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Competing interests statement

The author declares that he has no competing financial interests.

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OPINION

NKT cells: what's in a name?

Dale I. Godfrey, H. Robson MacDonald, Mitchell Kronenberg, Mark J. Smyth and Luc Van Kaer

Recent years have seen so-called natural killer T (NKT) cells emerge as important regulators of the immune response. The existence of NKT-cell subsets, and other types of T cell that resemble NKT cells, is an ongoing source of confusion in the literature. This perspective article seeks to clarify which cells fall under the NKT-cell umbrella, and which might be best considered as separate.

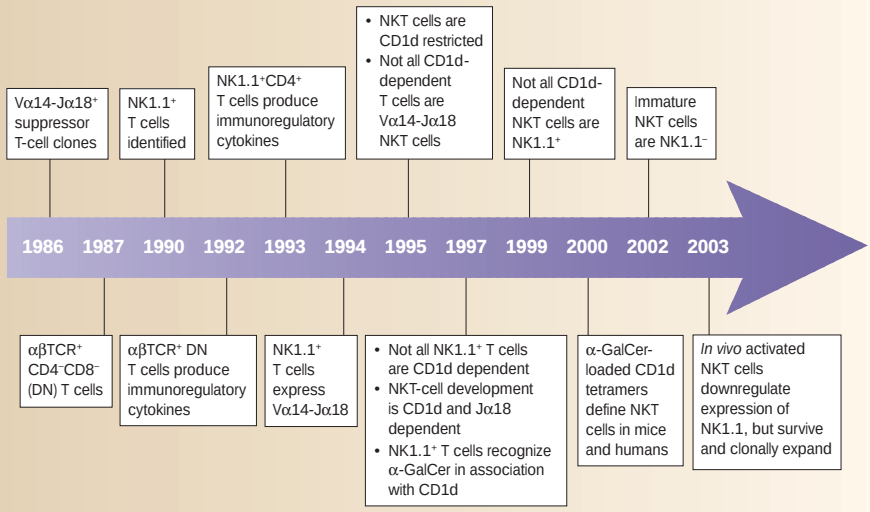
The term 'NK T cells' was first published in 1995 (REF. 1) and was used broadly to define a subset of mouse T cells that share some characteristics with NK cells, particularly expression of the NK1.1 marker (Nkrp1c or CD161c). The term NKT cells is now well accepted and is applied to mice, humans and other species. However, the classification of NKT cells has always been complicated by the fact that most commonly used mouse strains (apart from C57BL/6) do not express the NK1.1 marker. Furthermore, as the field of NKT-cell research develops, it is becoming increasingly clear that the simplistic definition that NKT cells are NK1.1$^+$ T cells (even in C57BL/6 mice) is not only inaccurate, but also potentially misleading. This Opinion article seeks to clarify some of the problems associated with the definition of NKT cells, and to compare and contrast the different types of T cell that are often considered as being within the broader NKT-cell family. The article mainly focuses on NKT-cell subsets in mice, with occasional references to humans and other species where appropriate.

A brief history of NKT cells

Research carried out in many laboratories over the past two decades led to the discovery and definition of NKT cells (TIMELINE). In 1987, three separate groups published studies showing the existence of a distinct subset of αβ-T-cell receptor (αβ-TCR)$^+$ T cells in mice that expressed intermediate rather than high levels of TCR, with a two- to three-fold higher frequency of Vβ8 expression than conventional T cells, and that lacked expression of CD4 and CD8 accessory molecules[2–4]. General interest in these double-negative (DN) αβ-TCR$^+$ lymphocytes increased when it was discovered that they were a potent source of immunoregulatory cytokines, including interleukin-4 (IL-4), interferon-γ (IFN-γ) and tumour-necrosis factor (TNF)[5]. Around the same time, other groups reported on the existence of a subset of αβ-TCR$^+$ T cells that expressed NK1.1, which was previously considered to be only expressed by NK cells[6,7]. These NK1.1$^+$ T cells also expressed intermediate levels of TCR with a bias towards Vβ8.2 expression, and included two subsets — CD4$^+$ T cells and DN T cells. Similar to NK1.1$^+$αβ-TCR$^+$ DN T cells, NK1.1$^+$CD4$^+$ cells were also found to be a potent source of immunoregulatory cytokines[8,9]. Others had similarly found that subsets of CD4$^+$ T cells in the thymus produced large amounts of cytokines[10–12]. However, at the time that these studies with thymocytes and peripheral T cells were published, it was unclear whether they were all dealing with the same cell type. Considering that NK1.1$^-$CD4$^+$ thymocytes were not a potent source of cytokines[8], it was probable that most CD4$^+$, high cytokine-producing T cells in the thymus were included in the NK1.1$^+$ fraction. Collectively, these studies indicated the existence of a unique subpopulation of T cells, defined by NK1.1 expression, that were potent cytokine producers and therefore likely to be effective immunoregulatory cells.

