Innate-like CD8+ T-cells and NK cells: converging functions and phenotypes

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Summary

New data in the worlds of both innate-like CD8+ T-cells and natural killer (NK) cells have, in parallel, clarified some of the phenotypes of these cells and also their associated functions. While these cells are typically viewed entirely separately, the emerging innate functions of T-cells and, similarly, the adaptive functions of NK cells suggest that many behaviours can be considered in parallel. In this review we compare the innate functions of CD8+ T-cells (especially mucosal-associated invariant T-cells) and those of NK cells, and how these relate to expression of phenotypic markers, especially CD161 and CD56.

Keywords: CD8+ T-cell; innate-like T-cell; mucosal-associated invariant T-cell; NK cell.

Introduction

Natural killer (NK) cells belong to the innate lymphoid cell (ILC) family, which share a dependency on the common cytokine receptor γ chain and the transcriptional repressor, inhibitor of DNA binding 2 (Id2) for their development.1,2 These cells lack somatically rearranged antigen receptors like T- and B-cell receptors, and are able to rapidly respond to microbial products, cytokine stimulation and contact with other leukocytes.3 NK cells form a distinct lineage of the family of ILCs and are often described as the cytotoxic arm of ILCs, or the innate counterpart of CD8+ T-cells.4–8

CD8+ T-cells have traditionally been studied in the context of their memory status, whether they are naïve or memory. However, in recent years a number, a large fraction of the human CD8+ T-cell population has been identified as mucosal-associated invariant T-cells (MAIT cells)9–11; an innate-like T-cell population that is classically defined by its expression of a semi-invariant T-cell receptor (TCR), Vα7.2-Jα23, and restriction by the major histocompatibility complex (MHC) class Iβ molecule, MHC-class I-related protein 1 (MR1).12,13 These cells share a functional phenotype with a family of other CD8+ T-cells that express high levels of the C-type lectin-like receptor CD161.14 CD161 expression divides the CD8+ T-cell population into three distinct functional subsets.15,16 These subsets show varying degrees of innate (or NK-like) activity according to their CD161 expression – thus CD161 expression can act as a marker of ‘innateness’ in T-cell populations.

Recent insights into these subsets of CD8+ T-cells as well as subsets of NK cells have highlighted interesting similarities between these populations. In this review, we will discuss what is currently known about each of the subsets of NK cells and CD8+ T-cells, using the segregation of CD8+ T-cells through CD161 expression as a starting point. We will, through this specific prism, explore shared functional features between these populations, as an alternative way of viewing these innate and adaptive counterparts. While there are many ways to approach this comparison through different individual

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CMV, CYTOMEGALOVIRUS; IFN, interferon; ILC, innate lymphoid cell; KIR, killer immunoglobulin-like receptors; MAIT cell, mucosal-associated invariant T-cell; MR1, MHC-class I-related protein 1; NK cell, natural killer cell; PLZF, promyelocytic leukaemia zinc finger protein; RORγ, RAR-related orphan receptor gamma; TCR, T-cell receptor; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; UCB, umbilical cord blood

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surface markers, the consistent nature of the CD161-associated functional effect in T-cells, and the set of co-expressed molecules that track with it, make this a simple and attractive focus.

**The ILC family**

Three groups of ILCs have been defined with distinct patterns of cytokine production that mirrors that of T helper cell subsets, called group 1 ILCs (ILC1), group 2 ILC (ILC2) and group 3 ILC (ILC3). ILC2s produce IL-5 and IL-13 in response to epithelial cell-derived IL33 and/or IL-25. Their production of IL-13 is critical for helminth expulsion, while they can also drive atopic dermatitis and allergic inflammations. ILC3s in turn are characterized by their innate ability to secrete IL-17A, IL-17F, IL-22 and GM-CSF. IL-22 production by ILC3s is critical in containing commensal bacteria, and IL-23-responsive ILC3s also mediate colitis and accumulate in the inflamed intestine of CD patients. ILC3s also include the LTi cells involved in organizing tertiary lymphoid structures and tissue repair.

Group 1 ILCs were initially proposed to include NK cells and non-NK cells, which secrete interferon (IFN)γ, and express T-bet. ILC1 cells are important anti-bacterial cells, involved for example in the protection against *Salmonella enterica*. Furthermore, ILC1s accumulate in the inflamed intestine of CD patients. There also seems to be some plasticity between ILC1 and ILC3 cells. Thus, ILCs secreting both IFNγ and IL-17 in response to IL-23, or IFNγ and IL-22 in response to IL-12+IL-18 have been reported. It is thought that both NK cells and ILC1s depend on IL-15 for their development, particularly due to the presence of tissue-resident NKp46+ ILC3s. NK cells are often described as the immunoregulatory population, based on their ability to secrete cytokines including IFNγ, and are considered to be poorly cytotoxic. CD56dim NK cells, on the other hand, have a highly cytotoxic phenotype and can efficiently lyse virus-infected and tumour cell lines without prior activation. However, this dichotomy based on cytokine production and cytotoxicity may be too simplistic, as CD56bright NK cells can also be cytotoxic, while CD56dim NK cells can also produce abundant cytokines and chemokines following activation.

