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Transcriptome analysis reveals similarities between human blood CD3−CD56bright cells and mouse CD127+ innate lymphoid cells

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For many years, human peripheral blood natural killer (NK) cells have been divided into functionally distinct CD3−CD56brightCD16− and CD3−CD56dimCD16+ subsets. Recently, several groups of innate lymphoid cells (ILC), distinct from NK cells in development and function, have been defined in mouse. A signature of genes present in mouse ILC except NK cells, defined by Immunological Genome Project studies, is significantly over-represented in human CD56bright cells, by gene set enrichment analysis. Conversely, the signature genes of mouse NK cells are enriched in human CD56dim cells. Correlations are based upon large differences in expression of a few key genes. CD56bright cells show preferential expression of ILC-associated IL7R (CD127), TNFSF10 (TRAIL), KIT (CD117), IL2RA (CD25), CXCR3, DPP4 (CD26), GPR183, and MHC class II transcripts and proteins. This could indicate an ontological relationship between human CD56bright cells and mouse CD127+ ILC, or conserved networks of transcriptional regulation. In line with the latter hypothesis, among transcription factors known to impact ILC or NK cell development, GATA3, TCF7 (TCF-1), AHR, SOX4, RUNX2, and ZEB1 transcript levels are higher in CD56bright cells, while IKZF3 (AIOLOS), TBX21 (T-bet), NFIL3 (E4BP4), ZEB2, PRDM1 (BLIMP1), and RORA mRNA levels are higher in CD56dim cells.

Natural killer (NK) cells are an important component of the innate immune system that serve the dual functions of direct cellular cytotoxicity and early secretion of regulatory cytokines. Phenotypically, human NK cells have been distinguished by their expression of CD56 (NCAM1), and the absence of CD3. For almost 30 years, human NK cells have been further classified into two sub-populations based upon surface levels of CD56 and CD16 (FCGR3A)1,2. The first population, composed of CD56bright cells, make up approximately 10% of circulating blood NK cells, and are characterized by high-density expression of CD56 and low or negative levels of CD16. The second population, CD56dim cells, make up the remaining ~90% of blood NK cells, and are characterized by low-density expression of CD56 and high levels of CD16. These two populations show distinguishing differences in expression of inhibitory NK cell receptors, cytokine and chemokine receptors, as well as differential functional responses (reviewed in ref. 2). For instance, CD56dim cells exert greater cytotoxic effects on target cells1, but produce lesser quantities of cytokines3. On the other hand, ex vivo CD56bright cells are weakly cytotoxic1, and produce high levels of immunoregulatory cytokines, such as IFN-γ, lymphotoxin-α and GM-CSF3.

The developmental relationship of the CD56bright and CD56dim populations remains unclear. Some reports have provided evidence that CD56bright NK cells may be developmental precursors of CD56dim NK cells4-6. Upon in vitro culture with synovial fibroblasts or cytokines, CD56bright NK cells were reported to undergo multiple changes in cell surface phenotype and function to resemble CD56dim NK cells4,5. Another study observed
acquisition of CD16 on sorted CD56\textsuperscript{bright} cells, but not other features of CD56\textsuperscript{dim} cells, upon culture in IL-15; this process could be regulated by TGF-\textbeta. CD56\textsuperscript{bright} cells were shown to have longer telomeres than CD56\textsuperscript{dim} cells, perhaps consistent with a more immature status\textsuperscript{6,4}. On the other hand, studies following clonal hematopoiesis in vivo in rhesus macaques revealed that analogous CD56\textsuperscript{\textminus} CD16\textsuperscript{\textminus} and CD56\textsuperscript{\textplus} NK cell populations showed differences in progenitor cell origin for many months after stem cell transplant; a large number of rhesus CD16\textsuperscript{\textplus} NK cells were derived from highly biased progenitor clones that did not substantially give rise to other lineages, while many CD56\textsuperscript{\textminus} CD16\textsuperscript{\textplus} cells shared progenitors with T, B, and myeloid cells\textsuperscript{5}. This provides evidence that human CD56\textsuperscript{\textplus} and CD56\textsuperscript{\textminus} populations may follow distinct developmental pathways. Interestingly, some patients deficient in GATA2 lack CD56\textsuperscript{\textplus} blood cells, while they retain some CD56\textsuperscript{\textminus} NK cells, possibly arguing against a simple precursor-progeny relationship\textsuperscript{6}. Mouse NK cells do not express CD56, making assignment of analogous mouse populations more challenging.

In the past few years, a number of related subsets of innate lymphoid cells (ILC), distinct from NK cells, have been described in mouse and human. These include: (i) Ror-\gamma-t-dependent group-3 ILC (ILC3), which produce IL-22 and play key roles in bacterial and fungal defence, mucosal homeostasis, regulation of immune tissue development, and modulation of adaptive immune responses; (ii) group-2 ILC (ILC2), expressing high levels of GATA2, which produce Th2-associated cytokines, including IL-5 and IL-13, and profoundly impact allergic responses and parasite defence; and (iii) T-bet (Bx21)-dependent, group-1 ILC (ILC1) cells, which produce IFN-\gamma and are reported to be distinct from NK cells in development and function (reviewed in ref. 10). Using fluorescent Id2 or Zbtb16 (PLZF) reporter mice, along with surface marker staining, two groups defined common lymphoid progenitor (CLP)-like precursor cells with the capacity to differentiate in vivo or in vitro into ILC3, ILC2, and ILC1 cells, but not conventional mouse NK cells\textsuperscript{11,12}. The developmentally distinct mouse ILC1 subset appears to include TRAIL\textsuperscript{\textplus} (NK510\textsuperscript{\textplus}) DX5\textsuperscript{\textminus} (CD49b\textsuperscript{\textminus}) CD49a\textsuperscript{\textminus} cells (previously described to display an immature NK phenotype\textsuperscript{13} or termed tissue resident NK cells\textsuperscript{4,14-16}. Genomic lineage-tracing/fate-mapping studies showed that most NK1.1\textsuperscript{\textplus} DX5\textsuperscript{\textminus} ILC1 cells had developed via a PLZF\textsuperscript{\textplus} precursor, while most NK1.1\textsuperscript{\textminus} DX5\textsuperscript{\textplus} liver and spleen NK cells had not, supporting different developmental pathways\textsuperscript{11}. In mice, Eomes is reported to distinguish conventional NK cells from ILC1, with lack of Eomes correlating with a TRAIL\textsuperscript{\textplus} DX5\textsuperscript{\textminus} phenotype in the liver\textsuperscript{17,18}, and CD127\textsuperscript{\textminus} DX5\textsuperscript{\textplus} phenotype in the small intestine\textsuperscript{12,17,18}. Both Eomes\textsuperscript{+} NK cells and Eomes\textsuperscript{+} ILC populations can express the typical mouse NK cell markers, NK1.1, Nkp46, and Nkg2D, complicating their separation\textsuperscript{12,17,18}. It has been proposed that all “helper ILC” (ILC3, ILC2, and ILC1) express CD127 (Il7r), implying that ILC1 may be distinguished from NK cells by CD127 expression. However, both ILC1 and NK cells depend mainly upon IL-15 for their development (rather than IL-7), and both subsets develop in thymectomy mice\textsuperscript{12,17,18}. Genetic lineage-tracing experiments also suggest that mouse Ror-\gamma-t ILC3 can lose Ror-\gamma-t expression to acquire an ILC1-like phenotype, producing IFN-\gamma and expressing NK1.1 and Nkp46\textsuperscript{15,19}; these transitioned cells have been referred to as “ex-ILC3 cells.” In human tonsil, non-NK ILC1 cells have been identified with a CD56\textsuperscript{\textplus} CD127\textsuperscript{\textplus} Nkp44\textsuperscript{\textplus} CD117\textsuperscript{\textminus} perforin\textsuperscript{\textminus} CRTH2\textsuperscript{\textminus} phenotype\textsuperscript{20}.