Further evidence for the unique character of NK1.1$^+$αβ-TCR$^+$ cells came from observations that their development was independent of MHC class II expression, but required β_2-microglobulin (β_2m), although they did not express CD8 (REFS 13–15). This led to the realization that NKT cells are reactive to the MHC class-I-like molecule CD1d[16]. In addition to the marked bias in TCR Vβ-chain usage

Timeline | Key events in the field of NKT-cell research



This timeline depicts some of the most important events that have shaped our understanding of classical natural killer T (NKT)-cell biology in mice. It does not incorporate any of the important studies that show the functional significance of these cells in various disease models. α-GalCer, α-galactosylceramide; DN, double negative.

towards Vβ8.2, most NK1.1$^+$ T cells were found to use an invariant TCR α-chain consisting of Vα14-Jα281 (now known as Jα18)[17]. Curiously, this TCR α-chain was previously recognized for its predominant expression by a panel of suppressor T-cell hybridomas[18,19] and overrepresentation in healthy unprimed mice[20]. This is arguably the first evidence for the immunoregulatory function that is now known to be associated with NKT cells. CD1d and the invariant TCR α-chain were both found to be essential for the normal development of NKT cells[21–24]. Moreover, there was growing evidence that CD1 molecules present hydrophobic/lipid antigens[25], and a model CD1d-reactive glycolipid antigen — the marine-sponge-derived agent (2S, 3S, 4R)-1-O-(α-D-galactopyranosyl)-N-hexacosanoyl-2-amino-1,3,4-octadecanetriol), commonly referred to as α-GALACTOSYLCERAMIDE (α-GalCer) — was identified as a potent stimulatory factor for NKT cells[26]. Further evidence that these cells are a separate T-cell lineage came from studies indicating unique requirements for their development[27–30]. Most notable was the finding that NKT cells are positively selected by β_2m- and CD1d-expressing bone-marrow-derived cells[13–15,31,32], most probably CD4$^+$CD8$^+$ DOUBLE-POSITIVE THYMOCYTES[33,34], rather than the CORTICAL THYMIC EPITHELIAL CELLS that positively select conventional T cells.

Taken together, these observations meant that by the mid 1990s, it was widely accepted that mouse NKT cells (both CD4$^+$ and DN) are a separate T-cell lineage characterized by CD1d reactivity, an invariant Vα14-Jα18 TCR α-chain, a TCR β-chain biased towards Vβ8.2, Vβ2 and Vβ7, glycolipid-antigen reactivity, expression of NK-cell and memory T-cell markers, and potent cytokine-producing capacity. A similar population of cells has now been identified in other species, including humans[35,36], other primates[37,38] and rats[39]; and in humans, these cells are characterized by an invariant Vα24-Jα18 TCR α-chain co-expressed with the Vβ11 TCR β-chain, which are the human homologues of the mouse Vα14-Jα18 and Vβ8.2 chains.

