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whereas CD56^{bright} cells are believed to be immunoregulatory actors (10, 11). Here, we present studies that characterize the effects of IL15 infusions on various aspects of NK-cell function in cancer patients. We show that IL15 infusions were associated with over 350-fold expansion of the CD56^{bright} NK-cell subpopulation and a 20-fold expansion of the CD56^{dim} NK subpopulation. The CD56^{bright} cells showed increased proliferation that may have been caused by higher CD122 expression. Population expansion rates were more than 10-fold higher in the CD56^{bright} subset following treatment when compared with other NK-cell subsets resulting in nearly equal numbers of both types of cells present in the peripheral blood after 10-day IL15 infusions. In addition, CD56^{bright} NK cells acquired the capability to respond to target cell activation by both increased cytokine production and cytotoxicity as a result of the treatment. These data suggest that CD56^{bright} NK cells generated by IL15 infusions have a potential to respond to tumors sensitive to NK cells.

We analyzed cytokine production of NK-cell subsets. CD56^{bright} NK cells respond to cytokine or PMA/Ionomycin stimulations with the generation of several cytokines (12, 25, 26, 29). We observed that this activity was even more pronounced after IL15 infusions. The effect could be explained by increased cytokine receptor expressions, but additional intracellular changes are likely to exist as suggested by the improved responses to PMA/Ionomycin that did not require receptor expression. In addition, cytokine generation was only observed after coincubations with NK target cells after IL15 infusions. This suggests that treatment induced expression of receptors, normally absent on CD56^{bright} NK cells, that could recognize ligands on tumor cells. In the case of antibody-coated target cells, this improved recognition may involve the Fc receptor CD16, expression of which was upregulated after IL15 infusion. IL15 treatment–induced CD56^{bright} NK cells also responded to K562 cells. The receptors that recognize this cell line appear to be more complex (27, 28) and may involve NKp30, NKp46, and NKG2D, expression of which had also been increased after IL15 infusions. Thus, multiple mechanisms are implicated in increased cytokine responses in IL15 infusion-induced CD56^{bright} NK cells.

We analyzed surface marker expression changes that were induced by IL15 infusions. We observed that changes in both subpopulations contributed to an increased phenotype similarity between them. We believe that this is most consistent with activities that were induced by higher concentrations of IL15 that had been achieved after infusions when compared with physiological steady-state levels. Such higher cytokine concentrations could have activated a signaling pathway with similar signaling strength in both cell types to result in similar expressions of some surface molecules. Infusions also caused an increase of CD62L^{-} and a decrease of CD57^{-}expressing populations among CD56^{dim} cells as well as a decrease of the CD27^{+} population among CD56^{bright} cells, suggesting that IL15 treatments induced expansion of less mature NK-cell populations (30–34).

Many phenotypic changes that were induced by IL15 infusions are also found after IL15-induced *ex vivo* cultures of NK cells (14, 15, 35–37). These include increases of NKp30, NKp46, NKG2D, Trail, and CXCR3, and decreases of CD57, CD27, and CCR7. One exception, however, exists. Cultured NK cells typically upregulate markers of activation including CD69, CD25, NKp44, and DNAM1 (14, 15, 38). We did not observe inductions of these markers after IL15 infusions. Because bona fide activating ligands are unlikely to be induced by culturing, the discrepancy between culture and infusions is most consistent with inhibitory mechanisms *in vivo* that are lost during culturing resulting in NK-cell activation *in vitro* only. A lack of NK-cell activation in IL15-infused cancer patients also suggests that no encounters between cancer and the analyzed blood NK cells had occurred that would have caused their activation.

The relationship between CD56^{dim} and CD56^{bright} NK cells remains a matter of debate. Most publications suggest CD56^{bright} NK cells represent a developmentally precursor stage (10). Others describe the ability to derive CD56^{bright} from CD56^{dim} via NK-cell activation (39, 40). Along this line, the increased presence of CD56^{bright} NK cells following IL15 treatments could have been caused by either different expansion rates of both cell subsets or a conversion of CD56^{dim} into CD56^{bright} NK cells. Most of our findings support the former: Analyses *ex vivo* showed increased proliferation rates in the CD56^{bright} subset (Fig. 1B). In addition, *in vitro* proliferation assays point to an increased ability of CD56^{bright} NK cells to proliferate in response to IL15 (Supplementary Fig. S1). Moreover, one would expect that descendants of a recent CD56^{dim} to CD56^{bright} conversion would retain some of the phenotypical markers of their precursor cells. Most of our data appear to suggest the opposite in that the majority of surface markers that are distinct between both subsets were expressed at similar levels on CD56^{bright} NK cells before and after treatments. These include CXCR3, CX3CR1, CD16, NKG2A, and CD158b (Fig. 2; Supplementary Fig. S2). CD25 and CCR7 are exceptions in that their expressions were downregulated on posttreatment CD56^{bright} NK cells to levels similar of CD56^{dim} NK cells. In summary, our data suggest that the predominant appearance of CD56^{bright} NK cells in response to IL15 treatments resulted mainly from a superior ability of this subset to proliferate.

