Membrane-bound IL-15 stimulation on peripheral blood natural kiler progenitors leads to the generation of an adherent subset co-expressing dendritic cells and natural kiler functional markers

Simone Negrini,[1,3,4]* Massimo Giuliani,[1,3]* Deniz Durali,[3,5] Salem Chouaib,[2,3] and Bruno Azzarone[1,3]

[1]UMR 1014 Inserm, Paul Brousse Hospital, Villejuif France; [2]UMR 753 Inserm, Institut Gustave Roussy PR2, Villejuif, France; [3]Université Paris-Sud Paris XI, France; [4]Department of Internal Medicine University of Genoa, Genoa, Italy; [5]TUBITAK Marmara Research Center Genetic Engineering and Biotechnology Institute (GEBI) Gebze / KOCAELI, Turkey

ABSTRACT

Human peripheral blood natural killer progenitors represent a flexible, heterogeneous population whose phenotype and function are controlled by their membrane-bound IL-15. Indeed, reciprocal membrane-bond IL-15 *trans*-presentation commits these cells into NK differentiation, while membrane-bound IL-15 stimulation with its soluble ligand (sIL-15Rα) triggers a reverse signal (pERK1/2 and pFAK) that modifies the developmental program of at least two subsets of PB-NKPs. This treatment generates: i) the expansion of an immature NK subset growing in suspension; ii) the appearance of an unprecedented adherent non-proliferative subset with a dendritic morphology co-expressing marker, cytokines and functions typical of myeloid dendritic cells ($CD1a^+$/$BDCA1^+$/$IL\text{-}12^+$) and NK cells ($CD3^-$/$NKp46^+$/$CD56^+$/$IFN\gamma^+$). The generation of these putative NK/DCs is associated to the rapid inhibition of negative regulators of myelopoiesis (the transcription factors STAT6 and GATA-3) followed by the transient upregulation of inducers of myeloid development, such as the transcription factors (PU.1, GATA-1) and the anti-apoptotic molecule (MCL-1)

Key words: NK progenitors, peripheral blood, membrane-bound IL-15.

Citation: Negrini S, Giuliani M, Durali D, Chouaib S, and Azzarone B. Membrane-bound IL-15 stimulation on peripheral blood natural kiler progenitors leads to the generation of an adherent subset co-expressing dendritic cells and natural kiler functional markers. Haematologica 2011;96(05):762-766. doi:10.3324/haematol.2010.033738

Introduction

Hematopoietic cells arise from a common stem cell. However, progenitor cells with more restricted capacity for differentiation emerge generating different specialized cell lineages. Studies in mice have indicated that NK cells emerge from lymphoid but not from myeloid precursors *in vivo*[1] and that interleukin-15 (IL-15) is essential for their development.[2] In contrast, in human umbilical cord blood, a myeloid-like adherent population ($CD34^-$/$CD14^+$/$CD11b^+$/$CD13^+$/$CD33^+$) has the potential, under the influence of flt3 ligand (FL) and IL-15, to differentiate *in vitro* into mature functional NK cells.[3] Moreover, IL-15 treatment of human cord blood progenitors, induces the contemporary generation of dendritic (DC) and NK cells, suggesting that human DC and NK progenitors may share a common cytokine requirement and interdependence.[4] In this regard, the commitment of human tissue-derived hematopoietic progenitors into the NK pathway is rapidly irreversible[5] and is strictly dependent on IL-15 *trans*-presentation by the IL-15Rα specifically expressed on CD11c(hi)-DCs.[6-8] These data reveal another outcome of NK-DC cross-talk that not only influences both innate and adaptive immune responses inducing NK-cell activation and DC maturation or apoptosis,[9] but is also required for promoting human NK cell development and differentiation *in vivo*.