In light of the discovery of these varied ILC subsets, the nature of the human peripheral blood CD3\textsuperscript{\textminus} CD56\textsuperscript{bright} subset is re-examined here bioinformatically, using transcriptome-wide expression data. Transcription factors distinguishing this subset are also identified.

**Results**

**Signature transcripts of mouse ILC are enriched in human CD56\textsuperscript{bright} cells, while mouse NK signature gene products are enriched in human CD56\textsuperscript{dim} NK cells.** We undertook a re-evaluation of the nature of the human blood CD56\textsuperscript{bright} and CD56\textsuperscript{dim} cell subsets. Microarray results from an Affymetrix HTA2.0 dataset\textsuperscript{21} was used to compare peripheral blood CD3\textsuperscript{\textminus} CD56\textsuperscript{bright} CD3\textsuperscript{\textplus} CD56\textsuperscript{dim} CD16\textsuperscript{\textplus} and CD3\textsuperscript{\textminus} CD56\textsuperscript{dim} CD16\textsuperscript{\textminus} cells, with results confirmed, where indicated, with data from an independent microarray dataset based upon HG-U133 A and B arrays\textsuperscript{22}. Robinette and colleagues previously examined multiple mouse ILC lineages by microarray in the context of the Immunological Genome Project (ImmGen), and used these data to define expression signatures: Core ILC signature genes were expressed at higher levels in multiple mouse ILC subsets, but were lower in conventional NK cells\textsuperscript{23}; Core NK signature transcripts were selectively over-expressed in mouse NK cells\textsuperscript{23}. After conversion of these signatures to homologous human genes, selective enrichment was examined in human CD56\textsuperscript{bright} and CD56\textsuperscript{dim} subsets using the Gene Set Enrichment Analysis (GSEA) algorithm (which calculates scores based on positions of signature genes within a rank-ordered list according to differential expression\textsuperscript{24}; Fig. 1A, B). Interestingly, the mouse Core ILC signature was significantly enriched in human CD56\textsuperscript{bright} cells (p = 0.003) (Fig. 1A). However, this enrichment was largely driven by a few key signature genes that displayed expression highly biased towards CD56\textsuperscript{bright} cells. Comparing CD56\textsuperscript{bright} with CD56\textsuperscript{dim} cells, IL7R (CD127) was 22.9-fold higher; GPR97, 2.6-fold; and IL2RA (CD25), 1.6-fold. Independent human microarray data, from the second microarray platform, confirmed enrichment of the Core ILC signature in CD56\textsuperscript{bright} cells due, in part, to higher expression of IL7R and GPR97 (an orphan G protein-coupled receptor) (Suppl. Figure 1A). In mouse, it has been proposed that surface CD127 (Il7r) distinguishes helper ILC lineages (ILC3, ILC2, and ILC1) from conventional NK cells. Strikingly, IL7R represented the transcript with the second highest fold-change between human CD56\textsuperscript{bright} and CD56\textsuperscript{dim} subsets, greater than CD16 (FCGR3A, 15.4-fold) and CD56 (NCAM1, 1.8-fold), the defining phenotypic markers of the subsets. At the protein level, CD127 appears to be bimodal on the surface of CD56\textsuperscript{bright} cells (Fig. 1C), perhaps indicating the presence of distinct subsets of cells within the CD56\textsuperscript{bright} population, or down-regulation upon recognition of IL-7. CD127 expression on CD56\textsuperscript{bright} cells has been reported previously\textsuperscript{25}.

CD56\textsuperscript{dim} cells appeared to be mostly negative for CD127 (Fig. 1C). Some CD56\textsuperscript{bright} cells also show low-level cell surface expression of CD25 (IL2RA) (Fig. 1C) as noted previously\textsuperscript{26,27}. Although not part of the bioinformatically-defined signature, CD27 is another marker that Klose and colleagues have reported to distinguish mouse ILC1 from conventional NK cells and ex-ILC3\textsuperscript{12}. Interestingly, CD27 is also selectively expressed on the cell surface of CD56\textsuperscript{bright} cells in a bimodal fashion (Fig. 1C), again highlighting the possibility of distinct subsets.
Figure 1. Signature genes, higher in mouse ILC subsets, are enriched in human blood CD3\(^{-}\)CD56\(^{bright}\) cells, while mouse NK cell signature genes are enriched in human CD3\(^{-}\)CD56\(^{dim}\) cells. (A, B) Transcriptional signatures of mouse NK cells (Core NK) and other mouse ILC (Core ILC) were defined by Robinette and colleagues as part of the ImmGen project\(^\text{23}\). Enrichment of corresponding homologous human genes was examined by Gene Set Enrichment Analysis in human blood CD56\(^{bright}\)CD16\(^{-}\)CD3\(^{-}\) and CD56\(^{dim}\)CD16\(^{+}\)CD3\(^{-}\) populations characterized by Affymetrix HTA2.0 microarray\(^\text{21}\). Deviations of cumulative scores (green line) above zero indicate enrichment in CD56\(^{bright}\) cells, while negative scores indicate enrichment in CD56\(^{dim}\) cells. Samples of each subset from three individuals were used for analysis. Signature genes without human homologues were not included in the analysis. (C) Flow cytometry staining of gated human blood CD56\(^{bright}\)CD16\(^{-}\)CD3\(^{-}\) or CD56\(^{dim}\)CD16\(^{+}\)CD3\(^{-}\) populations from three donors. Quadrant gates were set using fluorescence minus one (FMO) stains, of which one is shown. Intracellular staining for EOMES was performed on three blood samples distinct from those used to acquire the other results.
However, CD56\textsuperscript{bright} cells did not display substantially biased expression of other characteristic mouse ILC genes such as CXCR6 (Fig. 1A).

Conversely, mouse Core NK signature genes were highly enriched in the human CD56\textsuperscript{dim} subset (p < 0.001) (Fig. 1B). This was largely driven by 5 genes within the signature: KLRG1 (3.7-fold higher in CD56\textsuperscript{dim}), CMKLR1 (3.1-fold), ZEB2 (2.7-fold), KLRAP1 (1.8-fold), and SCIMP (1.5-fold). KLRAP1 is the single human (pseudogene) homologue of the mouse Ly49 receptors, several of which are part of the mouse Core NK signature. Functional equivalents of the Ly49 in human, the killer-cell immunoglobulin-like receptors (KIR), are also highly differentially expressed between the human subsets. Twelve of thirteen KIR genes that were interrogated displayed extremely biased expression in CD56\textsuperscript{dim} cells, ranging from 3.2-fold to 13.4-fold higher in CD56\textsuperscript{dim} cells (the exception being KIR2DL4, which was similar in both subsets) (data not shown). Using mAb recognizing several KIR, selective staining of CD56\textsuperscript{dim} NK cells has been reported for many years\textsuperscript{7, 28}. Examination of the second human microarray dataset also showed an enrichment trend of the mouse Core NK signature genes in CD56\textsuperscript{dim} cells (Suppl. Figure 1B). Reports have defined the transcription factor, Eomes, as critical in distinguishing mouse NK cells (which express Eomes) from ILC1 (which do not)\textsuperscript{12, 17}. However, the human CD56\textsuperscript{bright} and CD56\textsuperscript{dim} subsets do not differ significantly in transcript levels of EOMES (measured on both microarray platforms). Similarly, intracellular flow cytometry staining revealed high levels of EOMES protein in blood CD56\textsuperscript{bright} and CD56\textsuperscript{dim} cells\textsuperscript{9, 16}, irrespective of CD127 expression (Fig. 1C).