The search for a name

The term NK1.1$^+$ T cells is problematic, because it is really only appropriate for mouse strains, such as C57BL/6, that express the NK1.1 marker, whereas most other commonly used mouse strains (such as BALB/c, CBA and non-obese diabetic, NOD) do not, and because expression of NK1.1 and its human homologue (CD161) is not limited to the CD1d-dependent T cells with the properties described earlier. Furthermore, even in C57BL/6 mice, some cells with the invariant TCR α-chain rearrangement and α-GalCer reactivity do not express NK1.1 (see later). Several groups have proposed new names for these cells, including NK1.1$^+$ (like) T cells[40], natural T cells[41,42], NK T or NKT cells[1,43], Vα14 invariant (Vα14i) T cells[17] and iNKT cells[44]. Arguably, the term NKT cells has been most widely accepted and is now used to refer to these cells in all species in which they have been characterized. A curiosity of more recent literature is that NKT is now often explained as an abbreviation of natural killer T; yet this was not the basis for the use of this term, as it referred to NK1.1 expression. Furthermore, natural cytotoxicity does not seem to be a key effector mechanism of NKT cells[45], although these cells clearly can express perforin and FASL (CD95L), as well as other receptors (such as NKG2D) that tend to mediate cytotoxicity. Nonetheless, defining NKT cells as NK1.1$^+$ T cells is problematic, and perhaps the most accurate, if not the most handy or mellifluous definition, is CD1d-dependent natural killer-like T cells.

Subsets of NKT cells

Subsets of CD1d-dependent T cells that either express or do not express the invariant Vα14-Jα18 (Vα24-Jα18 in humans) TCR and/or NK1.1 (CD161 in humans) have been identified. Each of these T-cell subsets is commonly referred to as NKT cells, but they probably represent functionally distinct cell types.

Vα14-Jα18$^+$NK1.1$^+$ CD1d-dependent NKT cells. An interesting and largely unresolved question relates to the existence of subsets of Vα14-Jα18$^+$NK1.1/CD161$^+$ NKT cells. It is well known that at least two subsets of these cells exist in mice, differing in their expression of CD4 (CD4$^+$ and DN), but it is unclear whether these are functionally distinct subpopulations. Indeed, the purpose of CD4 expression by cells that seem to be completely MHC class-II-independent is a mystery. However, the significance of CD4 expression is suggested by conservation of this feature between mice and humans, and some studies have provided data indicating that mouse CD4$^+$ and DN NKT cells have distinct effector functions. They exist in different proportions in different tissues[46–48] and the CD4$^+$ fraction seems to produce higher levels of IL-4 in response to stimulation with CD3-specific antibody *in vitro*[46], although this is not apparent after α-GalCer stimulation *in vivo*[49]. Ablation of CD4$^+$ CD1d-restricted T cells in mice seems to be sufficient to overcome suppression of tumour rejection in at least one tumour model[50], supporting the possibility that the CD4$^+$ and CD4$^-$ NKT-cell subsets might be functionally distinct *in vivo*. Mouse NKT cells generally do not express the CD8 molecule, which seems to be a consequence of intrathymic selection[17]. By contrast, up to 50% of human NKT cells express CD8α[51], although only a small fraction express CD8β[52,53], and most macaque[37,38] and rat[39,54] NKT cells express CD8α.

Different functional phenotypes have also been observed with Vα24$^+$ NKT cells defined by the presence or absence of CD4 expression in human peripheral blood[51,55]. The CD4$^+$

NKT-cell subset produced higher levels of IL-4, IL-13, granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-2 than did the CD4⁻ NKT cells, whereas both populations produced IFN-γ and TNF at high levels. These subsets also varied in their pattern of expression of chemokine receptors, NK-cell receptors, CD95L and perforin. Taken together, these studies support the concept that Vα14-Jα18 (Vα24-Jα18 in humans) CD4⁺ and CD4⁻ NKT cells, and possibly CD8⁺ NKT cells in some species, might have distinct physiological functions *in vivo*.