Alternatively, these subsets can be functionally divided based on their responsiveness to specific signals. CD56bright NK cells express high levels of cytokine receptors, which enables these cells to produce abundant IFNγ and proliferate in response to cytokines such as IL-2, IL-15, IL-12, IL-18 and IFNαβ. As CD56bright NK cells are the dominant NK cell population in tissues including lymph nodes and inflammatory sites, this allows them to interact with DCs and T-cells at these sites. In contrast, CD56bright NK cells secrete little IFNγ in response to target cell recognition mediated by receptors such as NKG2D, even though the receptor is equally expressed by CD56bright and CD56dim cells. In turn, CD56dim NK cells are the earliest and dominant IFN+ cells in response to activating receptor ligation. Furthermore, CD56dim NK cells are able to form more conjugates with infected or transformed cells, and the expression of low-affinity receptor III (CD16) is largely restricted to CD56dim NK cells. These features,

**Subsets of NK cells**

**CD56bright and CD56dim NK cells**

Two NK cell subsets have been well characterized in human peripheral blood, based on the expression level of CD56: CD56bright and CD56dim NK cells. The majority of peripheral blood NK cells are CD56dim NK cells, while the ratio is inverted in most organs including the lymphoid tissues where the CD56bright NK cells dominate, particularly due to the presence of tissue-resident CD56bright NK cells (as discussed later). CD56bright NK cells are often described as the immunoregulatory population, based on their ability to secrete cytokines including IFNγ, and are considered to be poorly cytotoxic.

CD56dim NK cells, on the other hand, have a highly cytotoxic phenotype and can efficiently lyse virus-infected and tumour cell lines without prior activation. However, this dichotomy based on cytokine production and cytotoxicity may be too simplistic, as CD56bright NK cells can also be cytotoxic, while CD56dim NK cells can also produce abundant cytokines and chemokines following activation.
together with high expression of cytolytic molecules, allow CD56\textsuperscript{dim} NK cells to efficiently lyse target cells either directly or indirectly through CD16-mediated antibody-dependent cellular cytotoxicity (ADCC). The expression of a family of receptors called killer immunoglobulin-like receptors (KIRs), which modulate the responsiveness of NK cells to activating receptor ligation,\textsuperscript{62,63} is also restricted to CD56\textsuperscript{dim} NK cells.

Traditionally it has been thought that there is a linear developmental relationship between CD56\textsuperscript{bright} and CD56\textsuperscript{dim} NK cells. This is supported by studies showing that CD56\textsuperscript{bright} NK cells have longer telomeres.\textsuperscript{64} As murine NK cells do not express CD56, RAG2\textsuperscript{−/−} γc\textsuperscript{−/−} mice transplanted with human haematopoietic stem cells have been used to show that CD56\textsuperscript{bright} NK cells differentiated linearly into CD56\textsuperscript{dim} NK cells, acquiring CD16 and KIR expression.\textsuperscript{65,66} This has also been shown in vitro by culturing CD56\textsuperscript{bright} NK cells in the presence of synovial or skin fibroblasts, or cytokines.\textsuperscript{52,67}

Recent evidence from rhesus macaques, however, has suggested that the lineage origin of macaque NK cell homologues of CD56\textsuperscript{bright} NK cells (CD56\textsuperscript{+} CD16\textsuperscript{−}) may be different from CD56\textsuperscript{dim} homologues (CD56\textsuperscript{−} CD16\textsuperscript{+}).\textsuperscript{58} Furthermore, patients with mutations in the GATA2 gene lead to the absence of CD56\textsuperscript{bright} NK cells while CD56\textsuperscript{dim} NK cells are preserved.\textsuperscript{69,70} Thus, whether CD56\textsuperscript{bright} and CD56\textsuperscript{dim} NK cells should be considered cells with independent lineages needs to be re-examined.

**‘Adaptive’ CD56\textsuperscript{dim} NK cells**

Recently, a terminally differentiated population of NK cells with memory-like properties has been described in the context of CMV.\textsuperscript{71–73} Primary MCMV infection has been shown to induce the clonal expansion of NK cells expressing the Ly49H receptor, which interacts with the m157 protein of MCMV, which persist in tissues for months after infection and, upon re-challenge, undergo secondary expansion with enhanced effector functions.\textsuperscript{72} These NK cells thus exhibit memory-like properties that were previously only attributed to cells of the adaptive immune system.

In humans, CMV infections are asymptomatic in healthy individuals, but immunosuppressed individuals, such as patients with human immunodeficiency virus (HIV), are at high risk of developing disease. CMV also skews the NK cell receptor repertoire in humans, with cells expressing the activating heterodimer NKG2C/CD94 expanding in recipients of solid organ\textsuperscript{74} or umbilical cord blood (UCB) transplantation\textsuperscript{75} during primary CMV infection or reactivation. These cells have an enhanced ability to secrete IFNγ in response to target cells or, even more so, upon CMV reactivation.\textsuperscript{74–76} Therefore, it has been suggested that these cells represent the human counterparts of Ly49H+ NK cells with memory-like properties.