Transcripts varying between mouse splenic CD127\textsuperscript{+} ILC and CD127\textsuperscript{−} NK cells show commonalities with differential transcripts observed between human blood CD56\textsuperscript{bright} and CD56\textsuperscript{dim} subsets. In order to more closely examine these similarities, we examined the overall correlation of gene expression differences between the human blood subsets with differences between mouse ILC and NK cell subsets. Robinette and colleagues examined ILC and NK cell populations in several mouse tissues by microarray. In spleen, ILC1 were isolated as NK1.1\textsuperscript{+} Nkp46\textsuperscript{−} CD127\textsuperscript{−} CD27\textsuperscript{+}, while NK cells were NK1.1\textsuperscript{+} Nkp46\textsuperscript{+} CD127\textsuperscript{−} CD27\textsuperscript{+} (CD27\textsuperscript{+/−})\textsuperscript{31}; thus CD127 and to some extent CD27 were the defining differential markers. To compare across species, homologues were matched using the NCBI homologene database. This allowed analysis of 16,351 transcripts between human and mouse. Among these transcripts, 355 were found to differ by ≥ 2-fold in expression between human blood CD56\textsuperscript{bright} and CD56\textsuperscript{dim} subsets. Similarly, 184 differed by ≥ 2-fold between mouse splenic ILC1 and NK cells. Only 26 gene products overlapped between these two comparisons, showing that most differences between the subsets were species-specific. However, 20 of these 26 overlapping gene products (77%) differed in the same direction; that is, higher together in ILC1 and CD56\textsuperscript{bright} cells or higher together in mouse NK and CD56\textsuperscript{dim} cells. This is illustrated in Fig. 2A in the larger number of gene products in the upper right and bottom left, compared with the other two quadrants (p = 0.02 when compared by Fisher’s exact test). Although the overall relationship was not monotonic (Fig. 2A), Spearman correlation analysis was performed considering only the subset of gene products that differed ≥ 2-fold in expression between mouse splenic ILC1 and NK cells (p = 0.0005). However, the correlation coefficient was low (r = 0.25). Examined a different way, using the GSEA algorithm, mouse genes that were ≥ 2-fold higher in spleen CD127\textsuperscript{+} ILC1 showed significantly enriched expression in human CD56\textsuperscript{bright} cells (p < 0.001), while mouse genes ≥ 2-fold higher in spleen NK showed enrichment within the human CD56\textsuperscript{dim} population (p < 0.001) (Suppl. Figure 2). However, it is visually evident that this was due to a limited number of key transcripts (Fig. 2A).

Mouse liver and small intestine ILC1/NK cell subsets were also part of the ImmGen analysis, although isolated in a slightly different fashion. Again, both subsets had been sorted NK1.1\textsuperscript{+} Nkp46\textsuperscript{−}, but liver ILC1 were isolated as DX5\textsuperscript{−} TRAIL\textsuperscript{−}, while NK cells were DX5\textsuperscript{−} TRAIL\textsuperscript{−} \textsuperscript{23}. In the small intestine lamina propria, ILC1 were separated as ROR\textgreek{t}\textsuperscript{−} CD127\textsuperscript{+}, while NK cells were ROR\textgreek{t}\textsuperscript{+} CD127\textsuperscript{−} \textsuperscript{23}. A ROR\textgreek{t}\textsuperscript{−}GFP reporter had been utilized to exclude ILC3, which are predominant in intestinal tissue. No significant associations were found when similar Fisher’s exact test or Spearman correlation analyses were performed comparing human CD56\textsuperscript{bright}/CD56\textsuperscript{dim} subsets with mouse ILC1/NK cells from either liver or small intestine (Suppl. Figure 3). However, some genes displayed similar patterns of expression in multiple tissues. Two genes were expressed ≥ 2-fold higher in human CD56\textsuperscript{bright} cells and mouse ILC1 subsets from all three tissues examined (IL7R and GPR97, both part of the ILC Core signature; see blue labels, Fig. 2A, Suppl. Figure 3). Five additional genes showed levels ≥ 2-fold higher in CD56\textsuperscript{bright} cells and ILC1 subsets from two of the mouse tissues (GPR183, AHR, CXCR3, XCL1, and INPP4B; green labels, Fig. 2A, Suppl. Figure 3). In the opposite direction, four genes showed higher levels in CD56\textsuperscript{dim} NK cells and mouse NK subsets from all three tissues (KLRG1, ZEB2, PRRS1, and CMKLR1; blue labels, Fig. 2A, Suppl. Figure 3). Seven were higher in CD56\textsuperscript{dim} cells and NK subsets from two of the mouse tissues (S1PR5, OZMA, PRF1, FCCR2B, SLAMF6, ANXA2, GNPTAB; green labels, Fig. 2A, Suppl. Figure 3). If the stringency is lessened to ≥ 1.5-fold difference, LTB, DTX1, CAMK4, GPR34, and IL2RA transcript levels are higher in CD56\textsuperscript{bright} cells and mouse ILC1 subsets from all three tissues, while RAP1GAP2, NDRG1, KLF12, and SCIMP are higher in CD56\textsuperscript{dim} cells and all three mouse tissue subsets (data not shown). Similar analyses were performed using the second independent microarray dataset comparing human blood CD56\textsuperscript{bright} and CD56\textsuperscript{dim} subsets and revealed a similar pattern and many of the differentially expressed genes (Suppl. Figure 4). Interestingly, LTB (Lymphotixin \textgreek{b}) and TNFSF10 (TRAIL) were preferentially expressed in CD56\textsuperscript{bright} cells in this analysis (Suppl. Figure 4). For a number of the genes that showed selective expression in mouse ILC1 cell surface protein was confirmed on human CD56\textsuperscript{bright} cells (Fig. 2B). CD56\textsuperscript{bright} cells showed considerable staining with monoclonal antibodies recognizing CXCR3, CD26 (DP44), and TRAIL (TNFSF10) plus some reactivity with anti-CD117 (KIT) and anti-GPR183; in each case, staining of CD56\textsuperscript{dim} cells was lower or negative (Fig. 2B).

Some of these observations have also been reported by others\textsuperscript{30–32}. TRAIL expression is particularly striking, as TRAIL is used as a defining marker of ILC1 in mouse.

Although not necessarily matched between species by homologene, it is interesting to note that some MHC class II molecules were higher in both mouse splenic ILC1 and human CD56\textsuperscript{bright} cells (Fig. 2A). Expression of
Figure 2. Expression differences between mouse splenic CD127⁺ NK1.1⁺ NKp46⁺ ILC and CD127⁺ NK1.1⁺ NKp46⁺ NK cells show commonalities with differences between human blood CD56bright and CD56dim cells. ImmGen microarray data examining mouse splenic NK1.1⁺ NKp46⁺ CD127⁺ CD27⁺ cells (annotated as ILC1) and NK1.1⁺ NKp46⁺ CD127⁺ CD27⁻⁺ NK cells is analysed to show fold-change difference between subsets (x-axis). For all mouse genes with corresponding human genes in the NCBI Homologene database, this is contrasted with microarray expression differences observed between human blood CD56bright and CD56dim populations (y-axis) (HTA2.0 microarray). Transcripts differing by ≥ 2-fold between both human and mouse subsets are labelled, and their numbers are shown. Transcripts similarly varying by ≥ 2-fold in mouse ILC1/NK comparisons in both liver and small intestine (in addition to spleen) are shown in blue; those differing by ≥ 2-fold in 2-of-3 comparisons of mouse tissues are in green (see Suppl. Figure 3). Bold names also show ≥ 2-fold differences in an independent microarray dataset comparing human CD56bright and CD56dim cells (see Suppl. Figure 4). Although not matched by Homologene, human HLA-DRA (paired with mouse H2-Aa) is shown (with asterisk) but not included in transcript counts. (B) Flow cytometry staining of gated human blood CD56bright CD16⁻ CD3⁻ or CD56dim CD16⁺ CD3⁻ populations from three donors. Quadrant gates were set using fluorescence minus one (FMO) stains.
HLA-DR surface protein on some CD56 bright cells, but not CD56 dim cells, was observed (Fig. 2B) as has selective expression of HLA-DR on human ILC3. Transcript levels of KLRB1 (CD161, NKR-P1A) were comparable between human blood subsets (1.3 fold higher in CD56 dim).

