phenotype could not be induced by culture in synovial fluid, cytokines (data not shown), or IL-2/IL-15/LBL721.221 mix (Fig. 1*B*). CD56^{dim}CD16$^+$ cells (Fig. 1*A*, gate G2), cultured in parallel under identical conditions, modestly increased CD56 expression but did not adopt any other phenotypic features of CD56^{bright} cells, maintaining high CD16 and KIR expression (data not shown). CD56^{dim}CD16$^-$ cells were KIR$^+$ before culture and expressed CD16 as well as KIR by end of culture (data not shown).

CD56^{dim} cells generated from CD56^{bright} NK cells have increased cytotoxicity

Fig. 2*B* shows that CD56^{bright} cells, cocultured with synovial fibroblasts, acquired greater cytotoxicity against K562 target cells than CD56^{bright} cultured alone (although not as great as purified CD56^{dim} cells). This effect, consistent with the generation of

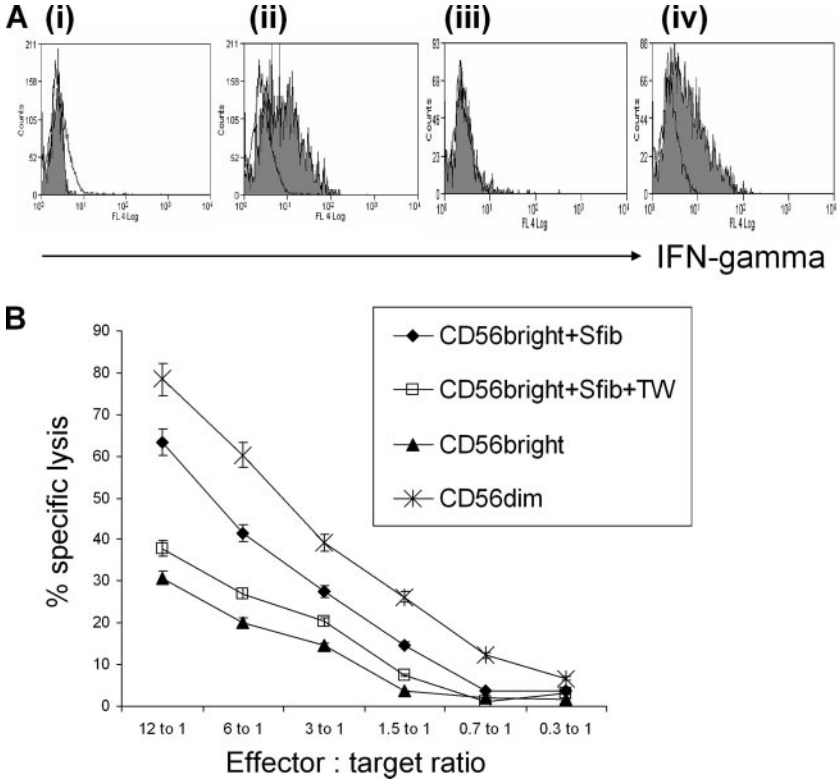

FIGURE 2. CD56^{dim} cells generated from CD56^{bright} cells adopt the functional properties of sorted CD56^{dim} NK cells. *A*, IFN-γ production after stimulation with PMA/ionomycin of (i) CD56^{dim} cells generated from CD56^{bright} NK cells by culture for 7 days with synovial fibroblasts (gate G3, Fig. 1*B*), (ii) undifferentiated CD56^{bright} NK cells (gate G4, Fig. 1*B*), (iii) sorted CD56^{dim}, and (iv) CD56^{bright} NK cells cultured in medium alone for 7 days. *B*, Cytotoxicity of CD56^{bright} NK cells cultured for 7 days in various conditions and CD56^{dim} NK cells against ^{51}Cr-labeled K562 cells in a 4 h assay. Experiments were repeated four times each in duplicate. Sfib, synovial fibroblasts; TW, Transwell.

CD56^{dim} NK cells expressing increased perforin levels, was substantially reduced by the presence of a Transwell.