These NKG2C+ cells can be identified by their high expression of CD57, and they express inhibitory KIRs specific for self-MHC class I molecules.\textsuperscript{74,77,78}

**Subsets of CD8+ T-cells**

**MAIT cells and CD161-expressing CD8+ T-cells**

Mucosal-associated invariant T-cells were first identified in 1993 by virtue of their expression of a unique TCR α rearrangement,\textsuperscript{79} which was subsequently ascribed to a novel subset of T-cells restricted by MR1.\textsuperscript{80} MAIT cells have been shown to detect a variety of microbes through the recognition of vitamin B metabolites presented by MR1.\textsuperscript{13,81,82} Furthermore, these cells were found to largely overlap with a unique subset of CD8+ T-cells expressing CD161 at a high level, CD161\textsuperscript{++} CD8+ T-cells.\textsuperscript{11,15}

Human peripheral blood CD8+ T-cells can be divided into three distinct populations according to their relative expression level of the C-type lectin-like receptor CD161.\textsuperscript{15} These subsets are clearly present at birth, suggesting that they are distinct populations with pre-programmed functional differences. MAIT cells are only a small fraction of the CD161\textsuperscript{++} CD8+ T-cell population in cord blood,\textsuperscript{83} but slowly expand with age, making up more than 90% of the CD161\textsuperscript{++} CD8+ T-cell population in adults.\textsuperscript{11,14} Both MAIT and non-MAIT CD161\textsuperscript{++} CD8+ T-cells share functional features, most notably the ability to respond to innate cytokines in the absence of TCR stimulation, due to their high expression of cytokine receptors such as IL-18R and IL-12R.\textsuperscript{9,84} This function is likely driven by the shared expression by MAIT and non-MAIT CD161\textsuperscript{++} CD8+ T-cells of the master transcription factor promyelocytic leukaemia zinc finger protein (PLZF).\textsuperscript{14,85}

Cells expressing CD161 at an intermediate level, CD161\textsuperscript{+/int} CD8+ T-cells, also have a distinct pre-programmed transcriptional profile that is shared between cord blood cells and their adult counterparts.\textsuperscript{16} The majority (96%) of these polyclonal cells in adults are memory cells, containing both effector memory and terminally differentiated memory cells as defined by CCR7 and CD45RA co-expression. They constitutively express the cytotoxic mediators granzyme B and perforin. In contrast, a quarter of the cells that lack CD161-expression, the CD161\textsuperscript{−} CD8+ T-cell population, are naïve CD8+ T-cells, and even within the memory population express a lower level of granzyme B and perforin compared with the CD161\textsuperscript{+/int} CD8+ T-cell population. The ability of a CD8+ T-cell to respond to innate cytokines is correlated with the expression level of CD161, where it is only weakly associated with the CD161\textsuperscript{+/int} CD8+ T-cell population and almost negligible in the CD161− CD8+ T-cells.\textsuperscript{14}
Functional similarities between NK cell and CD8+ T-cell subsets

Cytokine responsiveness and proliferation

CD56\textsuperscript{bright} NK cells and CD161++ CD8+ /MAIT cells both constitutively express high levels of cytokine receptors, such as IL-7R (CD127)\textsuperscript{98} and IL-18R (Fig. 1). IL18R signalling induces the secretion of IFN\textgamma; in response to IL-12+ IL-18, compared with their respective ‘dim’ counterparts. CD161++ CD8+ T-cells are uniformly high in IL-18R expression regardless of whether they are MAIT cells or non-MAIT, CD161++ V\alpha7-2–, cells,\textsuperscript{74} and this is already pre-programmed in fetal and cord blood cells.\textsuperscript{33,87} Additionally, CD56\textsuperscript{bright} NK cells are known for their proliferative capacity in response to cytokines;\textsuperscript{88} for example, liver-resident CD49a++ NK cells have been found to express high levels of CD25, and were able to proliferate in response to low doses of IL-2.\textsuperscript{89} CD161++ CD8+ T-cells are also highly proliferative upon stimulation with IL-12 and IL-15 compared with the CD161+ CD8+ T-cell counterparts.\textsuperscript{90,91}

In turn, CD161++ CD8+ T-cells have been described to be hyporesponsive to TCR signalling compared with their CD161+ and CD161– CD8+ T-cell counterparts.\textsuperscript{92} Indeed, MAIT cells did not proliferate in response to PHA, which cross-links the TCR/CD3 complex, but proliferated extensively when TCR stimulation was supplemented with anti-CD28 and anti-CD2, or with cytokines such as IL-12 and IL-18 during \textit{Escherichia coli}-induced proliferation. CD56\textsuperscript{dim} NK cells also have a heightened ability to respond to the cross-linking of activating receptors such as NKp30 and CD16.\textsuperscript{59,66} In particular, the terminally differentiated CD57++ NKG2C+ CD56\textsuperscript{dim} NK cells proliferate extensively in response to ligation of activation receptors such as NK2G2C, and have a heightened capacity to perform ADCC through CD16.\textsuperscript{92} Thus, both CD161++ CD8+ T-cells and CD56\textsuperscript{bright} NK cells have high cytokine responsiveness compared with other CD8+ and NK cell subsets, which is associated with a comparatively lower ability to respond to activating receptors alone.