An issue for all NK-cell–based cancer immunotherapy targeting solid tumors is their ability to migrate from blood to tumor sites. We had analyzed the expression of several chemokine receptors whose involvement in tumor-directed migration has been reported (5, 41, 42). No clear pattern emerged. Expression of CXCR3 and CX3CR1 increased, expression of CCR7 decreased, and expression of CXCR1 remained unchanged. Further studies are necessary to delineate migration patterns of IL15 infusion–induced NK cells.

Figure 3.

IL15 infusions sensitize NK cells to respond with cytokine production. PBMCs were stimulated with PMA/Ionomycin (**A**), IL12/IL18 (**B**), or coincubated with NK target cells (**C**), and intracellular cytokine amounts were determined by FACS on gated CD56^{dim} (CD3^{-}/CD16^{+}/CD56^{dim}) or CD56^{bright} (CD3^{-}/CD56^{bright}) NK cells. IL15 infusions caused increases of IFNγ, TNFα, and GM-CSF productions in PMA/Ionomycin- or IL12/IL18-responding NK cells within the CD56^{bright} subset. Within the CD56^{dim} subset, IFNγ production was increased after IL12/IL18 stimulation. CD56^{bright} NK cells also acquired the ability to respond to target cell exposure by cytokine production after IL15 infusions, whereas little change was seen for target cell–exposed CD56^{dim} NK cells. **D** shows that IL15 infusions had increased the expression of surface IL18 receptor on CD56^{dim} NK cells to levels lower than those on CD56^{bright} NK cells. We observed no IL12 receptor expression changes. Analyses were done once on each of 5 patients. Graphs depict mean + SD. *, $P < 0.05$ and **, $P < 0.01$.

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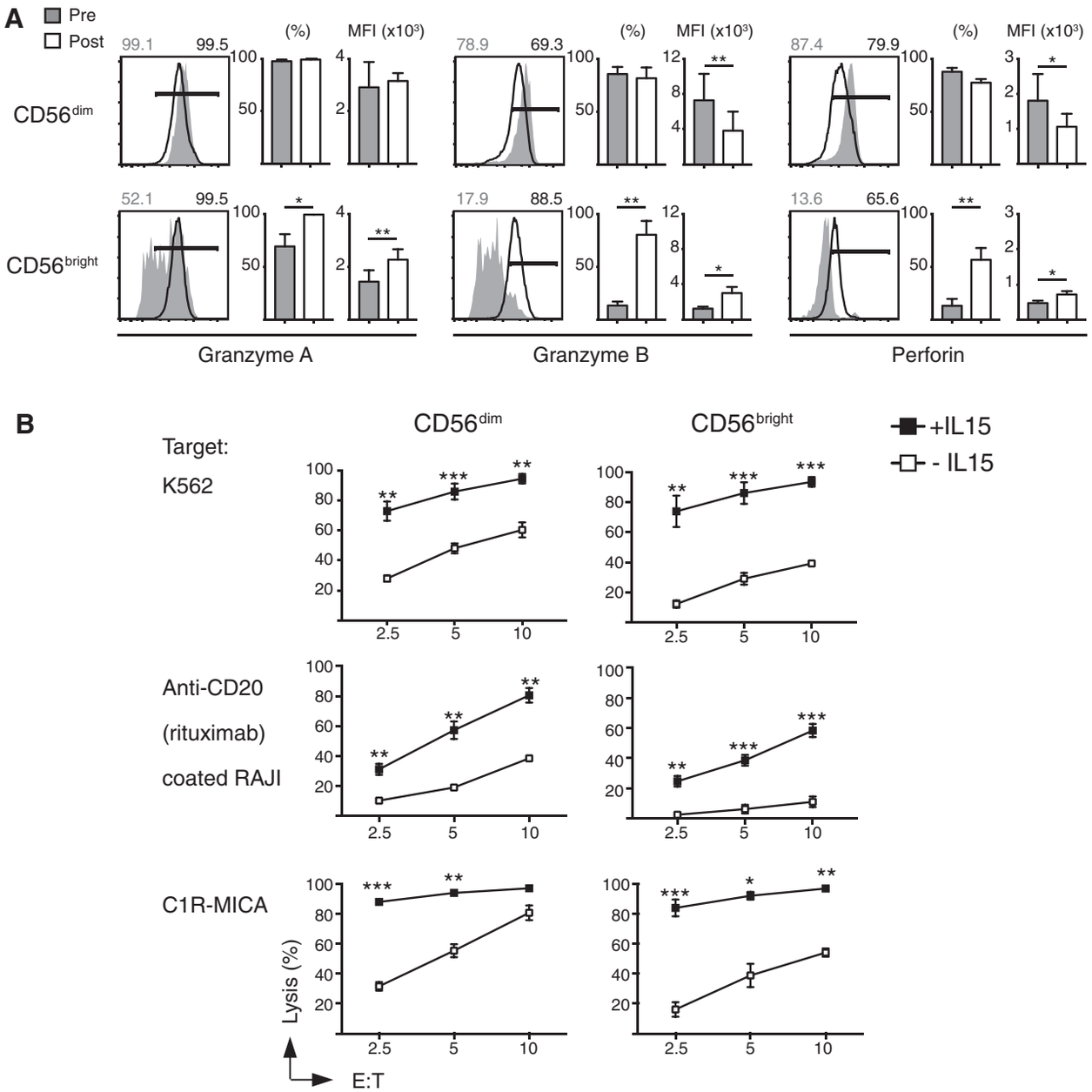

