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The Notch Ligands Jagged2, Delta1, and Delta4 Induce Differentiation and Expansion of Functional Human NK Cells from CD34$^+$ Cord Blood Hematopoietic Progenitor Cells

Rose C. Beck,[1] Mallika Padival,[1] David Yeh,[1] Justine Ralston,[1] Kenneth R. Cooke,[2] John B. Lowe[1]

Notch receptor signaling is required for T cell development, but its role in natural killer (NK) cell development is poorly understood. We compared the ability of the 5 mammalian Notch ligands (Jagged1, Jagged2, Delta1, Delta3, or Delta4) to induce NK cell development from human hematopoietic progenitor cells (HPCs). CD34$^+$ HPCs were cultured with OP9 stromal cell lines transduced with 1 of the Notch ligands or with OP9 stromal cells alone, in the presence of IL-7, Flt3L, and IL-15. Differentiation and expansion of CD56$^+$CD3$^-$ cells were greatly accelerated in the presence of Jagged2, Delta-1, or Delta-4, versus culture in the absence of ligand or in the presence of Jagged1 or Delta3. At 4 weeks, cultures containing Jagged2, Delta1, or Delta4 contained 80% to 90% NK cells, with the remaining cells being CD33$^+$ myelogenous cells. Notch-induced NK (N-NK) cells resembled CD56^{bright} NK cells in that they were CD16$^-$, CD94$^-$, CD117$^+$, and killer immunoglobulin-like receptors (KIR$^-$). They also expressed NKp30, NKp44, NKp46, 2B4, and DNAM-1, with partial expression of NKG2D. The N-NK cells displayed cytotoxic activity against the K562 and RPMI-8226 cell lines, at levels similar to activated peripheral blood (PB) NK cells, although killing of Daudi cells was not present. N-NK cells were also capable of interferon (IFN)-γ secretion. Thus, Notch ligands have differential ability to induce and expand immature, but functional, NK cells from CD34$^+$ HPCs. The use of Notch ligands to generate functional NK cells in vitro may be significant for cellular therapy purposes.

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INTRODUCTION

Natural killer (NK) cells are lymphocytes that arise from common lymphogenous progenitor cells during hematopoiesis. Unlike T and B lymphocytes, however, NK cells do not express rearranged antigen receptors and are therefore considered part of the innate immune system. NK cells have the unique ability to kill tumor or virally infected cells without prior immunization. Another major function of NK cells is to secrete cytokines, such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which augment inflammatory immune responses and also influence adaptive immunity. Human NK cells are defined as CD56$^+$CD3$^-$ lymphocytes, which morphologically are indistinguishable from CD8$^+$ granular T cells. The circulating pool of NK cells in humans consists of 2 populations: CD56^{dim}CD16^{bright} and CD56^{bright}CD16^{dim}. The former population is predominant (>95% of circulating NK cells), has more cytotoxic than cytokine-producing activity, and may represent a mature NK cell population, which evolves from CD56^{bright}CD16^{dim} cells [1]. Accordingly, CD56^{bright} NK cells express CD117, higher levels of CD94 (which heterodimerizes with NKG2A (Natural Killer Group 2, member A) or NKG2C to form HLA-E recognition receptors), and CCR7, which may allow for NK cell trafficking to lymph nodes [2]. In contrast, CD56^{dim} NK cells express higher levels of killer immunoglobulin-like receptors (KIRs) and the natural cytotoxicity receptors (NCRs), which include NKp30, NKp44, and NKp46, important for tumor cell recognition and lysis. CD56^{dim} NK cells downregulate expression of CCR7 and express CXCR1, which binds IL-8 and is postulated to

From the [1]Departments of Pathology; and [2]Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio.
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Correspondence and reprint requests: Rose C. Beck, MD, PhD, Department of Pathology, WRB 6526, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106 (e-mail: rose.beck@case.edu).
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aid in the $CD56^{dim}$ NK cell response to inflammation [2]. Members of the KIR family are critical for transmission of inhibitory signals to NK cells upon binding of self-MHC class I molecules, thus mediating self-tolerance of $CD56^{dim}$ NK cells. Expression of CD16, also known as the low-affinity IgG Fc receptor, also allows $CD56^{dim}$ NK cells to mediate antibody-dependent cell-mediated cytotoxicity in the presence of the antibody specific to target cells. Both types of NK cells express NKG2D, a homodimeric activating receptor that binds stress-inducible ligands on tumor cells.