Vα14-Jα18⁺NK1.1⁻ CD1d-dependent NKT cells. The CD1d-dependent fraction of NKT cells is now known to include a subset of NK1.1⁻ cells that exist in both the thymus and the periphery of mice. A similar population of CD1d-dependent Vα24⁺ NKT cells that lacks CD161 expression has been identified in humans. These cells, most of which express CD4, are clearly identified when using α-GALCER-LOADED CD1D TETRAMERS[47,49,55,56], although their existence was strongly hinted at in earlier studies[57,58]. The ability of these cells to recognize α-GalCer in association with CD1d, in itself strongly suggests a link with the better-known NK1.1⁺ fraction, although some clear differences exist, at least in mice. In particular, after *in vitro* stimulation, NK1.1⁻ CD1d-dependent NKT cells in the thymus produce higher levels of IL-4 and lower levels of IFN-γ than their NK1.1⁺ counterparts[59–61]. Three recent studies showed that, at least in the thymus, NK1.1⁻ CD1d-dependent NKT cells are precursors of NK1.1⁺ NKT cells. This was directly shown by the observation that intrathymically injected NK1.1⁻ NKT cells (isolated from the thymus of congenically marked donor mice), when isolated one week later, had given rise to NK1.1⁺ NKT cells[59–61]. Furthermore, most NKT cells that emigrate from the thymus do so at the NK1.1⁻ stage, and presumably continue their maturation in the periphery[59,60] (FIG. 1).

It is unlikely that all NK1.1⁻ NKT cells are immature precursors, as downregulation of NK1.1 expression has been observed after *in vitro* stimulation of NK1.1⁺ NKT cells[62]. Until recently, it was not clear whether this was of relevance to the NKT-cell response to stimulation *in vivo*, as most NKT cells were thought to undergo antigen-induced cell death within hours of stimulation with CD3-specific antibody, IL-12 (REF. 63) or α-GalCer[49,64–68]. Two recent studies[69,70] have now shown that most NKT cells survive α-GalCer-mediated stimulation *in vivo*. This phenomenon has probably been overlooked in studies to date owing to the simultaneous downregulation of both NK1.1 and αβ-TCR expression, and although the αβ-TCR is re-expressed within 24–48 hours, NK1.1 expression remains reduced for at least 12 days after stimulation. Using α-GalCer-loaded CD1d tetramers to identify NKT cells independently of NK1.1 expression, NKT cells were observed to undergo a wave of clonal expansion in the spleen, liver and bone marrow after *in vivo* stimulation, which peaked at 6–10 times their normal steady state number about 3 days after stimulation, and gradually declined to normal levels by days 9–12. This wave of clonal expansion was associated with sustained cytokine production, highlighting the fact that NKT cells are likely to be participating in the immune response for a lot longer than previously appreciated. So, although NK1.1⁻ NKT cells are immature in the thymus, a marked fraction of NK1.1⁻ NKT cells in other organs might be antigen-experienced cells, and these may vary widely in number depending on the immune status of the animal[69,70] (FIG. 1). The extent to which NKT-cell death versus NKT-cell marker downregulation contributes to the disappearance of these cells in response to CD3-specific antibody or IL-12 *in vivo* is not entirely clear[63], although downregulation of NK1.1 expression has been observed on some α-GalCer–CD1d tetramer⁺ NKT cells following IL-12 administration *in vivo*[71].

There are six *Nkrp1* genes in mice, and recently Nkrp1d and Nkrp1f were shown to interact with C-type lectin-related (Clr) molecules expressed by dendritic cells and osteoclasts[72]. As a member of the Nkrp1 family, NK1.1 might interact with Clr molecules, and this raises the interesting possibility that expression of different Nkrp1-family members by NKT-cell subsets might determine their reactivity with dendritic-cell subsets. The expression pattern of other Nkrp1-family members by NKT-cell subsets is unknown, and this is clearly an area that warrants further

