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The Transcription Factor AHR Prevents the Differentiation of a Stage 3 Innate Lymphoid Cell Subset to Natural Killer Cells

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SUMMARY

Accumulating evidence indicates that human natural killer (NK) cells develop in secondary lymphoid tissue (SLT) through a so-called "stage 3" developmental intermediate minimally characterized by a CD34$^-$CD117$^+$CD94$^-$ immunophenotype that lacks mature NK cell function. This stage 3 population is heterogeneous, potentially composed of functionally distinct innate lymphoid cell (ILC) types that include interleukin-1 receptor (IL-1R1)-positive, IL-22-producing ILC3s. Whether human ILC3s are developmentally related to NK cells is a subject of ongoing investigation. Here, we show that antagonism of the aryl hydrocarbon receptor (AHR) or silencing of AHR gene expression promotes the differentiation of tonsillar IL-22-producing IL-1R1^{hi} human ILC3s to CD56^{bright}CD94$^+$ interferon (IFN)-γ-producing cytolytic mature NK cells expressing eomesodermin (EOMES) and T-Box Protein 21 (TBX21 or TBET). Hence, we demonstrate the lineage plasticity of human ILCs by identifying AHR as a transcription factor that prevents IL-1R1^{hi} ILC3s from differentiating into NK cells.

INTRODUCTION

Natural killer (NK) cells are large granular lymphocytes whose roles in immunity include the production and release of immunomodulatory chemokines and cytokines as well as the direct cytolytic killing of malignant or pathogen-infected cells. NK cells are distinct from T and B lymphocytes in that NK cells do not rearrange T cell receptor or immunoglobulin (Ig) receptor genes, and for many years, NK cells were considered to represent the only non-T/B lymphocyte population (Spits et al., 2013; Walker et al., 2013). However, a wealth of recent data now indicates that NK cells represent only one subset of a much larger population of non-T/B lymphocytes now collectively described as innate lymphoid cells (ILCs) (Spits et al., 2013; Walker et al., 2013). ILC subsets vary in terms of their surface immunophenotypes, transcription factor expression, and functional attributes, and NK cells are currently classified as group 1 ILCs. Non-NK group 1 ILCs (designated ILC1 cells) have also been described (Bernink et al., 2013; Spits et al., 2013; Walker et al., 2013), and whereas non-NK ILC1s can produce interferon (IFN)-γ, they are not cytolytic (Bernink et al., 2013) and do not express the transcription factor eomesodermin (EOMES), which is selectively expressed in NK cells (Gordon et al., 2012; Klose et al., 2013; Spits et al., 2013). Given their diverse roles in immunity and human disease, gaining an understanding of how these various ILC populations develop is of high clinical relevance.

Within human secondary lymphoid tissue (SLT), NK cells appear to proceed through four discrete stages of maturity as they progress from oligopotent CD34$^+$CD45RA$^+$ progenitor cells to functionally competent CD56^{bright}CD94$^+$ NK cells (Freud et al., 2005, 2006). These four "lineage negative" (lacking CD3, CD14, and CD19 expression) lymphoid populations may be distinguished by their surface expression patterns of CD34, CD117, and CD94 such that stage 1 cells are CD34$^+$CD117$^-$CD94$^-$, stage 2 cells are CD34$^+$CD117$^+$CD94$^-$, stage 3 cells are CD34$^-$CD117$^+$CD94$^-$, and stage 4 cells, which bear immunophenotypic and functional features that most closely resemble peripheral blood CD56^{bright} NK cells, are CD34$^-$CD117$^{+/-}$CD94$^+$ (Freud and Caligiuri, 2006). Stage 3 cells were originally classified as "immature NK cells" because unlike stage 1 and stage 2 cells, they do not retain T cell or dendritic cell developmental potential ex vivo, yet in response to in vitro interleukin-15 (IL-15) stimulation or coculture with autologous T cells or OP9 stroma, at least a subset of stage 3 cells differentiates into stage 4 NK cells (Freud and Caligiuri, 2006). In addition, stage 3 cells lack expression of certain receptors expressed by mature (stage 4) NK cells, and they also lack two hallmark functions of mature NK cells: the capacities to produce IFN-γ and to perform perforin-mediated cytotoxicity (Freud et al., 2006). Although the role of IL-15 in driving human NK cell development (Mrózek et al., 1996), survival (Cooper et al., 2002), and effector function (Carson et al., 1994) has been well documented, ex vivo culture assays show that stage 3-to-stage 4 cell maturation in response to IL-15 is inefficient in vitro (Freud et al., 2006; Hughes et al., 2010). This suggests that the stage 3 population may be functionally heterogeneous, and/or IL-15 on its own may be inadequate to drive optimal progression from stage 3 to stage 4 in vitro (Ahn et al., 2013; Freud et al., 2006; Hughes et al., 2010).