It has been reported that some ILC3 can exhibit lineage-plasticity, losing Ror\[\gamma\]t expression and acquiring a phenotype similar to ILC1; the resulting cells are called “ex-ILC3” (12, 19). Therefore, the mouse cells isolated as CD127[+]NK1.1[+]NKp46[+] (and termed “ILC1” above) may also encompass or include these ex-ILC3 populations. Thus, it is possible that the human CD56 bright cell transcriptome (or subset(s) therein) may show commonalities with either mouse ILC1 or ex-ILC3 populations.

Human CD56 bright cells express higher levels of some transcripts that are also selectively expressed in mouse ILC1 and ex-ILC3. Klose and colleagues employed a different strategy to examine the diversity of mouse NK1.1[+] NKp46[+] cells, utilizing a dual-reporter mouse. A heterozygous knock-in of GFP at the Eomes locus (EomesGFP/+ ) reported Eomes expression, while transgenic Rorc\[\gamma\]tc(γt)-Cre x Rosa26R Yfp/+ provided fate mapping for prior Ror\[\gamma\]t expression characteristic of ILC3 (12). The authors used this system to define three NK1.1[+] NKp46[+] populations as follows: (i) Eomes[+] classical NK cells, (ii) Ror\[\gamma\]t fate-map[+] ex-ILC3 (that formerly expressed Ror\[\gamma\]t), and (iii) ILC1 that expressed neither (from ref. 12). Enrichment of corresponding homologous human genes was examined by Gene Set Enrichment Analysis in human CD56 bright and CD56 dim populations characterized by Affymetrix HTA2.0 microarray, using the following signatures: (A-B) Mouse ILC1/ex-ILC3 signature (transcripts detected at \( \geq 2\)-fold higher levels in both ILC1 versus NK and ex-ILC3 versus NK comparisons) and (C-D) Mouse Eomes[+] NK cell signature (genes with \( \geq 2\)-fold higher expression in both NK versus ILC1 and NK versus ex-ILC3 comparisons). (B,D) Fold difference in expression of these transcripts between human CD56 bright and CD56 dim cells is shown for comparison. Genes differing by \( \geq 2\)-fold between the human subsets are labeled (in order by fold change difference) and emboldened if conserved in similar analyses with a second human microarray dataset (see Suppl. Figure 5).

**Figure 3.** Transcripts with higher expression in mouse ILC1 and ex-ILC3 subsets show enrichment in human CD56 bright cells. Lists of signature transcripts were compiled from published microarray data comparing three mouse NK1.1[+] NKp46[+] subsets: (i) Eomes[+] NK cells, (ii) Ror\[\gamma\]t fate-map[+] ex-ILC3 (that formerly expressed Ror\[\gamma\]t), and (iii) ILC1 that expressed neither (from ref. 12). Enrichment of corresponding homologous human genes was examined by Gene Set Enrichment Analysis in human CD56 bright and CD56 dim populations characterized by Affymetrix HTA2.0 microarray, using the following signatures: (A-B) Mouse ILC1/ex-ILC3 signature (transcripts detected at \( \geq 2\)-fold higher levels in both ILC1 versus NK and ex-ILC3 versus NK comparisons) and (C-D) Mouse Eomes[+] NK cell signature (genes with \( \geq 2\)-fold higher expression in both NK versus ILC1 and NK versus ex-ILC3 comparisons). (B,D) Fold difference in expression of these transcripts between human CD56 bright and CD56 dim cells is shown for comparison. Genes differing by \( \geq 2\)-fold between the human subsets are labeled (in order by fold change difference) and emboldened if conserved in similar analyses with a second human microarray dataset (see Suppl. Figure 5).

- **Human CD56 bright cells express higher levels of some transcripts that are also selectively expressed in mouse ILC1 and ex-ILC3.** Klose and colleagues employed a different strategy to examine the diversity of mouse NK1.1[+] NKp46[+] cells, utilizing a dual-reporter mouse. A heterozygous knock-in of GFP at the Eomes locus (EomesGFP/+ ) reported Eomes expression, while transgenic Rorc\[\gamma\]tc(γt)-Cre x Rosa26R Yfp/+ provided fate mapping for prior Ror\[\gamma\]t expression characteristic of ILC3 (12). The authors used this system to define three NK1.1[+] NKp46[+] populations as follows: (i) Eomes[+] classical NK cells, (ii) Ror\[\gamma\]t fate-map[+] ex-ILC3 (that formerly expressed Ror\[\gamma\]t), and (iii) ILC1 that expressed neither (from ref. 12). Enrichment of corresponding homologous human genes was examined by Gene Set Enrichment Analysis in human CD56 bright and CD56 dim populations characterized by Affymetrix HTA2.0 microarray, using the following signatures: (A-B) Mouse ILC1/ex-ILC3 signature (transcripts detected at \( \geq 2\)-fold higher levels in both ILC1 versus NK and ex-ILC3 versus NK comparisons) and (C-D) Mouse Eomes[+] NK cell signature (genes with \( \geq 2\)-fold higher expression in both NK versus ILC1 and NK versus ex-ILC3 comparisons). (B,D) Fold difference in expression of these transcripts between human CD56 bright and CD56 dim cells is shown for comparison. Genes differing by \( \geq 2\)-fold between the human subsets are labeled (in order by fold change difference) and emboldened if conserved in similar analyses with a second human microarray dataset (see Suppl. Figure 5).

- **Figure 3.** Transcripts with higher expression in mouse ILC1 and ex-ILC3 subsets show enrichment in human CD56 bright cells. Lists of signature transcripts were compiled from published microarray data comparing three mouse NK1.1[+] NKp46[+] subsets: (i) Eomes[+] NK cells, (ii) Ror\[\gamma\]t fate-map[+] ex-ILC3 (that formerly expressed Ror\[\gamma\]t), and (iii) ILC1 that expressed neither (from ref. 12). Enrichment of corresponding homologous human genes was examined by Gene Set Enrichment Analysis in human CD56 bright and CD56 dim populations characterized by Affymetrix HTA2.0 microarray, using the following signatures: (A-B) Mouse ILC1/ex-ILC3 signature (transcripts detected at \( \geq 2\)-fold higher levels in both ILC1 versus NK and ex-ILC3 versus NK comparisons) and (C-D) Mouse Eomes[+] NK cell signature (genes with \( \geq 2\)-fold higher expression in both NK versus ILC1 and NK versus ex-ILC3 comparisons). (B,D) Fold difference in expression of these transcripts between human CD56 bright and CD56 dim cells is shown for comparison. Genes differing by \( \geq 2\)-fold between the human subsets are labeled (in order by fold change difference) and emboldened if conserved in similar analyses with a second human microarray dataset (see Suppl. Figure 5).

HLA-DR surface protein on some CD56 bright cells, but not CD56 dim cells, was observed (Fig. 2B) as has selective expression of HLA-DR on human ILC3. Transcript levels of KLRB1 (CD161, NKR-P1A) were comparable between human blood subsets (1.3 fold higher in CD56 dim).

It has been reported that some ILC3 can exhibit lineage-plasticity, losing Ror\[\gamma\]t expression and acquiring a phenotype similar to ILC1; the resulting cells are called “ex-ILC3” (12, 19). Therefore, the mouse cells isolated as CD127[+] NK1.1[+] NKp46[+] (and termed “ILC1” above) may also encompass or include these ex-ILC3 populations. Thus, it is possible that the human CD56 bright cell transcriptome (or subset(s) therein) may show commonalities with either mouse ILC1 or ex-ILC3 populations.