In contrast, circulating progenitors, at least *in vitro*, seem to display a much more flexible developmental program.[3,4] In this context, we have recently shown that human hematopoietic progenitors derived from peripheral blood (PB-HP), but not from other sources, express a membrane-bound IL-15 (mb-IL-15) that acting through reciprocal *trans*-presentation mimics *in vitro* the IL-15 trans-presentation by DC cells *in vivo*, inducing the generation of non-cytolytic NK *subset* displaying regulatory functions.[10] Herein, we show that this PB-NKp maintain throughout their differentiation process the expression of an mb-IL-15 able to deliver a bi-directional signal whose stimulation with the soluble IL-15Rα chain triggers a reverse signal leading to the appearance of an adherent subset with a DC morphology. These cells display both specific NK (NKp46) and myeloid dendritic (CD1a, BDCA1) markers, cytokine production and functions illustrating another possible chapter of the NK/DC functional

*Both authors equally contributed to this paper.
The online version of this article has a Supplementary Appendix.
Funding: this work was supported by grants from the Association pour la Recherche sur le Cancer (ARC, N° 3690), NRB-Vaincre le Cancer. SM had an ARC post-doctoral fellowship. MG had a post-doctoral fellowship from DIM STEM POLE.
Manuscript received on September 16, 2010. Revised version arrived on December 23, 2010. Manuscript accepted on January 11, 2011.
Correspondence: Bruno Azzarone, INSERM, UMR 1014, Bâtiment Lavoisier. Hôpital Paul Brousse, 14, Avenue Paul Vaillant Couturier. 94807 Cedex. Villejuif, France. Phone: international +33.0145595343. Fax: international + 33.0145595344. E-mail: bazzarone@hotmail.com

interplay whose *in vivo* significance has not yet been explored.

Design and Methods

Purification of CD34+ PB-HP and commitment to the NK pathway

CD34+ cells were purified (>95%) from healthy donors' buffy coats (CD34+ PB-HP) as previously described (12 by a direct immunomagnetic method; Miltenyi Biotech, Bergisch Germany). PB-HP were then committed to the NK pathway expanding the cells at 2×10^5cells/mL, in STEMα–A medium (Stem Alpha, Saint Clement les Places, France) supplemented with 100 ng/ml of SCF and FLT-3L (Immunotools, Friesoythe, Germany).

Information about mRNA extraction and cDNA synthesis can be found in the *Online Supplementary Appendix*.

Cell phenotype and cytokine detection by flow cytometry

Cell surface antigen expression was evaluated on suspensions of living cells by cytofluorimetry analysis as previously described.[11] In some experiments, acidic shock was performed incubating human PB-HP in ice-cold 0.1 M sodium acetate (pH 3.5) for 15 min at 4°C in order to determine the properties of the membrane-bound IL-15 expressed by these cells. Cytokine production was validated by intracellular detection using the Cytofix/Cytoperm reagent according to the manufacturer's instructions (R&D Systems). Flow cytometric analysis was performed using FACScalibur (Becton Dickinson, Saint Quentin Yvelines, France) using CellQuest (BD Biosciences) and FlowJo (Tree Star, San Carlos, California, USA) softwares. At least 5,000 events were analyzed in each test.

Cell morphology was assessed on cytospin preparations after using a RAL 555 staining Kit (Abcells, Tampere, France). Pictures were obtained with an Olympus microscope (Arcueil, France) equipped with a 40x objective lens using a CoolPix 995 digital camera (Nikon Paris, France).

Mixed lymphocyte reaction (MLR)

Allogeneic PBL were labeled with 1 μM of 5,6-carboxyfluorescein diacetate-succinimyl ester (CFSE) in PBS/0.1% BSA for 10 min at 37°C and washed with complete medium. Cells were then seeded in 96-well flat bottom microtiter plates (Costar) at 1×10^6/mL in complete culture medium with mitomycin treated NK-DC (NK-DC/PBL ratio 1/10). Proliferation was analyzed by flow cytometry after five days.

Flow cytometry and cytolytic degranulation

NK-DC cells were tested for their cytolytic potential after NCR or CD16 cross-linking by quantifying surface expression of CD107a, and intracellular IFN-γ, as previously described.[10] Monensin (10 μM) was added for the last 4 h to inhibit cytokine secretion. Cells were analyzed using a FACScan flow cytometer using CellQuest Analysis software (BD Biosciences).

Confocal laser-scanning microscopy

Nuclear localization of phospho-STAT6 and GATA3 was analyzed by confocal microscopy on PB-NKp. Cell permeabilization, intracellular staining and processing for confocal microscopy were performed as previously described.[11] The slides were examined by confocal laser microscopy (Leica TCS Confocal System, Wetzler, Germany).