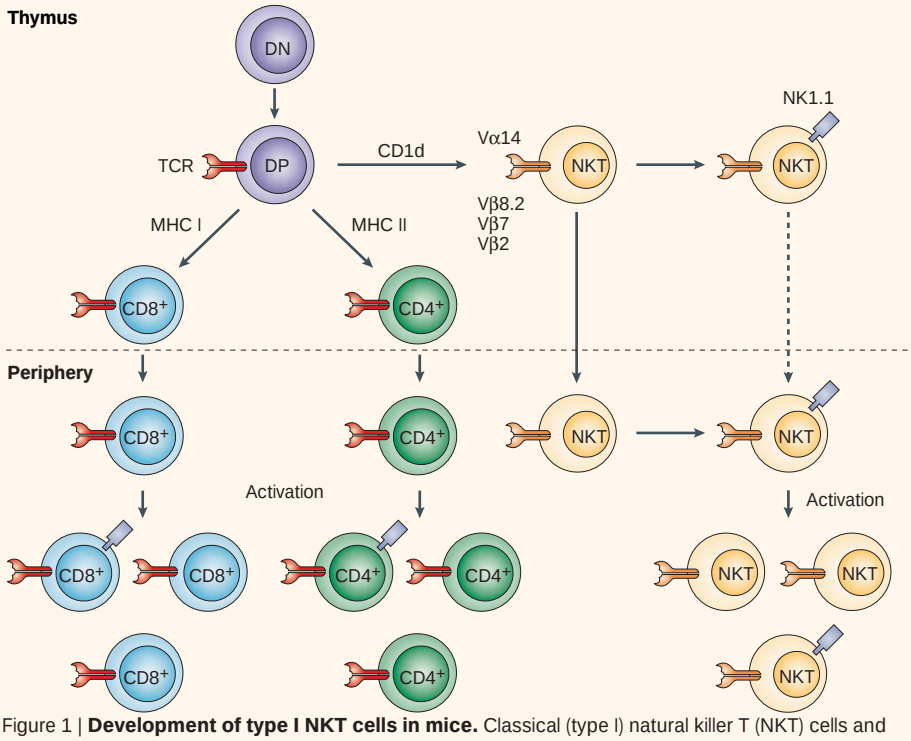


Figure 1 | **Development of type I NKT cells in mice.** Classical (type I) natural killer T (NKT) cells and conventional CD8⁺ and CD4⁺ T cells originate from a common precursor (CD4⁺CD8⁺ double-positive, DP) thymocytes[100]. Random T-cell receptor (TCR)-gene rearrangement leading to expression of Vα14-Jα18 in conjunction with either Vβ8.2, Vβ7 or Vβ2 leads to the CD1d-dependent selection and branching of the NKT-cell lineage. NK1.1 expression is a downstream event in the maturation of NKT cells, and most NKT cells migrate from the thymus to the periphery before this stage, although some (as indicated by the dashed arrow) NK1.1⁺ NKT cells can also emigrate[59–61]. Mature NK1.1⁺ NKT cells undergo clonal expansion in the periphery after activation (after α-galactosylceramide challenge *in vivo*). This clonal expansion is associated with the downregulation of NK1.1 expression, which is gradually re-expressed by at least some of these cells[69,70]. By contrast, mature conventional CD8⁺ and CD4⁺ T cells are typically NK1.1⁻, but under certain activation conditions (such as after infection with virus) some of these cells upregulate NK1.1 expression[85]. The kinetics of this process and the fate of these cells is unclear. CD1d-dependent, non-α-galactosylceramide-reactive (type II) cells are not included in this diagram as little is known about the development of these cells. DN, double negative.

investigation as it might provide important new insight into the physiological relevance of NK-cell receptor expression by NKT cells.

Vα14-Jα18-independent, CD1d-dependent, NKT cells. In addition to CD1d-dependent Vα14-Jα18$^+$ NKT cells, non-Vα14-Jα18-expressing CD1d-dependent T cells have also been identified in mice[73–77]. These cells have been less well characterized, mainly because they cannot be identified using α-GalCer-loaded CD1d tetramers, and therefore the exact prevalence of these cells and the extent to which they express NK1.1 is not precisely known. They seem, however, to be a marked fraction of the CD4$^+$ T cells in MHC class-II-deficient mice[75].