One of the key transcription factors regulating the cytokine responsiveness of both NK cells and T-cells is thought to be PLZF. PLZF is critical for the function of innate-like T-cells, including MAIT cells,\textsuperscript{85} γδ T-cells\textsuperscript{93} and iNKT cells.\textsuperscript{94,95} Furthermore, newly described murine innate T-cells such as the PLZF+ ROR\textgamma;+ natural Th17 cells\textsuperscript{96} and PLZF+ T-CD4 T-cells\textsuperscript{97} also express PLZF, and are highly responsive to cytokines. CMV-specific terminally differentiated NK cells, or ‘adaptive’ NK cells, have extensively downregulated PLZF expression, due to hypermethylation of the ZBTB16 intronic sequence.\textsuperscript{98} PLZF was shown to directly bind the promoters of genes encoding signalling adaptor molecules, such as the SAP family receptor adaptor molecule EAT-2, as well as CD161, IL-12R and IL-18R.\textsuperscript{98} The regulation of signalling adaptor molecules by PLZF not only affects cytokine responsiveness, but also responsiveness to receptor stimulation, as adaptive NK cells show a heightened ability to respond to ligation of receptors such as CD16 and NKG2C.\textsuperscript{70,98} while NK cells from a PLZF-deficient patient showed increased responsiveness of activating receptor ligation.\textsuperscript{99} Thus, PLZF appears to be a direct regulator of cytokine receptor expression and signalling proteins in both NK cells and T-cells, and their differential expression level in subsets of these populations directly corresponds to their ‘innate-ness’ (Fig. 2).

Regulation of cytotoxicity

CD8+ T-cells and NK cells constitute the cytotoxic arms of T-cells and ILCs, respectively and, thus, share the expression of granzymes and perforin. Resting CD161++ CD8+ T-cells, in particular the MAIT cells, express no GrB and little perforin, and instead are uniquely enriched for GrK expression.\textsuperscript{90} In turn, CD161+ CD8+ T-cells are enriched for GrB and perforin expression, even compared with their CD161– CD8+ T-cell counterparts.\textsuperscript{16} The GrK+ GrB– perforin\textsuperscript{low} phenotype of CD161++ CD8+ T-cells was similar to the phenotype of CD56\textsuperscript{bright} NK cells (Fig. 1).\textsuperscript{81,100} However, both CD56\textsuperscript{bright} NK cells\textsuperscript{81} and CD161++ CD8+ T-cells (including the MAIT cells)\textsuperscript{90} can upregulate GrB and perforin, and become efficient killer cells, after activation. For example, the CD56\textsuperscript{bright} NK cell population can kill activated autologous CD4+ T-cells in MS.\textsuperscript{101,102} Furthermore, immature DCs are killed by CD56\textsuperscript{bright} NK cells in lymph nodes in a TRAIL-dependent manner,\textsuperscript{103,104} and IL-2-activated peripheral blood CD56\textsuperscript{bright} NK cells can become efficient killers.\textsuperscript{50–52} Similarly, CD69 + CXCR6 + tissue-resident CD56\textsuperscript{bright} NK cells in
Innate-like T-cells and NK cells

(a) CD8+ T cells vs NK cells

- CD161
- CD56
- IL-18R
- CD57
- CD16
- GrB
- GrK

(b) %IL-18R+
%CD57+
%CD16+
%GrB+
%GrK+

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lymphoid tissues have recently been shown to constitutively express perforin, but require pre-activation to express GrB and become cytotoxic, much like MAIT cells.

Co-expression of the transcription factors T-bet and Eomes is critical for the cytotoxic functions of both CD8+ T-cells and NK cells, and the similarities in granzyme and perforin expression in these cells is reflected by their expression of the T-bet and Eomes. Thus, both CD56dim NK cells and CD161+ CD8+ T-cells are enriched for Eomes+ T-bet++ cells, while CD56bright NK cells have a Eomes+ T-bet+ phenotype, which is mirrored by CD8+ MAIT cells. Of note, CD161+ CD8+ T-cells express higher levels of GrB and perforin even compared with their CD161/C0/nantly differentiated CD8+ T-cells. Bright/dim/– cells reflect by their expression of the T-bet and Eomes. Thus, both CD161 bright/MAIT and CD56 bright NK cell populations express chemokine receptors associated with inflammation such as CCR1, CCR5, as well as CCR6, which directs cells to sites of inflammation. Thus, CD56bright NK cells are highly enriched and activated within synovial fluid from patients with psoriatic arthritis and rheumatoid arthritis (RA), as well as in inflamed tissues in tuberculosis. Similarly, MAIT cells also express GrB and become cytotoxic, much like MAIT cells. Of note, CD161 bright/MAIT and CD56 bright/CD8+ T-cells are enriched for Eomes+ T-bet++ cells, while CD56bright NK cells have a Eomes+ T-bet+ phenotype, which is mirrored by CD8+ MAIT cells. Of note, CD161+ CD8+ T-cells express higher levels of GrB and perforin even compared with their CD161– CD8+ T-cell counterparts, although both populations contain similar frequencies of effector memory T-cells and terminally differentiated CD8+ T-cells.