Western blotting

PB-NKps were stimulated for 15 min with 10 ng/ml of human soluble IL-15Rα/Fc Chimera (sIL-15Rα R&D Systems, Lille, France) for phosphorylation of the MAP kinase ERK1/2 and of the Focal Adhesion Kinase (FAK). Total cell lysates were subsequently processed for Western blot analysis as previously described.[11] Immunodetection of the protein blotted was determined using a Fujifilm Intelligent Dark Box II. ERK1/2 and FAK proteins were used as internal control.

Quantitative PCR

GATA-1, MCL-1, PU.1, IL-2Rβ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA levels were determined by using Light Cycler-based kinetic quantitative PCR (Roche Diagnostics). GATA-1, MCL-1, PU.1, IL-2Rβ and GAPDH PCR products were detected by using FastStart DNA Master hybridization probes (Roche Diagnostics). To correct for variations in RNA recovery and the reverse transcription yield, the amounts of GATA-1, MCL-1, PU.1, IL-2Rβ, cDNA were divided by the amount of GAPDH.[12] Results were expressed as fold increases of normalized values, over the level observed with untreated cells.

Results and Discussion

Human peripheral blood hematopoietic progenitors (PB-HP) express a membrane bound IL-15 (mbIL-15) able to deliver a bidirectional signaling

Flow cytometry analysis (*Online Supplementary Figure S1A*) shows that PB-HP display a membrane bound IL-15 (mb-IL-15) that cannot be removed after exposure to an acidic buffer (pH 3.5). This indicates that this form of mbIL-15 is retained at the membrane through an IL-15R-independent mechanism theoretically able to deliver a bi-directional signal.[13,14] Analysis of *Online Supplementary Figure S1B* confirms our previous results[10] showing that PB-HP, expanded at high cell density (2×10^5 cells/mL) in the presence of stem cell factor (SCF) and FL, progressively lose the expression of the early hematopoietic markers CD34 and CD38 through reciprocal mbIL-15 *trans*-presentation.[10] Moreover, this culture condition is necessary and sufficient to commit these cells into the NK differentiation pathway in the absence of exogenous lymphokines, as shown by the progressive acquisition of the different functional markers that define the mature NK cells.[10] The differentiation process is accomplished within three weeks. During this period the NK progenitors (PB-NKp) expand in suspension doubling their number (with less than 0.3% of adherent cells) and maintain the expression of the mbIL-15.

Analysis of *Online Supplementary Figure S1C* shows that stimulation of the mbIL-15, expressed by 18-day old NK progenitors, with the soluble IL-15Rα (sIL-15Rα) chain triggers a signal transduction represented by the phosphorylation of the MAP Kinase ERK1/2 and of the Focal Adhesion Kinase (FAK) confirming, therefore, the ability of this isoform of mbIL-15 to deliver a reverse signal in response to its soluble specific ligand.[13,14]

Mb-IL-15-dependent reverse signal induces in PB-NK progenitors the generation of an adherent subset sharing NK and DC specific markers

Subsequently we investigated if the induction of an mbIL-15-dependent reverse signal would modify the phenotype of these 18-day old NK progenitors and we observed within 3-5 days a double effect.

Indeed, flow cytometry analysis (Figure 1A left panel)

shows that about 80% of these 3-week old NK progenitors stay in suspension losing the expression of several NK markers (CD56, CD16, CD161, CD94), while they maintain the expression of CD11c, NKp44 and of NKp46 suggesting that they acquire a phenotype and morphology (Figure 1A, right panel) of immature NK progenitors. These cells lose their regulatory properties without acquiring lytic functions (*data not shown*).