This population is somewhat enriched for cells that express a TCR α-chain composed of Vα3-Jα9 or Vα8, in conjunction with Vβ8.2, but clearly their TCRs are more diverse than the classical Vα14-Jα18$^+$ CD1d-dependent NKT cells. In addition, CD1d-dependent Vα14-Jα18$^-$ cells might also include a subset of γδ-TCR$^+$ T cells[78]. The development and activation of CD1d-dependent Vα14-Jα18$^-$ T cells is distinct from Vα14-Jα18$^+$ NKT cells, as the Vα14-Jα18$^+$ NKT-cell subset, but not the Vα14-Jα18$^-$ T-cell subset, requires endosomal targeting of CD1d[74,79], indicating that they recognize different ligands in the context of CD1d. A TCR-transgenic mouse has been generated from a CD1d-dependent T-cell clone that expresses Vα3 and Vβ9 (REF. 77) and the main T-cell population that developed had similar characteristics to Vα14-Jα18$^+$ NKT cells. The Vα3, Vβ9-transgenic cells produced high levels of cytokines and were mainly, but not exclusively NK1.1$^+$. These CD1d-dependent Vα14-Jα18$^-$ T cells might have distinct functional capabilities; for example, CD1d-dependent NK1.1$^+$ T cells that are not detected by α-GalCer–CD1d tetramers are activated in response to hepatocytes expressing hepatitis B antigens. These cells mediate hepatitis when transferred to T-cell-deficient (recombinase-activating gene deficient) mice that transgenically express hepatitis B envelope antigens, whereas α-GalCer–CD1d tetramer-reactive NK1.1$^+$ T cells from the same donor mice did not[80].

CD1d-dependent T cells with diverse TCRs have also been found in humans. Some human NKT cells, defined by α-GalCer–CD1d tetramer reactivity, are Vα24$^-$Vβ11$^-$, most are CD8α$^+$CD8β$^+$ and exhibit CD8-dependent cytotoxicity *in vitro*[52]. Furthermore, most CD1d-dependent T-cell clones derived from a bone-marrow culture did not have the invariant TCR α-chain rearrangement and α-GalCer reactivity. It is possible, however, that the culture conditions promoted the selective expansion of this subset[81].

Classification of NKT-cell subsets. Taken together, it is probable that CD1d-dependent, Vα14-Jα18-independent T cells are a diverse population of lymphocytes. Importantly, the relationship of these cells to Vα14-Jα18$^+$ NKT cells is unclear; although they share some important characteristics, their distinct developmental/selection requirements indicate that they have distinct specificities. Clearly, there are two broad classes of cells that satisfy the criteria of being CD1d-dependent, NK-like T cells. For the purposes of this article, we classify these as type I NKT cells, being the Vα14-Jα18 (mouse) or Vα24-Jα18 (human) population (which can be further subdivided as discussed earlier), and type II NKT cells, which would include all other CD1d-dependent T cells (TABLE 1). As we learn more about the specificities and functions of distinct NKT-cell subsets, this simple classification might need revision.

Table 1 | Classification of NKT and NKT-like cells

	Type I cells (Classical NKT cells)	Type II cells (Non-classical NKT cells)	NKT-like cells (CD1d-independent NK1.1$^+$ T cells)
CD1d dependent	Yes	Yes	No
α-GalCer reactive	Yes	No*	No
TCR α-chain	Vα14-Jα18 (mice) Vα24-Jα18 (humans)	Diverse, but some Vα3.2-Jα9, Vα8 (mice)	Diverse‡
TCR β-chain	Vβ8.2, Vβ7 and Vβ2 (mice) Vβ11 (humans)	Diverse, but some Vβ8.2 (mice)	Diverse‡
NK1.1 (CD161)	+ (resting mature) –/low (immature or post-activation)	+/–	+
Subsets	CD4$^+$ and DN (mice) CD4$^+$, CD8$^+$ and DN (humans)	CD4$^+$ and DN (mice)	CD4$^+$, CD8$^+$ and DN
IL-4 production	Yes	Yes	No
IFN-γ production	Yes	Yes	Yes