Enrichment in tissues

Perhaps the most interesting similarity between CD56bright NK cells and CD16bright CD8+ T-cells is their enrichment in tissues. Although CD56bright NK cells only constitute 10% of peripheral blood NK cells, they are enriched in tissues such as lymphoid tissues, stomach, adrenal gland, colorectal, liver and adipose tissues. Although some of these may include organ-infiltrating NK cells, bona fide tissue-resident CD56bright NK cells have also recently been described (reviewed in). These cells are retained in the tissues through their expression of CD69, chemokine receptors such as CXCR6 and CCR5, as well as adhesion molecules such as CD49a. For example, more than 75% of the NK cells in lymph nodes express CD56 at high levels, the majority of which express CD69 and CXCR6, and have been shown to be lymphoid tissue-resident. Similar enrichment of both tissue-resident and circulating CD56bright NK cells has been found in the spleen, bone marrow and tonsils. The liver is also enriched for NK cells, half of which have the CD56bright phenotype. Liver-resident NK cells have been described to be CD56bright Eomeshigh, with high expression of CD69, CXCR6, CCR5, with Eomeshigh cells retained in the human liver without recirculation for up to 13 years. Finally, it is known that more than 70% of lymphocytes in the uterine decidua during pregnancy are CD56bright NK cells, expressing adhesion markers such as CD49a, CD69 and CD103.

Mucosal-associated invariant T-cells are also known to be highly enriched in peripheral tissues, as reviewed elsewhere. In particular, they may make up to 30%–50% of all intrahepatic T-cells, mediated by their expression of chemokine receptors such as CXCR6 and CCR6, which bind the chemokines CXCL16 and CCL20, respectively, that are constitutively expressed in the liver. Intrahepatic MAIT cells have been shown to be highly activated and express CD69, HLA-DR and CD38, and are positioned around bile ducts within hepatic portal tracts. Furthermore, as their name suggests, they are abundant within the gut, particularly in the jejunum and colon through their expression of gut-homing markers α4β7 and CCR9, as well as the lungs. In addition to their presence in tissues in the steady state, both CD161bright/MAIT and CD56bright NK cell populations express chemokine receptors associated with inflammation such as CCR1, CCR5, as well as CCR6, which directs cells to sites of inflammation. Thus, CD56bright NK cells are highly enriched and activated within synovial fluid from patients with psoriatic arthritis and rheumatoid arthritis (RA), as well as in inflamed tissues in tuberculosis.
Innate-like T-cells and NK cells

Lessons from NK cells to MAIT cells

A model summarizing the functional similarities between NK cell and CD8+ T-cell subsets discussed in this review is shown in Fig. 2. This, of course, disregards the specificity of the TCR, the developmental relationship between the subsets, and many differences are not noted here. For instance, the type-17 master transcription factor RORγt is expressed by MAIT cells, as well as non-MAIT CD8+ T-cells under inflammatory conditions, but not in NK cells. However, the general inverse correlation between cytokine responsiveness and responsiveness to activating receptors may in part explain the functional similarities between CD8+ T-cell and NK cell subsets. Furthermore, the tight regulation of cytotoxicity in both the CD56bright NK cells and MAIT cells may be associated with their abundance in tissues and the need to limit tissue damage that may be caused by their non-specific activation. These functions are associated with the shared expression of transcription factors that determine their effector functions. Interestingly, although these subsets of NK and T-cells can be clearly delineated using markers such as CD56 and CD161, both of these markers are not directly involved in determining the differential functions of these subsets; in fact, CD161 has been suggested to have opposing functions on T- and NK cells.

The similarities between NK cells and MAIT cells suggest that MAIT cells may share some functions already described in CD56bright NK cells. For instance, the expansion of CD56bright NK cells in patients with MS, receiving daclizumab treatment, is associated with better prognosis, due to their ability to kill pathogenic, highly activated CD4+ T-cells. Cytotoxicity is mainly mediated through the expression of Grk and NKG2D. MAIT cells are also enriched in MS lesions and indeed early papers suggested that they play a regulatory role. Whether MAIT cells are protective or pathogenic in MS may depend on their context of activation and the secretion of IL-17, but the regulatory role for MAIT cells could be further examined in the context of NKG2D-induced cytotoxicity. Additionally, it will be interesting to explore whether TRAIL-induced cytotoxicity utilized by CD56bright NK cells may also be used by MAIT cells.

Innate-like T-cells are so-called due to the fact that they share several functional features with NK cells. A recent study on iNKT cells demonstrated that iNKT cells share a broader transcriptional programme with NK cells than has been previously appreciated. In particular, many of the effector functions of innate-like T-cells can be shared with CD56bright NK cells. Furthermore, terminally differentiated/adaptive NK cells have been shown to have epigenetic modifications resembling memory CD8+ T-cells, particularly those affecting the expression of PLZF, IFNγ, cytokine receptors and signalling proteins. Thus, comparison of the epigenetic signature between immature NK cells and innate-like T-cells may also reveal important similarities associated with their innate programming, such as the expression of other members of the BTB-ZF protein family.