At the same time, we observed that the treatment with the sIL-15Rα chain causes the appearance of an adherent subset (Figure 1B, right panel) which represents about 20% of the total population, recalling previous results showing that ligation of mb-IL-15 induces monocyte adhesion.[13] Flow cytometry analysis (Figure 1B left panel) of the above mentioned adherent subset shows that these cells express the NK cell-specific marker NKp46[15] and myeloid DC specific markers CD1a[16] and BDCA1.[17] In addition, these cells express several other NK markers (CD56, CD16, CD161, NKp44 and perforin) as well as markers of immune activation (CD11c, HLA-DR, CD80, CD86, CD25) common to both cell types.[18] Dot plot analysis (Figure 1C left panel) shows that this adherent subset co-expresses CD80 and CD16 and, more importantly, CD1a and NKp46. Subsequently we analyzed the acquisition of cytotoxicity-associated functions in this "putative NK-DC" population activated by cross-linking of CD16 or NCR molecules. Flow cytometry shows that after CD16 cross-linking these cells become within three hours a source of cytolytic mediators, as shown by the surface expression of the degranulation marker CD107a, as well as within 24 h of IFNγ a powerful inducer of cytolytic activity of natural killer cells (Figure 1C, right panel). These cells do not proliferate and after detachment cannot be expanded, suggesting that they are terminally-differentiated cells. On the other hand, the adherent subset does not stem from the subpopulation that continues to grow in suspension after