*In humans, some CD1d-restricted Vα24/Vβ11$^-$, α-galactosylceramide (α-GalCer)-reactive T cells have been identified. ‡Although most natural killer T (NKT)-like cells express diverse T-cell receptors (TCRs), some subsets (for example, mucosal-associated invariant T cells) express semi-invariant TCRs. In mice, NKT cells have been traditionally defined as NK1.1$^+$ T cells. However, it is clear that expression of NK-cell markers is not unique to classical, CD1d-dependent NKT cells, which has resulted in confusion in the literature. We compare and contrast the different populations of cells that are often referred to as NKT cells, by dividing these into three different cell types: type I NKT cells, type II NKT cells and NKT-like cells. DN, CD4$^-$CD8$^-$ double negative; IFN-γ, interferon-γ; IL-4, interleukin-4.

T-cell subsets that resemble NKT cells

In addition to the type I and type II NKT-cell classes discussed above, other cell types are sometimes referred to as NKT cells in the literature. These include CD1d-independent NK1.1$^+$ cells and other T-cell subsets that express semi-invariant TCRs.

CD1d-independent NK1.1$^+$ T cells. Several studies have emerged over the past few years that have further complicated the simple classification of mouse NKT cells as CD1d-dependent, NK1.1$^+$ T cells. Studies with TCR-transgenic mice indicated that some NK1.1$^+$ T cells might be restricted to conventional MHC molecules[82–84]; and furthermore, analysis

Glossary

α-GALACTOSYLCERAMIDE
(α-GalCer). A synthetic or marine-sponge-derived glycolipid containing α-anomeric glycosidic linkage of the galactose residue to the sphingosine base. This, and structurally related lipids, potently activate CD1d-restricted natural killer T (NKT) cells that express the semi-invariant Vα14-Jα18 T-cell receptor (TCR) in mice (and the Vα24-Jα18$^+$ equivalent cells in humans)[101].

α-GALCER-LOADED CD1D TETRAMERS
A complex of four CD1d molecules loaded with α-GalCer that has sufficient affinity to detect cell-surface expression of the semi-invariant TCR expressed by NKT cells in mice (Vα14-Jα18$^+$) and humans (Vα24-Jα18$^+$) using flow cytometry.

CORTICAL THYMIC EPITHELIAL CELLS
Epithelial cells found in the outer region or cortex of thymic lobules. These cells are crucial for the positive selection of conventional MHC class-I- and class-II-restricted T cells, but not for the positive selection of CD1d-restricted NKT cells.

DOUBLE-POSITIVE THYMOCYTES
Immature T cells in the thymus that are characterized by the expression of both the CD4 and CD8 co-receptor proteins. They represent the majority (80–90%) of thymocytes. These cells express CD1d molecules and have been implicated in the positive selection of NKT cells.

Box 1 | NKT and NKT-like cells: the importance of classification

It is now clear that there are distinct NKT-cell subsets and other types of T cell that resemble NKT cells. In this article, we have therefore divided the cells under study as either type I or type II NKT cells, or NKT-like cells (TABLE 1). When studying NKT or NKT-like cells, the following points should be considered:

- Not all NK1.1$^+$ T cells are classical (type I) NKT cells. They include other CD1d-dependent (type II) NKT cells, as well as CD1d-independent (NKT-like cells) T cells, which might vary widely in function. The most reliable way to detect type I NKT cells in mice and humans is by using CD1d tetramers loaded with α-galactosylceramide (α-GalCer). This reagent is becoming more widely available as several laboratories are now producing it, and soluble recombinant CD1d molecules suitable for making tetramers are now being produced at the U.S. National Institutes of Health tetramer facility. Furthermore, a dimerized CD1d reagent is commercially available, which can also be used to identify type I NKT cells if loaded with α-GalCer[97].
- Not all type I NKT cells express NK1.1 (in C57BL/6 mice). Immature type I NKT cells do not express NK1.1, and expression of this marker is downregulated for at least several days after the activation of mature type I NKT cells *in vivo*. As above, the best way to avoid this problem is to use α-GalCer-loaded CD1d multimers to detect these cells.
- As both type I and type II NKT cells are CD1d dependent, any phenotype observed in CD1d-deficient mice might be due to a deficiency in type II NKT cells rather than type I NKT cells[78,98,99], and the functional relationship between these two cell types is unclear. In this sense, the combined use of TCR Jα18-deficient mice and CD1d-deficient mice is useful to distinguish between type I and type II NKT cells at the functional level *in vivo*.