CD56^{bright} cell differentiation into CD56^{dim} NK cells can occur without cell proliferation

Interestingly phenotypic differentiation (to CD56^{dim}) appeared relatively independent of cell proliferation, as costaining with CFSE and CD56 showed that ~50% of cells that had differentiated from CD56^{bright} to CD56^{dim} cells had not undergone cell

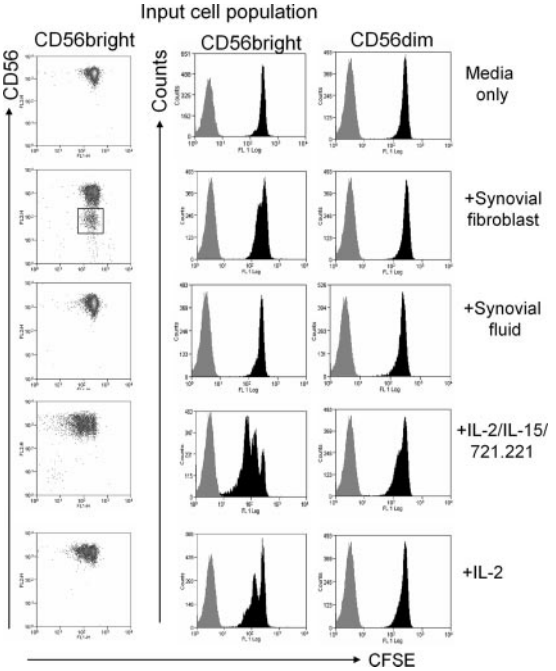

FIGURE 3. CD56^{bright} cell differentiation into CD56^{dim} NK cells can occur without cell proliferation. CFSE-labeled CD56^{bright} (*left* and *middle column*) and CD56^{dim} (*right column*) NK cells were cultured for 7 days in medium alone or with the addition of synovial fibroblasts, 10% synovial fluid, 100 IU/ml IL-2 + 10 ng/ml IL-15 + LBL721.221 mix, or 100 IU/ml IL-2. Co-staining with CD56 mAb of CFSE-labeled CD56^{bright} input NK cells (*left*). Proliferation of CD56^{bright} (*middle*) and CD56^{dim} (*right*) is shown as dilution of CFSE labeling (filled histogram). Unlabeled CD56^{bright} or CD56^{dim} NK cells are also shown (gray-filled histogram). One of five representative experiments is shown.

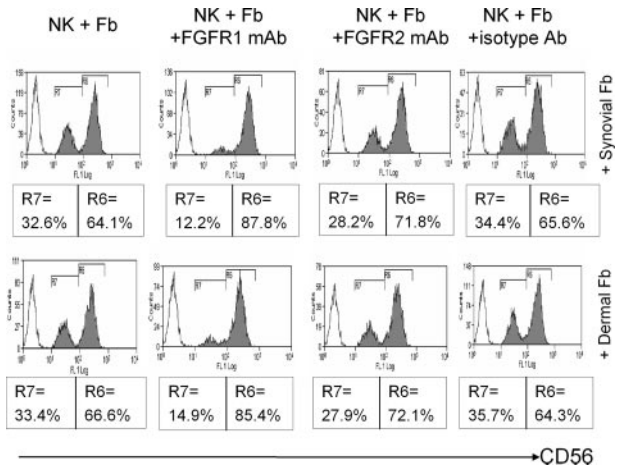

FIGURE 4. CD56^{bright} differentiation into CD56^{dim} is inhibited by FGFR1 blocking Ab. CD56 staining of NK cells; cells were first gated on lymphocytes and CD3$^-$. CD56^{bright} NK cells were cultured with allogeneic synovial fibroblasts (*top row*) or dermal fibroblasts (*bottom row*) for 7 days in medium alone or with the addition of FGFR1 blocking mAb, FGFR2 blocking mAb, or IgG1 isotype control Ab. Experiments were repeated three times each in duplicate. Both FLS and NK cell viability and number did not differ significantly between the three groups (data not shown).