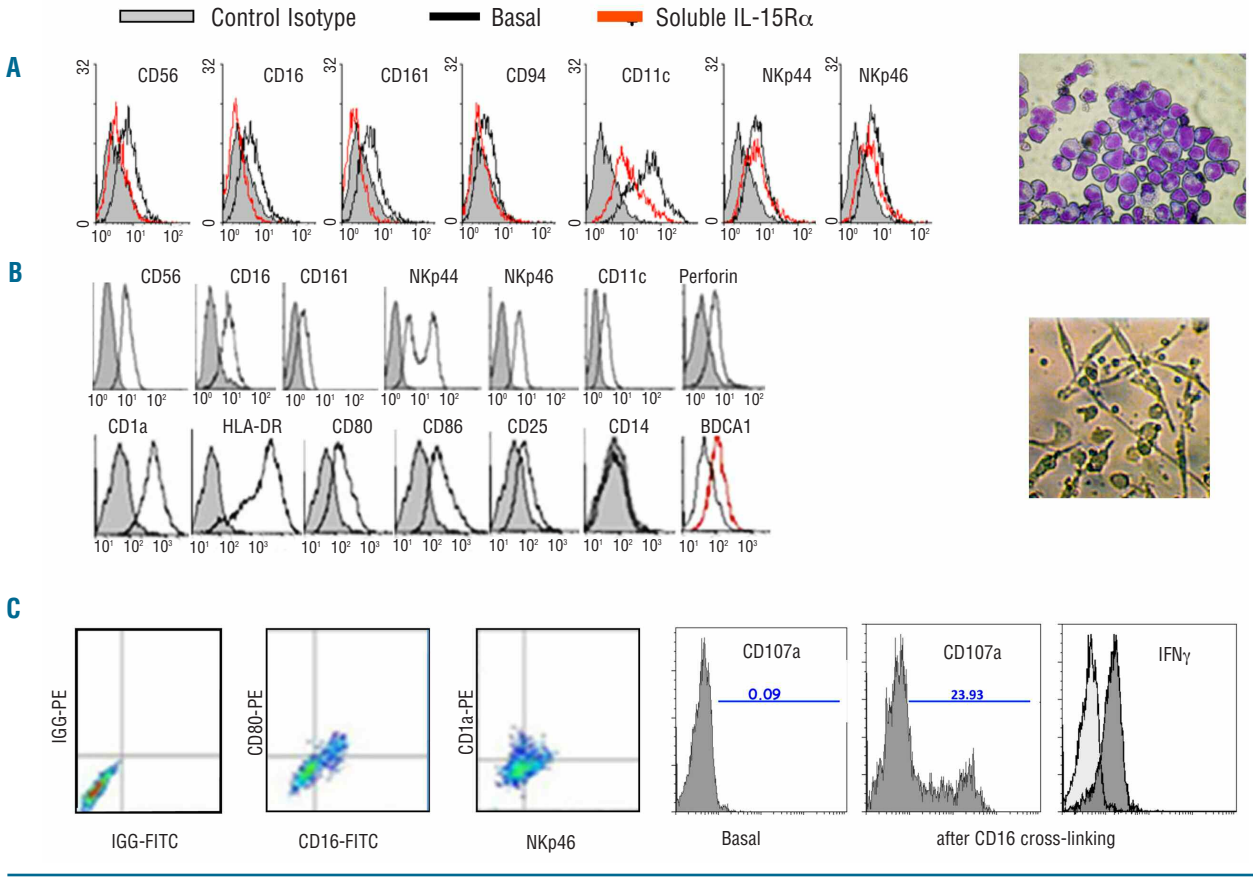

Figure 1. Mb-IL-15-dependent reverse signal induces in PB-NK progenitors the generation of an immature NK subset growing in suspension and of an adherent subset sharing NK and DC specific markers. (A) Left panel: effects of 96 h pre-incubation of 18-day old PB-NKp with the soluble IL-15Rα chain (10 ng/mL). Phenotype was evaluated by flow cytometry analyzing several NK markers (CD16, CD56, CD94, CD161, CD11c, NKp44, NKp46). Shadowed peaks represent staining with isotype control Abs. Black open peaks represent staining with the indicated Abs. red open peaks represent cultures treated with the sIL-15Rα chain. The data are representative of 3 separate experiments. Right panel: Ral 555 staining on these immature NK cells. (B) Left panel: Flow cytometric analysis of the adherent subset generated by 96 hours stimulation of 18-day old PB-NKp with sIL-15Rα (10 ng/mL). Phenotype was evaluated by flow cytometry analyzing several NK markers (CD56, CD16, CD161, NKp44, NKp46 CD11C, perforin) and myeloid DC markers (CD1a, HLA-DR, CD80, CD86, CD25, CD14, BDCA11). Shadowed peaks represent isotype control. Black open peaks represent staining with the indicated Abs. Red open peaks represent cultures treated with the sIL-15Rα chain. Right panel: Ral 555 staining of this adherent subset. One experiment representative of 5 is shown. (C) Left panel: Dot Plot analysis of CD16-FITC/CD80-PE and NKp46-FITC/CD1a-PE co-expression in the adherent cell subset generated by 96h stimulation of 18-day old PB-NKp with sIL-15Rα (10ng/mL). These data are representative of 3 independent experiments. Right panel: induction of cytolytic markers. Flow cytometric analysis shows that CD16 cross-linking performed overnight on the adherent cell subset generated by stimulation of 18-day old PB-NKp with sIL-15Rα, induces the surface expression of the degranulation marker CD107a and the intracellular production of IFNγ (gray peaks). These data are representative of 3 independent experiments.