Conclusions

Given the striking functional difference between CD56bright and CD56dim NK cells, defining which population is being investigated has been a requirement for NK cell research for a long time. However, bulk CD8+ T-cell research generally only uses specific memory markers to differentiate the naïve and memory populations. Particularly with the advent of high-dimensional analysis techniques, such as mass cytometry, inclusion of CD161 in panels, along with MAIT cell tetramers, may help to highlight true differences from differences accounted for by the variable frequency of MAIT' cells and CD161+ T-cells.

Overall, much effort in immunology has been focused on ‘splitting’ various subtypes, looking for differences in phenotype or function. There might be a reasonable argument made for the usefulness of ‘lumping’ cell types, i.e. looking for commonalities in phenotype and function. Because these cells do not work in isolation, such an integrative approach may help understand how responses to pathogens and tumours are mediated in vivo and how common pathways may be manipulated for patient benefit in disease.

Disclosures

The authors have no conflicts of interest to declare.

References

1. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa SI et al. Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. Nature 1999; 397:702–6.
2. Hoyler T, Klose CS, Soubani A, Turqueti-Neves A, Pfeiffer D, Rawlins EL et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. Immunity 2012; 37:634–48.

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preferential use of several V' beta genes and an invariant TCR alpha chain. J Exp Med 1993; 178:1–16.

Tilky F, Treiner E, Park SL, Garcia C, Lemonnier F, De La Salle H et al. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class IIb-restricted alpha/betalpha T cell subpopulation in mammoths. J Exp Med 1999; 189:1907–21.

Le Bourhis L, Martin E, Pégault I, Guioth A, Feux N, Cori M et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 2010; 11:701–8.

Gold MC, Cerri S, Smyk-Feareon S, Cander ME, Vogt TM, Delpeche J et al. Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol 2010; 8: e1000407.

Walker LJ, Kang YH, Smith MO, Tharmlaingham H, Ramasorthy N, Fleming VM et al. Human MAIT and CD8+ cells develop from a pool of type-17 precommitted CD8+ T cells. Blood 2012; 119:622–33.

USher JE, Bilton M, Attwood E, Shadwell J, Richardson R, Lara C et al. CD61++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12–IL-18 in a TCR-independent manner. Eur J Immunol 2014; 44:195–203.

Koay HF, Gherardin NA, Enders A, Lob L, Mackay LK, Almeida CF et al. A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. Nat Immunol 2016; 17:1300. https://doi.org/10.1038/ni.3565.

Zwirner NW, Domaica CI. Cytokine regulation of natural killer cell effector functions. BioFactors 2010; 36:274–9.

Leosnayah E, Lob L, Nixon DF, Sandberg JK. Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. Nat Commun 2014; 5:3143.

Punt J, Owen J, Caligiuri MA. The biology of human natural killer cell subsets. Trends Immunol 2001; 22:633–40.

Matturri G, Kautz T, Lunemann S, Richert L, Gála L, Sailerberger W et al. Proliferative capacity exhibited by human liver-resident CD4+8+ CD56+ NK cells. PLoS ONE 2017; 12:e0182532.

Kuroska A, USher JE, Csogrove C, Clough C, Ferguson JR, Smith K et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. Mucosal Immunol 2015; 8:429–40.

Turtle CJ, Dellow J, Jolly RC, Swanson HM, Basem R, Tablinelli L et al. Invariant sigleukin overcome acquired TCR signaling pathway regulation and govern the fate of human CD161(b+) CD8+ semi-invariant T cells. Blood 2011; 118:2752–62.

Rollé A, Pullmann J, Ewen EM, Le VT, Halesius A, Hengel H et al. IL-12-producing monocytes and HLA-E-control HCMV-driven NKGC+ NK cell expression. J Clin Invest 2014; 124:5055–16.

Kreislovsky T, Savage AK, Hobbs R, Gouani F, Bronron R, Perete P et al. TCR-inducible PLZF transcription factor required for innate phenotype of a subset of γδ T cells with restricted TCR diversity. Proc Natl Acad Sci USA 2009; 106:12453–8.

Savage AK, Constantinides MG, Han J, Picard D, Martin E, Li B et al. The transcription factor PLZF directs the effector program of the NK cell lineage. Immunology 2008; 29:391–403.

Kovalsky D, Ussher JE, Csogrove C, Clough C, Ferguson JR, Smith K et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. Mucosal Immunol 2015; 8:429–40.

Turtle CJ, Dellow J, Jolly RC, Swanson HM, Basem R, Tablinelli L et al. Invariant sigleukin overcome acquired TCR signaling pathway regulation and govern the fate of human CD161(b+) CD8+ semi-invariant T cells. Blood 2011; 118:2752–62.

Rollé A, Pullmann J, Ewen EM, Le VT, Halesius A, Hengel H et al. IL-12-producing monocytes and HLA-E-control HCMV-driven NKGC+ NK cell expression. J Clin Invest 2014; 124:5055–16.

Kreislovsky T, Savage AK, Hobbs R, Gouani F, Bronron R, Perete P et al. TCR-inducible PLZF transcription factor required for innate phenotype of a subset of γδ T cells with restricted TCR diversity. Proc Natl Acad Sci USA 2009; 106:12453–8.