**Human CD56 bright cells express higher levels of some transcripts that are also selectively expressed in mouse ILC1 and ex-ILC3.** Klose and colleagues employed a different strategy to examine the diversity of mouse NK1.1[+] NKp46[+] cells, utilizing a dual-reporter mouse. A heterozygous knock-in of GFP at the Eomes locus (EomesGFP/+ ) reported Eomes expression, while transgenic Rorc\[\gamma\]tc(γt)-Cre x Rosa26R Yfp/+ provided fate mapping for prior Ror\[\gamma\]t expression characteristic of ILC3 (12). The authors used this system to define three NK1.1[+] NKp46[+] populations as follows: (i) Eomes[+] classical NK cells, (ii) Ror\[\gamma\]t fate-map[+] ex-ILC3, and (iii) ILC1, which expressed neither (with the minor caveats of monoallelic reporter expression and inappropriate transgene expression possible). Using microarray data characterizing these three mouse populations from small intestine (12), we created lists of signature genes selectively expressed in the different subsets. These were then contrasted with human CD56 bright and CD56 dim cells by GSEA. Mouse transcripts enriched \( \geq 2\)-fold in both ILC1 and ex-ILC3 relative to NK cells (mouse ILC1/ex-ILC3 signature) were significantly over-represented in the CD56 bright population (Fig. 3A). This was due to higher expression of several signature genes, including IL7R, GBP97, HOXA5, IRAK3, ZMAT4, and EPAS1 in human CD56 bright cells (Fig. 3B), all of which were also identified by previous analyses in either Fig. 2A or Suppl. Figure 3 using ImmGen data. A similar result was observed using the other human microarray dataset (Suppl. Figure 5A,B). On the other hand, signature transcripts of mouse Eomes[+] NK cells (\( \geq 2\)-fold higher in both NK versus ILC1 and NK versus ex-ILC3 comparisons) were reported by the GSEA algorithm to be enriched in human CD56 dim cells (Fig. 3C). However, inspection of the GSEA plot shows that the Eomes[+] NK signature transcripts are actually preferentially clustered at both ends of the scale, some enriched in the CD56 bright and some in the CD56 dim subsets (Fig. 3D, Suppl. Figure 5C,D). Signature transcripts selectively found in only Ror\[\gamma\]t fate-map[+] ex-ILC3 appeared to be enriched in expression in human CD56 bright cells; however, this was mostly due to expression of one gene, GPR183, shared by both CD56 bright cells and mouse ex-ILC3 (Suppl.
Figure 6A,B,E,F). GPR183 (EBI2), a G-protein-coupled migration receptor recognizing oxysterols 35 also features prominently in the Fig. 2A analysis using ImmGen data described above, although cell surface expression of GPR183 on CD56 bright cells appeared to be quite low (Fig. 2B). Enrichment patterns of mouse ILC1 signature transcripts were inconsistent between the two human microarray datasets (Suppl. Figure 6C,D,G,H). To gauge the magnitude of the GSEA enrichments that were observed here, the same mouse ILC signatures were also compared by GSEA with the other mouse microarray dataset from ImmGen (Suppl. Figure 7).

In summary, human CD56 bright cells shared the selective expression of a limited subset of genes with mouse spleen CD127+ NK1.1+ NKp46+ ILC and with ILC defined by other reporters. Two hypothetical interpretations of the preceding data include:

(a) Ontological/developmental/lineage similarity of CD127+ human blood CD56 bright cells with mouse spleen CD127+ NK1.1+ NKp46+ ILC, or

(b) Presence in both cell types of a similar module of gene regulation, perhaps due to expression of particular transcription factors.

To investigate the latter possibility, transcription factor expression in CD56 bright and CD56 dim cells was examined in greater detail.

**Figure 4.** Human CD56 bright and CD56 dim subsets differ in expression of many transcription factors known to affect development of ILC and NK cells. (A) Fold-change differences in expression between CD56 bright and CD56 dim cells analysed by two Affymetrix microarray platforms: HTA2.0 (black bars) and HG-U133 A/B (grey bars). Three samples of each subset were analysed on each microarray, for a total of 6 independent sample pairs. Transcription factors showing >2-fold difference on both array platforms are shown, ordered by mean fold change. Genes labelled in purple italics have been shown to affect development of ILC and/or NK in mouse models (see references in text). (B) Quantitative real-time RT-PCR validation of several differences in transcription factor expression (including some that showed >2-fold change in only one microarray dataset). RNA was isolated from human blood CD56 bright CD16− cells, CD56 dim CD16+ cells, and the “intermediate population” displaying CD56 bright CD16+ phenotype. Transcript levels are quantitated relative to ACTB in samples from three individuals (represented by triangle, circle and square symbols) with a bar depicting the geometric mean level of expression. Samples were distinct from those analysed by microarray. *p < 0.05, **p < 0.01, ***p < 0.001, by Tukey’s Multiple Comparison Test after one way ANOVA of Log10 transformed values. Trends were consistent if data was normalized to GAPDH transcript levels instead of ACTB.

Human CD56 bright and CD56 dim cells differ in expression of multiple transcription factors previously linked to ILC and NK cell development. Microarray analysis of transcription factors differing between human blood CD56 bright and CD56 dim populations is shown in Fig. 4A. This list includes differences verified on both microarray platforms with independent samples. CD56 bright cells showed higher levels of transcript for SCML1, RUNX2, SOX4, ZEB1, TCF7, HOXA5, AHR, AFF3, MYC, SSBP2, TCF4, and GATA3. On the other hand, CD56 dim cells had higher signal for IKZF3 (AIOLOS), MYBL1, RORA, PRDM1 (BLIMP1), PYHIN1, NFI13 (E4BP4), ZEB2, HIPK2, ADRB2, BNC2, BCL11B, TBL1X, CRY1, TCF7L2, and TBX21 (T-bet). The differences in a subset of transcription factors were additionally validated by real-time RT-PCR (Fig. 4B), in all cases confirming or trending with the microarray results. “Intermediate” cells with a CD56 bright CD16+ phenotype were very similar in transcription factor expression to CD56 bright CD16− cells (Fig. 4B), as observed previously for cell surface markers5.
Discussion

ImmGen studies have previously defined signature genes that are preferentially expressed in mouse ILC or NK cell subsets41. When these signatures were examined in microarray data comparing human peripheral blood subsets, several interesting patterns emerged. The mouse core ILC signature was enriched in human CD56bright cells (Fig. 1A); however, this was driven largely by transcript levels of only a few genes (IL7R, GPR97, and IL2RA).

More in depth comparisons revealed a small subset of genes that are expressed at higher levels in both human CD56bright cells and mouse ILC1 defined by different markers or reporters. This included IL7R (CD127), TNFSF10 (TRAIL), KIT (CD117), IL2RA (CD25), CD27, CXCR3, DPP4 (CD26), GRP183, AHR, XCL1, and MHC class II; most of which were also shown to be preferentially expressed at the cell surface of CD56bright cells (Fig. 2). On the other hand, the mouse core NK signature was enriched in CD56dim cells (Fig. 1B). Human CD56dim and mouse NK cells shared higher expression of KLRG1, PRF1 (perforin), GZMA (granzyme A), S1PR5, CMKLRI (chemerin receptor 23), and other genes (Fig. 2A). Interpreted in reverse, this indicates that both human CD56bright and mouse ILC1 have lower levels of these NK cell-associated transcripts.