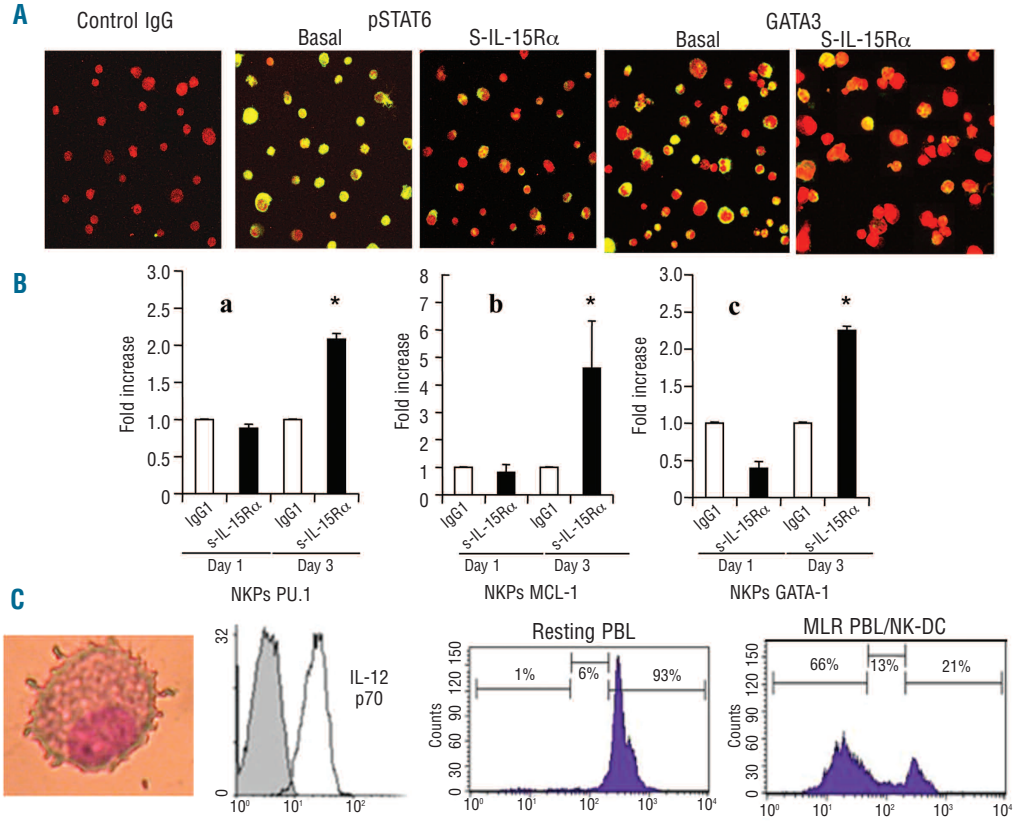

Figure 2. Mb-IL-15-dependent reverse signal induces in PB-NK progenitors the modulation of transcription factors involved in the control of myelopoiesis. (A) Effects of 24 h pre-incubation of 18-day old PB-NKp with the soluble IL-15Rα chain (10 ng/mL) on the nuclear localization (yellow staining) of phospho-STAT6 or GATA3 by confocal microscopy. These cells were fixed, permeabilized, and stained for phospho-STAT6 or GATA3 transcription factors (green). Nuclei were stained by propidium iodide (red). As negative controls, cells were incubated with rabbit IgG, second reagent, and propidium iodide. These data are representative of 3 independent experiments. (B) Quantitative RT-PCR analysis in PB-NKp of PU.1 (a), MCL-1 (b) and GATA-1 (c). Control IgG1 (empty bars), treatment with soluble IL-15R α chain (black bars); bars represent the mean±s.d. obtained from triplicate measurements. These data are representative of 3 independent experiments. * *P* values (*P*<0.05) between control and treated cultures were calculated with Student's t-test. (C) Left panel Ral 555 staining of an adherent cell exhibiting a typical a dendritic morphology. Central panel: flow cytometric analysis shows that pre-incubation of 18-day old PB-NKp with the soluble IL-15Rα chain (10 ng/mL/) induces within 96 h the intracellular production of IL-12 (open black peaks). Right panel Induction of mixed lymphocyte reaction (MLR). CFSE staining of human PBL co-cultured or not (resting) with NK-DC cells.

acquiring the phenotype of immature NK cells, since replanting of these cells does not generate adherent cells (*data not shown*). These data suggest that the PB-NK progenitors generated through reciprocal *trans*-presentation of their mbIL-15, though displaying after 18 days an homogenous NK phenotype, possibly represent a heterogeneous population that contains distinct subsets: the first is committed to NK differentiation and constitutes the majority, while the second exhibits a greater flexibility even at an advanced stage of NK differentiation, preserving a double differentiation potential.

Mb-IL-15-dependent reverse signal induces in PB-NK progenitors the modulation of transcription factors involved in myeloid differentiation

Finally, we tried to determine the molecular mechanisms by which mbIL-15 induces PB-NKp differentiation. Since myelopoiesis is regulated through the balance between negative and positive signals,[19-22] we analyzed whether the generation of the putative NK/DC subset was preceded by modifications of both negative and positive regulators of myeloid development.

Thus, the conversion of NKPs into myeloid-like cells induced by the soluble IL-15Rα chain was checked by confocal microscopy investigating the modulation of the transcription factors STAT-6 and GATA-3 which inhibit myeloid commitment.[19,20] Analysis of Figure 2A shows that stimulation of 18-day old NK progenitors with the sIL-15Rα chain causes, within 24 h, the loss of the constitutive nuclear localization of STAT-6 and GATA-3 (disappearance of the nuclear yellow staining), which obviously leads to the loss of the specific transcriptional functions.