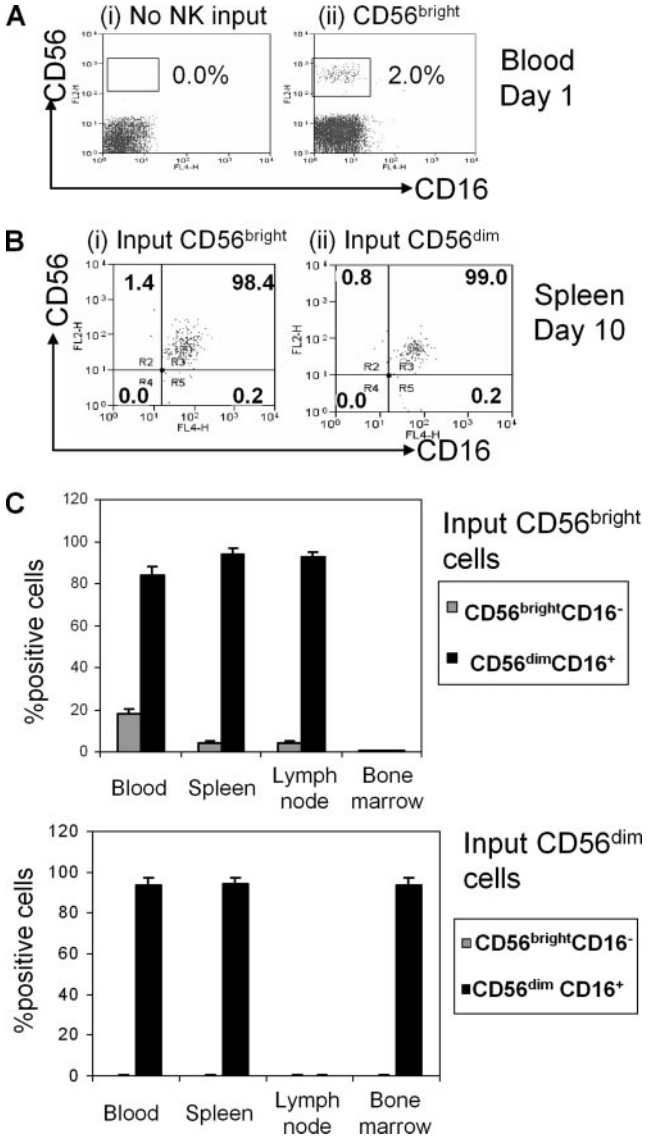

FIGURE 5. CD56^{bright} NK cells differentiate into CD56^{dim} cells in vivo. *A*, CD56 and CD16 staining of peripheral blood at day 1 from mice without human NK cells input (i) and following 5×10^5 CD56^{bright} NK cell transfer (ii). *B*, FACS analysis of spleen cells from NOD-SCID mice 10 days after CD56^{bright} (i) and CD56^{dim} (ii) input. CD56 and CD16 expression on transferred NK cells is given as the percentage in each quadrant shown; cells are first gated on CFSE-positive CD45$^+$ cells. *C*, Phenotypic analysis of transferred CFSE-labeled human CD56^{bright} and CD56^{dim} NK cells. Cells are gated on CFSE-positive cells. The percentage of positive CD56^{bright} CD16$^-$ (▨) and CD56^{dim}CD16$^+$ NK (■) cells in peripheral blood, spleen, lymph nodes, and bone marrow at day 10 in mice injected with CD56^{bright} CD16$^-$ (*top*) or CD56^{dim}CD16$^+$ (*bottom*) NK cells.

division (Fig. 3). Notably, proliferation was observed without phenotypic change in the presence of cytokines.

CD56^{bright} cell differentiation into CD56^{dim} NK cells is inhibited by FGFR1 receptor blocking mAb

Fig. 4 shows that, in the presence of FGFR1 mAb, there was a 60–70% reduction in the percentage of CD56^{dim} cells generated by coculture with allogeneic synovial fibroblasts (Fig. 4, *top panels*). By contrast, culture in the presence of FGFR2 blocking mAb only marginally reduced the percentage of CD56^{dim} NK cells generated. The mAbs to both FGFR1 and FGFR2 stained the FLS (data not shown). CD56^{bright} cells, cultured with allogeneic dermal

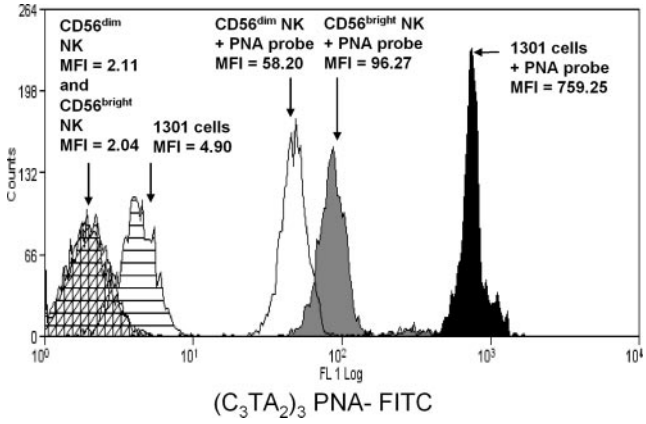

FIGURE 6. CD56^{bright} NK cells have longer telomeres than CD56^{dim} NK cells. Fluorescence of FITC-labeled PNA probe of CD56^{bright} (gray-filled histogram), CD56^{dim} (open histogram), and control 1301 cells (filled histogram) is represented. The fluorescence of these cell populations as well as of cells hybridized without PNA probe is shown. Data are representative of four independent experiments.