These similarities could indicate a developmental or lineage relationship between human blood CD56bright cells and mouse ILC1, or NK-like, or that cells defined primarily by CD127 expression in mouse spleen (with NK1.1 and Nkp46) could be analogous to human CD56bright cells rather than bona fide ILC1. As suggested recently, NK cells and ILC1 may represent two extremes of a broad spectrum of cells comprising the group-1 ILC population42. Human CD56bright cells may inhabit the middle of this spectrum. Strikingly, recent data from rhesus macaques showed that subsets similar to human CD56high and CD56dim cells predominantly arose from distinct progenitors, with limited overlap and little evidence of differentiation from one subset to the other. It is possible that human blood CD127+ CD56high cells develop from IL-22-producing ILC3 described to exhibit a “stage 2” signature (Lin− CD117+ CD161+ IL-1R1+ CD94+), whereas CD56dim NK cells may develop independently from distinct NK cell progenitors (such as those described in ref. 40). Future studies will be required to delineate their lineage relationships (including those with human liver ILC/NK cell subsets41, 42).

Alternatively, the subset of genes with common expression in CD56bright cells and mouse ILC1 may be the result of a similar module of gene regulation, due to actions of particular transcription factors. Strikingly, many transcription factors that differ between CD56bright and CD56dim human blood populations have been shown to affect ILC development and function in mouse knockout models. CD56bright cells show higher levels of GATA3, AHR, TCF7, and RUNX2 transcripts (Fig. 4A). Upon gene disruption in mouse hematopoietic cells, Gata3 was shown to be necessary for development of ILC3, ILC2, and CD127+ ILC1, while CD127− NK cells (and DX5− liver ILC) were largely maintained41, 43. Similarly, mice deficient in Ahr displayed largely intact NK cell populations, despite deficiencies in ILC3 subsets44–46 and liver ILC147. Tcf7 (encoding TCF-1 protein) affects all mouse ILC/NK lineages and is expressed in early ILC/NK progenitors48, 49. Among the Runx family of transcription factors, Runx3 impacts mouse ILC3, ILC1, and NK development48. Interestingly, Tcf7loth reporter mice and mice with WIPF inserted at the distal Runx3 promoter both appear to show higher levels of fluorescence in ILC1 and ILC3 versus NK cells44, 50. Human RUNX3 transcript levels were high in both CD56bright and CD56dim subsets (not shown) while CD56bright cells selectively expressed greater RUNX2 mRNA (Fig. 4A,B).

On the other hand, human CD56dim cells show higher levels of mRNA for RORA, BCL11B, and TBX21 (Fig. 4A), which have also been implicated in ILC development. Rora− and Bcl11b− mice have severe deficiencies in ILC244–45. Mice deficient in Tbx21 (T-bet) lack NK1.1− Nkp46− TRAIL− DX5− ILC1 cells in liver, while DX5− NK cells are partially retained but phenotypically altered12, 17, 18. In summary, the transcription factors that may control the differentiation of human CD56bright and CD56dim cells appear to be closely related with those that affect ILC versus NK cell differentiation in the mouse. One exception is Eomes, implicated in mouse studies as critical in the regulation and demarcation of NK cell versus ILC1 development12, 17, 18. EOMES did not score as significantly different between the human subsets in microarray. Both CD56bright and CD56dim cells showed high levels of EOMES protein by intracellular flow cytometry (Fig. 1C). Interestingly, the subset of human liver ILC/NK that express the characteristic mouse ILC gene CXCR6 are also Eomeshigh, suggesting differences between species41.

The CD56dim population also possesses higher levels of several other transcription factors associated with the formation or function of mature mouse NK cells, including NFIL3 (E4BP4), PRDM1 (BLIMP1), and IKZF3 (AILOLS) (Fig. 4A). Njil3−/− mice lack most NK cells56, 57, but Njil3 is also necessary for the formation of ILC progenitors and most mouse ILC lineages with the apparent exception of liver DX5− ILC153, 54, 55. Prdm1 deficiency has been shown to affect the proliferation and maturation phenotype of mouse DX5− NK1.1− NK cells, exhibiting increased CD27, CD117, and decreased KLRG155, molecules that are also differentially expressed between human CD56bright/CD56dim and mouse ILC1/NK cell subsets (Fig. 1D, 2). Selective expression of PRDM1 in human CD56dim cells has been additionally confirmed56, 57. Ikaros has also been shown to affect mouse DX5− NK1.1− NK cell maturation markers and function58.

Interestingly, human CD56dim cells express higher levels of transcripts for ZEB2, while CD56bright cells show greater amounts of message for the related molecule, ZEB1 (Fig. 1A,B). Zeb2 was also selectively expressed in mouse NK cells in comparisons versus ILC1 in all three tissues examined (Fig. 2, Suppl. Figure 3, 4), and is included in the ImmGen mouse Core NK signature.1 Targeted deletion of Zeb2 resulted in impaired NK cell maturation affecting several molecules observed to differ between CD56bright and CD56dim subsets (including CD27, KLRG1, S1PR5, and CXCR3) in a gene-dosage dependent manner52. It is intriguing to speculate that the Zeb1/Zeb2 balance could form a molecular switch controlling the differentiation fate of ILC/NK subsets. It is also striking that Zeb2, TBX21, PRDM1, and IKZF3, each expressed at higher levels in human CD56dim cells, all show some commonalities in mouse knockout models32, 46, 47.

Many of the transcription factors that differ between CD56bright and CD56dim cells also play roles in T cell development and differentiation. For example, Gata3, Tcf7, Lef1, Bcl11b (and Runx1/Runx3) were included in...
a regulatory network controlling mouse thymic T cell development, which also includes Kit (CD117) and IL7r (CD127)\(^9\). Similarly, Sox4, Tcf7, Prdm1, Runx2, Gata3, and Tbx21 (along with Eomes) are part of a bioinformatically determined transcription factor network associated with mouse T cell memory formation\(^9\). Furthermore, perturbation analyses performed by retrovirual transduction of each gene into T cells has suggested complex regulatory effects amongst these transcription factors\(^9\). For example, overexpression of Sox4 downregulated mRNA for Tcf7, Eomes, Runx2, and Tbx21, while overexpression of Tcf7 downregulated Sox4, Eomes, and Prdm1 transcripts in addition, transduction of Prdm1 repressed transcription of both Sox4 and Tcf7\(^9\). It is likely that a similar network of interactions (including additional factors from Fig. 4) governs differentiation into human blood CD56\(^{\text{bright}}\) and CD56\(^{\text{dim}}\) subsets. Future studies will determine if this network is analogous to that regulating ILC versus NK cell differentiation in the mouse, which in turn may explain the cross-species transcriptome similarities observed (Figs 1, 2 and 3).

**Methods**

**Microarray data analyses.** Affymetrix HTA2.0 chip CEL files from flow cytometry sorted human peripheral blood CD56\(^{\text{bright}}\) (CD3\(^{-}\) CD56\(^{\text{bright}}\) CD16\(^{-}\)) and CD56\(^{\text{dim}}\) (CD3\(^{-}\) CD56\(^{\text{dim}}\) CD16\(^{-}\)) subsets (from ref. 21) (NCBI GEO accession numbers GSM2278891-GSM2278896 inclusively) were normalized by robust multi-array average (RMA) algorithm using Expression Console version 1.4.1.46 with “Gene level RNA sketch” settings, and annotated with Affymetrix release 35.1 transcript cluster annotations. Unless otherwise noted, the shown fold change values are calculated from this normalized HTA2.0 data. HG-U133A/B CEL files from three additional independent samples of each of human blood CD56\(^{\text{bright}}\) and CD56\(^{\text{dim}}\) cells (from ref. 22) (GSM2108750-GSM2108755 inclusively) and GSM2108760-GSM2108765 inclusively) were normalized using Partek Genomics Suite 6.6 using the RMA algorithm with default settings and annotated with Affymetrix annotations (release 35).

CEL files encoding microarray data describing mouse spleen, liver, and small intestine ILC subsets from the ImmGen project\(^23\) were downloaded from NCBI GEO with accessions GSM1585312-GSM1585337 inclusively, then RNA normalized with Partek Genomics Suite 6.6 (with default settings) and annotated with Affymetrix release 35 transcript cluster annotations. Additional CEL files analysing small intestine ILC and NK populations were also downloaded from ArrayExpress (E-MTAB-2428)\(^12\) and similarly analysed using Partek Genomics suite. Human transcription factors were identified using a list compiled from three sources (see refs 68–70).