Concerning the positive regulation of myeloid development, it has recently been shown that Toll-like receptor (TLR) agonists shift the differentiation of lineage-committed hematopoietic progenitors to favor myelopoiesis through the upregulation of the transcription factors PU.1, and GATA-1.[21] Thus, we used real-time PCR (RT-PCR) to investigate if the stimulation of mbIL-15-with its soluble ligand (IL-15Rα) could generate similar effects in PB-NKps. Analysis of Figure 2B shows that NKPs constitutively express the transcripts for GATA-1, PU.1 and that stimulation with the soluble IL-15Rα chain induced within three days a transient increase in the transcription rates of PU.1 (2-fold) and of GATA-1 (2-fold). In these experiments, control IgG1 did not modify the expression of these markers. Interestingly, we also detected a strong increase (4-fold) in the expression of the anti-apoptotic factor MCL-1, whose ERKs-dependent upregulation, during the induction of monocytic differentiation provides a means through which enhancement of viability may be linked to the induction of myeloid development.[22]

After modulation of the transcription factors which favor myeloid development these non-classical adherent killer cells display a typical dendritic morphology (Figure 2C, left panel), as well as the constitutive production of IL-12: a typ-

ical cytokine secreted by professional antigen-presenting cells (APC), which is necessary for the activation of NK cells and for their production of IFNγ (Figure 2C, central panel) Finally, these cells acquire a typical APC function such as the induction, upon co-culture, of the proliferation of naïve allogeneic PBL (Figure 2C, right panel). Indeed, flow cytometry shows that, after vital CFSE staining, naïve resting PBL display a single peak of highly fluorescent cells. Upon co-culture with NK-DC cells, almost 80% of PBL proliferate undergoing one (13%) or two (66%) population doublings as shown by the appearance of peaks with decreased fluorescence intensity.

From a functional point of view, the existence of cells displaying both NK and DC properties could have a relevant impact on early defences against cancer and infections. In this context, there have been recent reports of the presence within mouse solid tumors of non-classical killer cells displaying both NK and DC properties and powerful tumor killing activity whose development depends on IL-15 *trans*-presentation.[23] On the other hand, there have been recent reports of the capacity of topical TLR7 agonists to mediate the recruitment and differentiation of human myeloid and plasmacytoid DC into killer cells displaying tumoricidal activity against human basal cell carcinomas.[24] Thus, our data showing the generation of adherent cells that display both myeloid DC and NK markers and functions suggest the possible existence of human "non-classical killer like cells" that could have relevance in anti-cancer response.[18,23]

However, it must be stated that this novel NK/DC subset is generated through a sequential mbIL-15-dependent double hit represented by a *trans*-presentation process and subsequently by the activation of a reverse signal triggered by the soluble IL-15Rα chain.

Therefore, the possibility of activating this double hit *in vivo* is certainly a very remote event since the mb-IL-15 competent for bi-directional signaling is only expressed by PB-HP; thus this type of commitment cannot take place at a tissular level. On the other hand, the very low frequency of PB HP (less than 0.1%) minimizes the chances of triggering their commitment into NK differentiation through an efficient reciprocal IL-15 *trans*-presentation.