fibroblasts, also underwent differentiation into CD56^{dim} cells, and this was reduced to a similar degree by a FGFR1 mAb (Fig. 4, *bottom panels*).

CD56^{bright} cells differentiate into CD56^{dim} cells in vivo

We next transferred resting human CD56^{bright}CD16$^-$ and CD56^{dim} CD16$^+$ NK subsets into NOD-SCID mice. CD56^{bright} cells could be detected in peripheral blood of NOD-SCID mice 1 day after transfer (Fig. 5*A*). However by day 10, the majority of transferred CD56^{bright} NK cells had acquired a CD56^{dim} phenotype (Fig. 5*C*) and resided in lymphoid tissue (spleen and lymph nodes). These cells now expressed CD16 (Fig. 5*B*). CD62L expression was also down-regulated (data not shown). A CD56^{dim}CD16$^+$ population was also seen in peripheral blood of mice that had received CD56^{bright} cells. By contrast, in mice engrafted with CD56^{dim} NK cells, no phenotypic change in CD56 or CD16 expression was seen. CD56^{dim} cells were present in both bone marrow and peripheral blood of NOD-SCID mice and maintained their CD56^{dim} CD16$^+$ expression (Fig. 5*C*).

CD56^{bright} cells have longer telomeres than CD56^{dim} NK cells

One prediction of the linear differentiation model is that peripheral blood CD56^{bright} NK cells would be more immature than CD56^{dim} and therefore have longer telomeres. We therefore measured telomere repeat lengths in peripheral blood CD56 NK subsets by flow cytometry with fluorescence in situ hybridization using a $(C_3TA_2)_3$ PNA probe. CD56^{bright} NK cells (Fig. 6, gray-filled histogram) had a consistently longer telomere length than CD56^{dim} NK cells (Fig. 6, open histogram).

Discussion

It has previously been widely assumed that the two populations of human NK cells, CD56^{bright}CD16$^-$ and CD56^{dim}CD16$^+$, constitute different lineages. In this study, we demonstrate that CD56^{bright} cells can differentiate into cells phenotypically and functionally identical with circulating CD56^{dim} NK cells. CD56^{bright} differentiation into CD56^{dim} cells occurs in vivo upon transfer into NOD-SCID mice, and in vitro upon coculture with peripheral tissue fibroblasts. In vitro, the resulting CD56^{dim} cells have identical function to human peripheral blood CD56^{dim} cells, with enhanced cytolytic function but reduced IFN-γ production. In

vitro maturation is dependent on cell contact and is inhibited by Abs to FGFR1. Lastly we show that human peripheral blood CD56^{dim} NK cells have shorter telomeres than CD56^{bright} cells, further supporting a model in which CD56^{dim} cells are more mature and arise by differentiation from CD56^{bright} cells.

CD56 is a glycoprotein that is involved in both homophilic and heterophilic adhesion and is implicated in neural development (13). NK cells predominantly express the 140-kDa isoform of CD56, also known as NCAM (16). In addition to homophilic binding, CD56 also has various heterophilic interactions with other ligands, including FGFR1 (17). The extracellular domain CD56 consists of five Ig and two fibronectin type III domains (16). The former is involved in homophilic adhesion, whereas the latter can bind to the third Ig domain of FGFR1 through its second fibronectin type III domain (17). FGFR1 is constitutively expressed on fibroblasts in both membrane-bound and secreted forms (9), and ligation by CD56 can induce a signal transduction cascade in cells expressing FGFR1 (18). The high density of NCAM on the surface of CD56^{bright} NK cells may thus promote adhesion to fibroblasts and aid differentiation to CD56^{dim} cells. This finding is evidenced by the inhibition of CD56^{bright} differentiation by a FGFR1 blocking mAb, suggesting that the CD56-FGFR1 interaction contributes to this process. Further evidence of the importance of cell contact is provided by coculture experiments in the presence of a Transwell. Thus when CD56^{bright} cells are separated from FLS, differentiation into CD56^{dim} cells, as evidenced by reduction in K562 cell killing, is reduced. We speculate that a similar mechanism may occur on murine transfer. It is not, however, known whether CD56 is a ligand for murine FGFR1 in either joints or perhaps secondary lymphoid tissue. Further investigation to define additional factors that promote this differentiation is required.