**Gene Set Enrichment Analysis** was performed with GSEA v2.2.0 or v2.2.3 (Broad Institute)\(^24\) using default settings with the exception of gene set randomization as the permutation type, due to the limited number of phenotypes analysed.

**Cross-species transcriptome comparisons.** Single microarray probesets were chosen to represent each gene, prioritizing: lack of cross reactivity, recognition of coding regions, and higher fold changes between subsets, when such information was annotated. Mouse and human gene expression results were synchronised using NCBI Homologene database (release 68) allowing examination of 16,351 mouse-human homologous gene pairs. Spearman correlations between human and mouse relative gene expression were performed on log\(_2\) transformed fold change values, examining only genes for which expression differed by more than two fold between the mouse subsets. Lists of signature mouse transcripts were also converted to human homologues via NCBI Homologene.

**Human blood samples.** Peripheral blood immune cell subsets were isolated from de-identified blood bank buffy-coat or leukopack samples. Depending on the site of the experiments and source of samples (i) NIH Office of Human Subjects Research Protections, and (ii) the Committee on the Use of Human Subjects (the Harvard institutional review board) determined that this use of this material is exempt from the requirements of IRB review, therefore not requiring written consent.

**Quantitative Real-time RT-PCR.** Leucopacks were treated using a negative enrichment antibody mixture (NK RosetteSep, Stem Cell Technologies). Enriched cells were then flow cytometry sorted in order to isolate CD56\(^{\text{bright}}\) (CD3\(^{-}\) CD56\(^{\text{bright}}\) CD16\(^{-}\) ), CD56\(^{\text{dim}}\) (CD3\(^{-}\) CD56\(^{\text{dim}}\) CD16\(^{-}\) ) subsets, as well as an intermediate population which is CD3\(^{-}\) CD56\(^{\text{bright}}\) CD16\(^{+}\). Cells were washed 3-4 times with PBS and cell pellets stored at -80°C. RNA was isolated using the RNeasy Plus Mini Kit (Qiagen). All isolated RNAs showed an RNA Integrity Number of >9.3 when tested by Agilent Bioanalyzer. First strand cDNA was synthesized from 100-200ng total RNA using RT\(^2\) First Strand Kit (Qiagen) and PCR reactions were performed with RT\(^2\) SYBR Green Mastermix (Qiagen) in triplicate. The PCR reaction profile consisted of 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C in BioRad CFX96 or Stratagene MX3000p thermocyclers. Primers were derived from custom designed RT\(^2\) Profiler PCR Arrays (SuperArray/Qiagen). Samples with multiple melting-curve peaks were not included in analyses.

**Flow cytometry.** After overnight at room temperature, NK cells were isolated from human blood buffy-coat samples with RosetteSep as above, and stained with the following mAb clones recognizing CD56 (NCAM16.2), CD16 (3G8), CD127 (HIL-7R-M21), HLA-DR (G46-6), CD25 (M-A251), TRAIL (RIK-2) from BD Biosciences, samples with Rosettesep as above, and stained with the following mAb clones recognizing CD56 (NCAM16.2), CD16 (3G8), CD127 (HIL-7R-M21), HLA-DR (G46-6), CD25 (M-A251), TRAIL (RIK-2) from BD Biosciences, and stained with the following mAb clones recognizing CD16 (3G8), CD127 (HIL-7R-M21), HLA-DR (G46-6), CD25 (M-A251), TRAIL (RIK-2) from BD Biosciences, and stained with the following mAb clones recognizing CD16 (3G8), CD127 (HIL-7R-M21), HLA-DR (G46-6), CD25 (M-A251), TRAIL (RIK-2) from BD Biosciences, and stained with the following mAb clones recognizing CD16 (3G8), CD127 (HIL-7R-M21), HLA-DR (G46-6), CD25 (M-A251), TRAIL (RIK-2) from BD Biosciences.

**References**

1. Lanier, L. L., Le, A. M., Civeit, C. I., Loken, M. R. & Phillips, J. H. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J Immunol.* **136**, 4480–4486 (1986).
2. Cooper, M. A., Fehniger, T. A. & Caligiuri, M. A. The biology of human natural killer-cell subsets. *Trends Immunol.* **22**, 633–640 (2001).

**Note:** This version includes the abstract, introduction, methods, results, discussion, and references of the original manuscript but not the references. Please refer to the original manuscript for a complete list of references.
3. Cooper, M. A. et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood. 97, 3146–3151 (2001).

4. Chan, A. et al. CD56bright human NK cells differentiate into CD56dim cells: role of contact with peripheral fibroblasts. J Immunol. 179, 89–94 (2007).

5. Huntington, N. D. et al. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. J Exp Med. 206, 35–34 (2009).

6. Romagnani, C. et al. CD56brightCD16- killer Ig-like receptor- NK cells display longer telomeres and acquire features of CD56dim NK cells upon activation. J Immunol. 178, 4947–4955 (2007).

7. Allan, D. S. et al. TGFB-beta affects development and differentiation of human natural killer cell subsets. Eur J Immunol. 40, 2289–2295 (2010).

8. Wu, C. et al. Clonal tracking of rhesus macaque hematopoiesis highlights a distinct lineage origin for natural killer cells. Cell Stem Cell. 14, 486–499 (2014).

9. Mace, E. M. et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. Blood. 121, 2669–2677 (2013).

10. Artis, D. & Spits, H. The biology of innate lymphoid cells. Nature. 517, 293–301 (2015).

11. Constantinides, M. G., McDonald, B. D., Verhoeef, P. A. & Bendelac, A. A committed precursor to innate lymphoid cells. Nature. 508, 397–401 (2014).

12. Klose, C. S. et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. Cell. 157, 340–356 (2014).

13. Takeda, K. et al. TRAIL identifies immature natural killer cells in newborn mice and adult mouse liver. Blood. 105, 2082–2089 (2005).

14. Peng, H. et al. Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. J Clin Invest. 123, 1444–1456 (2013).

15. Sojka, D. K. et al. Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. Elife. 3, e01659 (2014).

16. Tang, L. et al. Differential phenotypic and functional properties of liver-resident NK cells and mucosal ILC1s. J Autoimmun. 67, 29–35 (2016).

17. Daussy, C. et al. T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. J Exp Med. 211, 563–574 (2014).

18. Gordon, S. M. et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. Immunity. 36, 55–67 (2012).

19. Vonarbourg, C. et al. Regulated expression of nuclear receptor ROGammat confers distinct functional fates to NK cell receptor-expressing ROGammat(+) innate lymphocytes. Immunity. 33, 736–751 (2010).

20. Bernink, J. H. et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol. 14, 221–229 (2013).

21. Cavalli, R. C. et al. Induced Human Decidual NK-Like Cells Improve Utero-Placental Perfusion in Mice. PLoS One. 11, e0164353 (2016).

22. Kopcow, H. D. et al. Human decidual NK cells from gravid uteri and NK cells from cycling endometrium are distinct NK cell subsets. Placenta. 31, 334–338 (2010).

23. Robinette, M. L. et al. Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. Nat Immunol. 16, 306–317 (2015).

24. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 102, 15545–15550 (2005).

25. Hanna, J. et al. Novel insights on human NK cells’ immunological modalities revealed by gene expression profiling. J Immunol. 173, 6547–6563 (2004).

26. Caligiuri, M. A. et al. Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer cell subset with high affinity receptors. J Exp Med. 171, 1509–1526 (1990).

27. Nagler, A., Lanier, L. L. & Phillips, J. H. Constitutive expression of high affinity interleukin 2 receptors on human CD16-natural killer cells in vivo. J Exp Med. 171, 1527–1533 (1990).