Savage AK, Constantinides MG, Han J, Picard D, Martin E, Li B et al. The transcription factor PLZF directs the effector program of the NK cell lineage. Immunology 2008; 29:391–403.

Kovalsky D, Ussher JE, Csogrove C, Clough C, Ferguson JR, Smith K et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. Mucosal Immunol 2015; 8:429–40.

Turtle CJ, Dellow J, Jolly RC, Swanson HM, Basem R, Tablinelli L et al. Invariant sigleukin overcome acquired TCR signaling pathway regulation and govern the fate of human CD161(b+) CD8+ semi-invariant T cells. Blood 2011; 118:2752–62.

Rollé A, Pullmann J, Ewen EM, Le VT, Halesius A, Hengel H et al. IL-12-producing monocytes and HLA-E-control HCMV-driven NKGC+ NK cell expression. J Clin Invest 2014; 124:5055–16.

Kreislovsky T, Savage AK, Hobbs R, Gouani F, Bronron R, Perete P et al. TCR-inducible PLZF transcription factor required for innate phenotype of a subset of γδ T cells with restricted TCR diversity. Proc Natl Acad Sci USA 2009; 106:12453–8.

Savage AK, Constantinides MG, Han J, Picard D, Martin E, Li B et al. The transcription factor PLZF directs the effector program of the NK cell lineage. Immunology 2008; 29:391–403.
Human lymphoid tissues harbor a distinct CD69\(^+\) CXCR6\(^+\) NK cell population. J Immunol 2016; 197:4835–91.

104 Hayakawa Y, Screpanti V, Yagita H, Grandien A, Ljunggren HG, Smyth MJ et al. NK cell TRAIL eliminates immature dendritic cells in vivo and limits dendritic cell vaccination efficacy. J Immunol 2003; 172:123–9.

105 Lughart G, Melsen JE, Vervat C, van Oostaijen-ten Dam MM, Curver WE, Roelen DL et al. Human lymphoid tissues harbor a distinct CD3\(^69\) \^ CXCR6\(^+\) NK cell population. J Immunol 2016; 197:78–84.

106 Intlekofer AM, Takekomo N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR et al. Effector and memory CD8\(^+\) T cell fate coupled by T-bet and eomesodermin. Nat Immunol 2005; 6:1236–44.

107 Koss J, Cosma GL, Betts MB, McLane LM. Characterization of T-bet and eomes in peripheral human immune cells. Front Immunol 2014; 5:217.

108 Kurioka A, Jahun AS, Hannaway RF, Walker LJ, Fergusson JR, Sverremark-Ekström et al. Shared and distinct phenotypes and functions of human CD161\(^++\) V\(\alpha\)7.2\(^+\) T cell subsets. Front Immunol 2017; 8:1031.

109 Cuff AO, Robertson FP, Stegmann KA, Pallett LJ, Maini MK, Davidson BR et al. Human endometrial NK cells are special immature cells that await pregnancy. J Immunol 2016; 197:1272–81.

110 Melsen JE, Lugthart G, Lankester AC, Schilham MW. Human circulating and tissue-resident CD56\(^{bright}\) natural killer cell populations. Front Immunol 2016; 7:262.

111 Tang XZ, Jo J, Tan AT, Sandalova E, Chia A, Tan KC et al. IL-7 licenses activation of human liver intrahepatic mucosal-mucosal-associated invariant T cell T cells. J Immunol 2013; 190:3142–52.

112 Hudspeth K, Donadon M, Cimino M, Pontarini E, Tentorio P, Preti M et al. Human liver-resident CD3\(^{bright}\)/CD16\(^{neg}\) NK cells are retained within hepatic sinusoids via the engagement of CCR5 and CXCR6 pathways. J Autoimmun 2016; 66:40–50.

113 Stegmann KA, Robertson F, Hanuš N, Gil U, Pallant C, Christophides T et al. CXCR6 marks a novel subset of T-bet low Eomes bi na natural killer cells residing in human liver. Sci Rep 2016; 6:257.

114 Cuff AO, Robertson FP, Stegmann KA, Pallett LJ, Maini MK, Davidson BR et al. Foresight NK cells in human liver are long-lived and do not recirculate but can be replenished from the circulation. J Immunol 2016; 197:4835–91.

115 Manaster I, Mizrahi S, Goldman-Wohl D, Selu HY, Stern-Ginossar N, Lankey D et al. Endometrial NK cells are special immature cells that await pregnancy. J Immunol 2008; 180:1869–76.

116 Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. Hum Pathol 1991; 21:761–7.

117 Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: new guardians of the immune system. Front Immunol 2014; 5:217.

118 Jeffery HC, van Wilgenburg B, Kurioka A, Parekh K, Stirling K, Roberts S et al. MAIT cells: new guardians of the immune system. Front Immunol 2014; 5:217.

119 Croxford JL, Miyake S, Huang Y-Y, Shimamura M, Yamamura T. Invariant V\(\alpha\)19 T cell fate coupled by T-bet and eomesodermin. J Immunol 2014; 193:3891–901.

120 Nagalath, P., Gringois, J., Hall, S., Venter, N., Kiefet B et al. Mucosal-associated invariant T cell deletion in obese and type 2 diabetic patients. J Clin Invest 2015; 125:1753–62.