It has been traditionally postulated that human NK cells develop in the bone marrow (BM), but recent data suggest that NK cell precursors from the BM may migrate to secondary lymphogenous organs, where maturation of NK precursors into $CD56^{bright}$ NK cells can occur. The NK cell precursors identified in lymph nodes have been divided into subsets based on expression of CD34, CD94, and CD117 [3-5]. In line with this hypothesis, $CD56^{bright}$ NK cells are found preferentially in lymph nodes and can express CD16, KIRs, and NCRs, as well as acquire cytotoxic activity, upon activation [6,7].

In vitro, both mouse and human NK cells can differentiate from BM- or cord blood-derived hematopoietic progenitor cells (HPCs) in the presence of cytokines alone; these NK cells appear phenotypically immature and resemble $CD56^{bright}$ cells [8]. Culture of HPCs in the presence of both cytokines and stromal cells allows for further maturation of NK cells, including acquisition of KIRs or the murine equivalent, the Ly49 receptor family [9,10]. The signals mediated by stromal cells to induce NK cell maturation are unknown, but 1 potential signal may be from Notch ligands expressed by stromal cells.

The evolutionarily conserved Notch receptor pathway plays important roles in cell-fate decisions, and is essential for T cell development [11]. In vertebrates, 4 different Notch receptors, Notch 1-4, may bind up to 5 known ligands, Jagged1, Jagged2, Delta1, Delta3, and Delta4. Binding of different Notch ligands to distinct Notch receptors can variably influence T cell development [12-15]. In particular, the Notch1:Delta4 interaction has been shown to have greater capacity to induce murine T cell development versus Notch binding of Delta1 [12]. Although T and NK cells are postulated to derive from a common T/NK precursor, the Notch1 receptor is not absolutely required for NK cell development in vivo, as an inducible Notch1-knockout mouse has normal levels of $NK1.1^+$ cells [16]. The importance of signaling via other Notch receptors in NK cell development is unclear. In vitro, Notch signaling mediated by binding of Jagged2, in the presence of cytokines important for NK cell differentiation (IL-15 or IL-2), induces murine NK cell development from Lin-$Sca1^+$c-kit^+ HPCs [17], whereas Delta1 has recently been shown to cause NK cell differentiation from human $CD34^+$ HPCs [18]. Thus, there is in vitro evidence that Notch signaling can drive NK cell differentiation from the early hematopoietic precursors.

To further define the capacity of Notch ligands to modulate development of human NK cells, we determined the differential ability of the 5 different Notch ligands found in vertebrates to induce NK cell differentiation and expansion from $CD34^+$ HPCs isolated from umbilical cord blood (UCB). In addition, we analyzed the cell surface phenotype and function of Notch-induced NK (N-NK) cells and compared these characteristics to NK cells generated in vitro in the absence of Notch signaling, as well as to NK cells found in normal adult peripheral blood (PB). N-NK cells display a predominantly immature $CD56^{bright}$ surface phenotype, with no expression of CD16, and the capacity to secrete IFN-γ. Unlike physiologic $CD56^{bright}$ NK cells, however, they do not express CD94 and have only moderate levels of NKG2D expression. In addition, N-NK cells lack the inhibitory receptors NKG2A and KIRs, yet, they do express all 3 of the NCRs, NK-30, NKp44, and NKp46, and are able to lyse hematopoietic tumor cell lines in vitro. Thus, the receptor profile of N-NK cells is biased toward activating NK cell receptors. Finally, the presence of Notch ligand stimulates marked expansion of NK cells from HPCs, resulting in average yields of 700 to 1100 NK cells per input cell.

The use of Notch ligands to generate large numbers of functional, inhibitory receptor-negative NK cells may be useful for producing human NK cells for cell therapy purposes, as infusion of allogeneic NK cells is 1 strategy for tumor immunotherapy [19,20]. The absence on N-NK cells of inhibitory receptors (KIR and NKG2A), which recognize major histocompatibility complex (MHC) class I antigens would be advantageous in an allogeneic therapy setting, circumventing the need for KIR:HLA ligand mismatch between donor and recipient. Elucidation of the ability of different Notch ligands to induce NK cell differentiation and expansion, as well as evidence for the functionality of N-NK cells, allow for more optimal design of in vitro NK cell culture strategies for cell therapy.

METHODS

OP9 Cell Lines

The murine BM stromal cell line OP9 [21] was transduced with 1 of 5 different Notch ligands in a bicistronic message with green fluorescent protein (GFP) in the vector Ret-10, to generate the lines OP9-Jagged1, OP9-Jagged2, OP9-Delta1, OP9-Delta3, and OP9-Delta4, as previously described [22]. A control cell line, OP9-Ret-10, was transduced with vector containing GFP alone. The OP9 cell lines