Figure 4.

IL15 infusions increase cytotoxic activities in both NK-cell subsets. **A,** Intracellular stains of gated NK subsets indicate decreases of cytotoxic molecules in CD56^{dim} NK cells, whereas the amounts of granzymes A, B, and perforin increased in CD56^{bright} NK cells after IL15 infusions. **B,** IL15 infusions augmented cytolytic activities for both sorted NK-cell subsets against three different target lines. Analyses were done once on each of 5 patients. Graphs depict mean ± SD. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

We observed improved cytotoxic activities for both CD56^{bright} and CD56^{dim} NK cells against diverse target cell lines with various recognition requirements. Several changes in CD56^{bright} cells that had been caused by IL15 treatments could account for this increase: Besides improved recognition of target cells, these cells had increased intracellular amounts of the molecules Granzyme A, Granzyme B, and Perforin that are used for killing target cells. This was not the case for CD56^{dim} NK cells that also showed increased killing activities despite lower amounts of granzymes and perforin. However, increased amounts of membrane-bound death receptor ligand Trail may contribute to stronger cytotoxic responses as may the improved recognition via NKG2D for MICA-expressing targets, NKp30 and NKp46 for K562. Thus, the goal of inducing augmented cytotoxic NK-cell activities by IL15 infusions was achieved that may suggest a potential utility in cancer immunotherapy.

In summary, continuous infusions of IL15 in cancer patients is accompanied by a preferential expansion of populations of phenotypically immature CD56^{bright} NK cells with increased abilities to recognize tumor cells and react by cytokine production and cytotoxicity. On the basis of the present study, IL15, by increasing NK-cell numbers and their cytotoxic capacity, may increase the efficacy of anticancer therapies. IL15 could be useful in cases of tumors that have deleted Class I MHC where direct NK cytotoxicity may be effective. IL15 may be useful in combination with therapies that are based on antitumor antibodies of which the effects include tumor killing via ADCC. To translate this opportunity in the treatment of leukemia, we have initiated a clinical trial using IL15 with anti-CD52 alemtuzumab (NCT02689453), and we are planning to combine IL15 with anti-CCR4 mogamulizumab in patients with HTLV-1–associated adult T-cell leukemia/lymphoma (43).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Dubois, J.R. Müller, T.A. Waldmann
Development of methodology: S. Dubois, J.R. Müller
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Dubois, K.C. Conlon, J. Hsu-Albert, T.A. Waldmann
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Dubois, J.R. Müller
Writing, review, and/or revision of the manuscript: S. Dubois, K.C. Conlon, J.R. Müller, T.A. Waldmann
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Beltran, B.R. Bryant
Study supervision: T.A. Waldmann

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Cancer Immunology Research

IL15 Infusion of Cancer Patients Expands the Subpopulation of Cytotoxic CD56 ^{bright} NK Cells and Increases NK-Cell Cytokine Release Capabilities

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