121 Ito H, Nishiyama M, Tsuchiya T, Fujita T, Yone H, Shinoda K et al. Deficiency of mucosal-associat ed invariant T cells in multiple sclerosis. J Immunol 2016; 196:1671–8.

122 Schierloh P, Yokobori N, Alem E, Musella RM, Beigier-Bompadre M, Saab MA et al. Increased susceptibility to apoptosis of CD56dimCD16\^- NK cells induces the enrichment of IFN-gamma-producing CD56\(^{bright}\) cells in tuberculous pleurisy. J Immunol 2005; 173:6852–68.

123 De Matos CT, Berg L, Michaelsson J, Fellander-Tsai L, Kärre K, Söderström K. Activating and inhibitory receptors on synovial fluid natural killer cells of arthritis patients: role of CD94/NKG2A in control of cytokine secretion. Immunology 2007; 122:291–301.

124 Schinell F, Yokobori N, Almán M, Mauseia RM, Beigier-Bompadre M, Saab MA et al. Increased susceptibility to apoptosis of CD56dimCD16\^- NK cells induces the enrichment of IFN-gamma-producing CD56\(^{bright}\) cells in tuberculous pleurisy. J Immunol 2005; 173:6852–68.

125 Sarriera NE, Esche M, Lomette L, Lion J, Fumery M, Marcoli P et al. Immune mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. Clin Exp Immunol 2014; 176:266–74.

126 Gracey E, Qiyum Z, Almgloothi L, Lawson D, Karki S, Avvaru N et al. IL-17 primes IL-17 in mucosal-associated invariant T (MAIT) cells, which contribute to the Th17-axis in ankylosing spondylitis. Ann Rheum Dis 2016; 75:2124–32. https://doi.org/10.1136/annrheumdis-2013-208902.

127 Cho YN, Lee SJ, Kim TJ, Jin HM, Kim MI, Jung HJ et al. Mucosal-associated invariant T cell deficiency in systemic lupus erythematosus. J Immunol 2014; 193:3891–901.

128 Magalhães I, Pinguí K, Posticu C, Bessolos S, Venter, N., Kiefet B et al. Mucosal-associated invariant T cell deletion in obese and type 2 diabetic patients. J Clin Invest 2015; 125:1753–62.

129 Salou M, Nielc R, Garcia A, Barón D, Michel I, Elang-Ngono A et al. Neuropathologic, phenotypic and functional analyses of mucosal associated invariant T cells in multiple sclerosis. J Immunol 2016; 196:1671–8.

130 Annibali V, Ristori G, Angelini DF, Serafini B, Mechelli R, Cannoni S et al. CD161\^-high CD8\^ T cells bear pathogenetic potential in multiple sclerosis. Brain 2011; 134:542–54.

131 Willing A, Leach OA, Ufer F, Attfield KE, Steinbach K, Kursawe N et al. CD3\(^\text{+}\)MAIT cells infiltrate into the CNS and alterations in their blood frequencies correlate with IL-18 serum levels in multiple sclerosis. Eur J Immunol 2014; 44:3119–28.

132 Teunissen MB, Yermenenko NG, Barton DL, Chielei S, Spuls PI, De Rie MA et al. The IL-17A-producing CD8\^+ T-cell population in psoriatic lesional skin comprises mucosa-associated invariant T cells and conventional T cells. J Invest Dermatol 2014; 134:2898–907.

133 Mezon B, Ballick NJ, Walter GJ, Rajoéka Bh, Garwood T, Evans HG et al. Interleukin-17 + CD8 + T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. Arthritis Rheumatol 2014; 66:1272–81.

134 Rosen DR, Cao W, Avery DT, Tangri SG, Liu YJ, Houk CJ et al. Functional consequences of interactions between human NKR-P1A and its ligand LIT1 expressed on activated dendritic cells and B cells. J Immunol 2008; 180:6508–17.

135 Nielsen N, Østum N, Ursø B, Lanier LL, Speer C. Cytotoxicity of CD3\(^{bright}\) NK cells towards autologous activated CD4 + T cells is mediated through NKG2D, LFA-1 and TRAIL and dampened via CD94/NKG2A. PLoS ONE 2012; 7:e31959.

136 Miyazaki Y, Miyake S, Chiba A, Lantt O, Yamamura T. Mucosal-associated invariant T cells regulate Th1 response in multiple sclerosis. Int Immunol 2011; 23:329–35.

137 Crawford JL, Miyake S, Huang Y-Y, Shimamura M, Yamamura T. Invariant V\(\alpha\)19 T cells regulate autostimulation in inflammation. Nat Immunol 2006; 7:987–94.

138 Cohen NR, Brennan PJ, Shay T, Watts GF, Brilg M, Kang J et al. Shared and distinct transcriptional programs underlie the hybrid nature of iNKT cells. Nat Immunol 2013; 14:96–10.

139 Luecke-Eversloh M, Hammer Q, Derrick P, Nordstrom K, Gasparoni G, Pisk M et al. Human cytomegalovirus drives epigenetic imprinting of the IFN-g locus in NKG2Chi NK cell subsets. PLoS Pathog 2014; 10:e1004414.