These data support a model of human NK phylogeny in which CD56^{bright} NK cells are relatively immature precursors of CD56^{dim} NK cells and undergo maturation in peripheral tissues. This process can occur on contact with fibroblasts and may occur at sites of inflammation. We have shown, in vitro, that this differentiation can occur in contact with synovial and dermal fibroblasts. We propose that, upon differentiation from CD56^{bright} cells in the periphery, CD56^{dim} NK cells change chemokine receptor expression and consequently leave sites of inflammation and lymphoid tissue to (re)-enter the peripheral circulation. The expansion of CD56^{bright} NK cells at sites of inflammation (19) would support our model, as these cells are younger and more naive compared with the more mature CD56^{dim} NK cells.

It is also possible that different subpopulations of CD56^{bright} cells may differentiate at different sites or under different conditions in vivo. In this respect, fibroblasts have been shown to affect the retention, differentiation, and exit of a variety of infiltrating blood leukocytes (20). Peripheral tissue containing fibroblasts may thus provide a suitable microenvironment for the maturation of NK cells. Interestingly splenic fibroblasts have been shown to promote the differentiation of CD34$^+$ hemopoietic bone marrow progenitor cells into CD56^{bright}CD94$^+$ NK cells (21).

After transfer into NOD-SCID mice, almost all CD56^{bright} cells acquired CD16 and down-regulated CD56. By contrast only ~30% of CD56^{bright} cells differentiated to CD56^{dim} upon in vitro culture with synovial or dermal fibroblasts for a comparable time, and this only increased to a maximum of 40% after longer culture (data not shown). These data suggest that other cell types or factors may be important in this process in vivo. Our finding that the two CD56 subsets have different migratory properties and tissue distribution after in vivo transfer is consistent with their expression of different adhesion and costimulatory molecules (22–24).

Our data also show that human CD56^{bright} NK cells have longer telomeres and greater proliferative capacity, suggesting that they are more immature than CD56^{dim} cells. A number of lines of existing data are also consistent with a linear model of human NK cell differentiation. Thus CD56^{bright} NK cells have been generated from CD34$^+$ hemopoietic bone marrow progenitor cells in vitro (2), and predominate following allogeneic bone marrow transplant (25). The IL-7Rα-chain mRNA is up-regulated by ~11-fold in CD56^{bright} cells compared with CD56^{dim}. IL-7Rα ligation in this population induces specific proliferation and may have function in NK homeostasis and survival (26). In mice, IL-7Rα is present in immature NK cells and is lost upon terminal maturation (6). The importance of lymphoid tissue in NK development has been further highlighted by data that showed CD56^{bright} NK cells in lymph nodes and tonsils are able to up-regulate KIR, CD16, and perforin upon IL-2 stimulation (27).

Recently, it has been shown that human NK cells undergo four discrete stages of NK cell differentiation from CD34$^+$ hemopoietic bone marrow progenitor cells to CD56^{bright} cells that can be phenotypically differentiated using a combination of markers including CD34, CD94, and CD117 (28). Our study adds a terminal stage of differentiation and also emphasizes that CD56 staining must be combined with one or more of CD16, KIR, or CD62L to define two circulating human NK cell populations.

The implications of this model are that CD56^{bright} NK cells, although a minor population in peripheral blood, are critical in that they have greater proliferative and migratory capacity. In cases of human NK deficiency or to prevent graft-vs-host disease resulting from bone marrow transplantation, physiological NK function would be better reconstituted by transfer of CD56^{bright} cells that have the potential both to secrete cytokines and differentiate into CD56^{dim} cells. Lastly, NK cell maturation shows considerable similarity with T cells in expression of phenotypic markers of activation and Ag experience, including CD62L and KIRs. It is possible that the developmental patterns/pathways proposed in this study within peripheral tissues may have presaged the development and maturation pathways seen for T lymphocytes.

Disclosures

The authors have no financial conflict of interest.

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