28. Andre, P. et al. Modification of P-selectin glycoprotein ligand-1 with a natural killer cell-restricted sulfated lactosamine creates an alternate ligand for L-selectin. Proc Natl Acad Sci USA. 97, 3400–3405 (2000).

29. Knox, J. J., Cosma, G. L., Betts, M. R. & McClane, L. M. Characterization of T-bet and eomes in peripheral human immune cells. Front Immunol. 5, 217 (2014).

30. Campbell, J. J. et al. Unique subpopulations of CD56(+)NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. J Immunol. 166, 6477–6482 (2001).

31. Jacobs, R. et al. CD16–CD56+ natural killer cells after bone marrow transplantation. Blood. 79, 3239–3244 (1992).

32. Matos, M. E. et al. Expression of a functional c-kit receptor on a subset of natural killer cells. J Exp Med. 178, 1079–1084 (1993).

33. Nielsen, N., Odum, N., Urso, B., Lanier, L. L. & Spee, P. Cytotoxicity of CD56(bright) NK cells towards autologous activated CD4+T cells is mediated through NKGD2, LFA-1 and TRAIL and dampened via CD94/NKG2A. Proc Nat Acad Sci USA. 105, 4947–4955 (2008).

34. Hepworth, M. R. et al. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4(+) T cells. Science. 348, 1031–1035 (2015).

35. Hannedouche, S. et al. Oxyterols direct immune cell migration via EBI2. Nature. 475, 524–527 (2011).

36. Cortez, V. S. & Colonna, M. Diversity and function of group 1 innate lymphoid cells. Immunol Lett. 179, 19–24 (2016).

37. Freud, A. G. et al. Evidence for discrete stages of human natural killer cell differentiation in vivo. J Exp Med. 203, 1033–1043 (2006).

38. Hughes, T. et al. Interleukin-1beta selectively expands and sustains interleukin-22+ immature natural killer cells in secondary lymphoid tissue. Immunity. 32, 803–814 (2010).

39. Hughes, T. et al. The transcription factor AHR prevents the differentiation of a stage 3 innate lymphoid cell subset to natural killer cells. Cell Rep. 8, 150–162 (2014).

40. Renoux, V. M. et al. Identification of a Natural Human Natural Killer Cell Lineage-Restricted Progenitor in Fetal and Adult Tissues. Immunity. 43, 394–407 (2015).

41. Stegmann, K. A. et al. CXCRC6 marks a novel subset of T-bet(lo)/Eomes(hi) natural killer cells residing in human liver. Sci Rep. 6, 26157 (2016).

42. Marquardt, N. et al. Cutting edge: identification and characterization of human intrahepatic CD49a+ NK cells. J Immunol. 194, 2467–2471 (2015).

43. Yagi, R. et al. The transcription factor GATA3 is critical for the development of all IL-7Ralpha-expressing innate lymphoid cells. Immunity. 40, 378–388 (2014).

44. Kiss, E. A. et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science. 334, 1561–1565 (2011).

45. Lee, J. S. et al. AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. J Exp Med. 113, 144–153 (2012).

46. Qiu, J. et al. The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. Immunity. 36, 92–104 (2012).
47. Zhang, L. H., Shin, J. H., Haggadone, M. D. & Sunwoo, J. B. The aryl hydrocarbon receptor is required for the maintenance of liver-resident natural killer cells. *J Exp Med.* **213**, 2249–2257 (2016).
48. Yang, Q. et al. TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nat Immunol.* **16**, 1044–1050 (2015).
49. Mielke, L. A. et al. TCF-1 controls ILC2 and NKp46+RORgammaT+ innate lymphocyte differentiation and protection in intestinal inflammation. *J Immunol.* **191**, 4383–4391 (2013).
50. Ejihara, T. et al. Runx3 specifies lineage commitment of innate lymphoid cells. *Nat Immunol.* **16**, 1124–1133 (2015).
51. Halim, T. Y. et al. Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. *Immunity.* **37**, 463–474 (2012).
52. Wong, S. H. et al. Transcription factor RORalpha is critical for nuocyte development. *Nat Immunol.* **13**, 229–236 (2012).
53. Walker, J. A. et al. Bcl11b is essential for group 2 innate lymphoid cell development. *J Exp Med.* **212**, 875–882 (2015).
54. Yu, Y. et al. The transcription factor Bcl11b is specifically expressed in group 2 innate lymphoid cells and is essential for their development. *J Exp Med.* **212**, 865–874 (2015).
55. Califano, D. et al. Transcription Factor Bcl11b Controls Identity and Function of Mature Type 2 Innate Lymphoid Cells. *Immunity.* **43**, 354–368 (2015).
56. Kamizono, S. et al. Nfil3/E4bp4 is required for the development and maturation of NK cells *in vivo*. *J Exp Med.* **206**, 2977–2986 (2009).
57. Gascogne, D. M. et al. The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol.* **10**, 1118–1124 (2009).
58. Geiger, T. L. et al. Nfil3 is crucial for development of innate lymphoid cells and host protection against intestinal pathogens. *J Exp Med.* **211**, 1723–1731 (2014).
59. Seillet, C. et al. Nfil3 is required for the development of all innate lymphoid cell subsets. *J Exp Med.* **211**, 1733–1740 (2014).
60. Yu, X. et al. The basic leucine zipper transcription factor Nfil3 directs the development of a common innate lymphoid cell precursor. *Elife.* **3** (2014).
61. Male, V. et al. The transcription factor E4bp4/Nfil3 controls commitment to the NK lineage and directly regulates Eomes and Id2 expression. *J Exp Med.* **211**, 635–642 (2014).
62. Kallies, A. et al. A role for Blimp1 in the transcriptional network controlling natural killer cell maturation. *Blood.* **117**, 1869–1879 (2011).
63. Smith, M. A. et al. PRDM1/Blimp-1 controls effector cytokine production in human NK cells. *J Immunol.* **185**, 6058–6067 (2010).
64. Holmes, M. L. et al. Peripheral natural killer cell maturation depends on the transcription factor Aiolos. *EMBO J.* **33**, 2721–2734 (2014).
65. van Helden, M. J. et al. Terminal NK cell maturation is controlled by concerted actions of T-bet and Zeb1 and is essential for melanoma rejection. *J Exp Med.* **212**, 2015–2025 (2015).
66. Kueh, H. Y. & Rothenberg, E. V. Regulatory gene networks circuits underlying T cell development from multipotent progenitors. *Wiley Interdiscip Rev Syst Biol Med.* **4**, 79–102 (2012).
67. Hu, G. & Chen, J. A genome-wide regulatory network identifies key transcription factors for memory CD8+ T-cell development. *Nat Commun.* **4**, 2830 (2013).
68. Ravasi, T. et al. An atlas of combinatorial transcriptional regulation in mouse and man. *Cell.* **140**, 744–752 (2010).
69. Zhang, H. M. et al. AnimalTFDB: a comprehensive animal transcription factor database. *Nucleic Acids Res.* **40**, D144–149 (2012).
70. Zhang, H. M. et al. AnimalTFDB 2.0: a resource for expression, prediction and functional study of animal transcription factors. *Nucleic Acids Res.* **43**, D76–81 (2015).

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**Author Contributions**

D.S.J.A., A.R., and A.S.C. performed the experiments. D.S.J.A. and A.R. analyzed the data and prepared the figures. D.S.J.A., J.R.C., H.D.K., C.L.K., O.A.A., and M.T. wrote the paper. H.D.K. and A.S.C. provided unpublished microarray data. The project was initiated in the laboratory of J.L.S. by D.S.J.A. and H.D.K., continued in the laboratory of J.R.C. (with input from H.D.K.), and completed with support and input from R.W.C. and C.E.D.

**Additional Information**

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