Several recent studies provide additional evidence to suggest that the stage 3 population, minimally defined as $CD34^{-}CD117^{+}CD94^{-}$, may be comprised of a heterogeneous group of ILC subsets, potentially including bona fide stage 3 NK cell developmental intermediates that would fit into the aforementioned linear model of human NK cell development as well as other non-NK lineage ILC subsets that share the basic $CD34^{-}CD117^{+}CD94^{-}$ immunophenotype. In particular, the latter include group 3 ILCs (ILC3s), which can express T-Box Protein 21 (TBX21 or TBET), and are defined by expression of the transcription factors RAR-related orphan receptor C (RORC) and aryl hydrocarbon receptor (AHR) (Spits et al., 2013). According to the most recent classification of ILC subsets, ILC3s comprise at least two populations thought to be mutually exclusive in humans: (1) a population expressing natural cytotoxicity receptors (NCRs), including NKp44 and NKp46, as well as IL-1 receptor (IL-1R1), IL-23R, and IL-22 (Cella et al., 2009, 2010; Crellin et al., 2010; Hughes et al., 2010)—a population now referred to as NCR^{+} ILC3 (Spits et al., 2013; Walker et al., 2013); and (2) a lymphoid tissue-inducer (LTi) population expressing molecules required for the development of lymphoid tissues as well as CD117, CD127, CD161, IL-1R1, IL-23R, IL-22, and IL-17, but not CD56 or NCRs (Spits et al., 2013; Walker et al., 2013; and the references within). Recent fate-mapping studies in mice suggest that the analogous NCR^{+} ILC3s and NK cells, which require expression of the transcription factors TBX21 and EOMES for development (Gordon et al., 2012; Intlekofer et al., 2005; Spits et al., 2013), represent distinct lineages (Satoh-Takayama et al., 2010); however, adoptive transfer experiments show that some NCR^{+} ILC3s can adopt an NK-like phenotype in vivo (Lee et al., 2012; Qiu et al., 2012; Vonarbourg et al., 2010), potentially representing nonconventional NK cells or other ILC1 cells. Similar to these mouse data, clonal in vitro assays with human cells suggest some plasticity between the group 3 and group 1 ILC phenotypes (Cella et al., 2010; Crellin et al., 2010; Hughes et al., 2010). Therefore, whether an ILC3 phenotype is truly a transient attribute of at least some human NK cell precursors or whether ILC3s comprise a stable, mutually exclusive lineage apart from NK cells in humans is still unclear. The molecular mechanism(s) regulating these processes is also unknown.

AHR is a ligand-activated transcription factor (Hankinson, 1995; Swanson and Bradfield, 1993) that regulates differentiation of dendritic cells, regulatory T cells, T_H17 cells, intraepithelial intestinal $\gamma\delta$ T cells, and, notably, mouse NCR^{+} ILC3s (Kadow et al., 2011; Kremer et al., 1994; Lee et al., 2012; Platzer et al., 2009; Quintana et al., 2008; Veldhoen et al., 2008). AHR is required for IL-22 production by T_H17 cells (Veldhoen et al., 2008). AHR binds to halogenated aromatic hydrocarbons, and currently described ligands include naturally occurring dietary substances as well as synthetic substances and environmental pollutants (reviewed by Denison and Nagy, 2003). For example, upon exposure to light, the aromatic amino acid tryptophan can be metabolized in vitro to products including the AHR agonist, 6-formylindolo[3,2-b]carbazole (FICZ) (Rannug et al., 1995). Tryptophan is also metabolized to FICZ in vivo by skin keratinocytes upon UV light exposure (Wei et al., 1998). In contrast to many naturally occurring AHR antagonists that have been reported to act as either partial agonists or incomplete antagonists (reviewed by Stejskalova et al., 2011), the chemical CH-223191 lacks AHR agonist activity and efficiently antagonizes activation of AHR (Smith et al., 2011; Zhao et al., 2010).

In this study, we evaluated the impact of AHR modulation on the differentiation and maturation of a subset of human SLT-derived $CD34^{-}CD117^{+}CD94^{-}$ cells with constitutive IL-22 production and high expression of AHR and IL-1R1 ex vivo (previously referred to as stage 3 cells and herein referred to as IL-1R1^{hi} ILC3s) (Freud et al., 2006; Hughes et al., 2010). Whereas in vitro stimulation of purified IL-1R1^{hi} ILC3s with the AHR agonist, FICZ, acted to suppress differentiation into stage 4 mature NK cells, AHR blockade with CH-223191 or silencing of *AHR* gene expression via small hairpin RNA (shRNA) induced their differentiation into phenotypically and functionally mature NK cells. These data suggest that AHR likely plays an important regulatory role in the differentiation of human ILC populations and provide evidence that its constitutive expression in IL-1R1^{hi} ILC3s prevents human NK cell differentiation. Furthermore, these data indicate that inhibition of AHR activity represents a potentially novel means by which the generation of cytotoxic, IFN-γ-producing NK cells may be achieved pharmacologically for clinical benefit.

RESULTS

IL-1β Utilizes AHR to Maintain the IL-22^{+} Phenotype of Human IL-1R1^{hi} ILC3s

We purified fresh human stage 3 or IL-1R1^{hi} ILC3s and $CD56^{bright}CD94^{+}$ stage 4 NK cells from SLT to assess transcription factor expression in each population ex vivo via real-time RT-PCR. Although *RORC* and *AHR* were both absent, *TBX21* (*TBET*) and *EOMES* were highly expressed by $CD56^{bright}CD94^{+}$ stage 4 NK cells, providing additional support for their designation as NK cells (Spits et al., 2013). In contrast, IL-1R1^{hi} ILC3s lacked expression of *TBX21* and *EOMES* but constitutively expressed *RORC* and *AHR*, the latter of which was present at levels at least 15.50 ± 1.22-fold greater than that detected in $CD56^{bright}CD94^{+}$ stage 4 NK cells (Figure 1A).

We therefore reasoned that IL-1R1^{hi} ILC3s may respond to treatment with AHR ligands. We previously showed that these cells constitutively and selectively express abundant *AHR* and *IL-22* mRNA relative to earlier and later stages of human NK cell development and that exogenous IL-1β is required to maintain this phenotype in the presence of the cell's survival factor,