In conclusion, our data essentially highlight the existence of a subset of circulating NK progenitors that, owing to their great flexibility and plasticity, can theoretically be switched to an alternative differentiation program, even when they exhibit a phenotype of fully mature NK cells.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Akashi K, Traver D, Miyamoto T and Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature. 2000;404(6774): 193-7.
2. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. J Exp Med. 2000;191(5):771-80.
3. Perez SA, Sotiropoulou PA, Gkika DG, Mahaira LG, Niarchos DK, Gritzapis AD, et al. A novel myeloid-like NK cell progenitor in human umbilical cord blood. Blood. 2003;101(19):3444-50.
4. Bykovskaia SN, Buffo M, Zhang H, Bunker M, Levitt ML, Agha M, et al. The generation of human dendritic and NK cells from hemopoietic progenitors induced by interleukin-15. J Leukoc Biol. 1999;66(4):659-66.
5. Freud AG, Becknell B, Roychowdhury S, Mao HC, Ferketich AK, Nuovo GJ, et al. A human CD34(+) subset resides in lymph nodes and differentiates into CD56bright natural killer cells. Immunity. 2005;22(3): 295-304.
6. Guimond M, Freud AG, Mao HC, Yu J, Blaser BW, Leong JW, et al. In vivo role of Flt3 ligand and dendritic cells in NK cell homeostasis. J Immunol. 2010;184(6):2769-75.
7. Mortier E, Woo T, Advincula R, Gozalo S, Ma A. IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. J Exp Med. 2008;205(5):1213-25.
8. Huntington ND, Legrand N, Alves NL, Jaron B, Weijer Ket, Plet A, al. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. J Exp Med. 2009;206(1):25-34.
9. Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, et al. Effector and regulatory events during natural killer-dendritic cell interactions. Immunol Rev. 2006; 214:219-28.
10. Giuliani M, Giron-Michel J, Negrini S, Vacca P, Durali D, Caignard A, et al. Generation of a novel regulatory NK cell subset from peripheral blood CD34+ progenitors promoted by membrane-bound IL-15. PLoS One. 2008;3(5):e2241.
11. Giron-Michel J, Caignard A, Fogli M, Brouty-Boye D, Briard D, van Dijk M, et al. Differential STAT3, STAT5, and NF-kappaB activation in human hematopoietic progenitors by endogenous interleukin-15: implications in the expression of functional molecules. Blood. 2003;102(1):109-17.
12. Durali D, de Goer de Herve MG, Giron-Michel J, Azzarone B, Delfraissy JF, Taoufik Y. In human B cells, IL-12 triggers a cascade of molecular events similar to Th1 commitment. Blood. 2003;102(12):4084-9.
13. Neely GG, Epelman S, Ma LL, Colarusso P, Howlett CJ, Amankwah EK, et al. Monocyte surface-bound IL-15 can function as an activating receptor and participate in reverse signaling. J Immunol. 2004;172(7):4225-34.
14. Budagian V, Bulanova E, Orinska Z, Pohl T, Borden EC, Silverman R, et al. Reverse signaling through membrane-bound interleukin-15. J Biol Chem. 2004;279(40): 42192-201.
15. Walzer T, Jaeger S, Chaix J, Vivier E. Natural killer cells: from CD3(-)NKp46(+) to post-genomics meta-analyses. Curr Opin Immunol. 2007;19:(3):365-72.
16. Cernadas M, Lu J, Watts G, Brenner MB. CD1a expression defines an interleukin-12 producing population of human dendritic cells. Clin Exp Immunol. 2009;155(3):523-33.
17. Tsoumakidou M, Tzanakis N, Papadaki HA, Koutala H, Siafakas NM. Isolation of myeloid and plasmacytoid dendritic cells from human bronchoalveolar lavage fluid. Immunol Cell Biol. 2006;84(3):267-73.
18. Spits H and Lanier LL. Natural Killer or Dendritic: What's in a Name? Immunity 2007;27(1):11-6.
19. Bunting KD, Yu WM, Bradley HL, Haviernikova E, Kelly-Welch AE, Keegan AD, et al. Increased numbers of committed myeloid progenitors but not primitive hematopoietic stem/progenitors in mice lacking STAT6 expression. J Leukoc Biol. 2004;76(2):484-90.
20. Chen D, Zhang G. Enforced expression of the GATA-3 transcription factor affects cell fate decisions in hematopoiesis. Exp Hematol. 2001;29(8):971-80.
21. De Luca K, Frances-Duvert V, Asensio MJ, Ihsani R, Debien E, Taillardet M, et al. The TLR1/2 agonist PAM(3)CSK(4) instructs commitment of human hematopoietic stem cells to a myeloid cell fate. Leukemia. 2009;23(11):2063-74.
22. Townsend KJ, Zhou P, Qian L, Bieszczad CK, Lowrey CH, Yen A, et al. Regulation of MCL1 through a serum response factor/Elk-1-mediated 16 mechanism links expression of a viability-promoting member of the BCL2 family to the induction of hematopoietic cell differentiation. J Biol Chem. 1999;274(3):1801-13.
23. Bonmort M, Dalod M, Mignot G, Ullrich E, Chaput N, Zitvogel L. Killer dendritic cells: IKDC and the others. Curr Opin Immunol. 2008;20(5):558-65.
24. Stary G, Bangert C, Tauber M, Strohal R, Kopp T, Stingl G. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. J Exp Med. 2007;204(6):1441-51.