of CD1d-deficient mice revealed the existence of a residual population of CD1d-independent NK1.1$^+$ T cells. Two separate studies[46,48] examined NK1.1$^+$ T-cell subsets in more detail, and showed that in wild-type mice, NK1.1$^+$ T cells included a population of cells that is CD1d and Vα14-Jα18 independent, and produces comparatively low levels of cytokines. The CD1d-independent fraction was enriched for CD8$^+$NK1.1$^+$ T cells, although CD4$^+$ and DN NK1.1$^+$ T cells were also found in this population. Apart from NK1.1 expression, there is little evidence that these CD1d-independent NK1.1$^+$ T cells are related to NKT cells. The CD1d-independent cells seem to be quite heterogeneous, and might include: conventional T cells that have upregulated NK1.1 expression after activation[85] (FIG. 1); distinct invariant T cells that are reactive with MHC class-I-related molecules other than CD1d[86,87] (see later); and thymus-independent T cells of unknown origin and function[46,88,89]. Based on analysis of CD1d-deficient mice, CD1d-dependent NKT cells (including type I and II cells) seem to constitute at least 80% of the NK1.1$^+$ T cells in the thymus and liver of mice, although they are closer to 50% in the bone marrow and spleen.

Similar CD1d-independent NK-cell-marker-expressing T cells are present in humans, and type I NKT cells (defined by reactivity with α-GalCer-loaded CD1d tetramers) represent only a subset of T cells that express NK-cell markers such as CD161 or CD56 (REFS 90,91). Even co-expression of Vα24 and Vβ11 does not completely overlap with CD1d-tetramer reactivity in humans[52,91], although this might partly reflect a lack of specificity for α-GalCer rather than CD1d in general. Considering the dissimilarity of these CD1d-independent NK1.1$^+$ T cells with type I NKT cells, we suggest that they should not be called NKT cells. For the purpose of this article, we refer to them as NKT-like cells (TABLE 1).

Other semi-invariant T-cell subsets. In addition to NKT cells, several other T-cell subsets that express invariant or semi-invariant TCRs have been identified. For example, mucosal-associated invariant T (MAIT) cells express the canonical Vα19-Jα33 (Vα7.2-Jα33 in humans) TCR rearrangement, preferentially localize in the gut mucosa, and are selected and/or restricted by the MHC class-I-like molecule MR1 (REF. 86). Interestingly, cells that express the canonical Vα19 TCR are abundant among peripheral NK1.1$^+$ cells isolated from CD1d-mutant mice[87]. Similar to NKT cells, MAIT cells have an activated phenotype. However, although NKT cells are present in germ-free mice[92], MAIT cells are absent in these animals, indicating that commensal flora are required for their migration or clonal expansion in the gut. Several subsets of γδ-T cells also express canonical TCRs, and some of these express NK-cell markers such as NK1.1 (REFS 93–96). Although NK1.1$^+$ populations of γδ-T cells sometimes have been referred to as γδ-NKT cells, we suggest that this is potentially confusing, as these are generally not CD1d specific. These semi-invariant T-cell subsets would broadly fall under the category of NKT-like cells as outlined earlier, although clearly this category includes a diverse range of cell types.

Concluding remarks

Although there is little doubt about the significance of type I NKT cells to the field of immunology, this area has become progressively more complex since the discovery of these cells about 16 years ago. The traditional definition of NKT cells by NK1.1 and TCR co-expression is fraught with problems that might necessitate careful reinterpretation of results from some earlier studies. Important points to consider when studying these cells are outlined in BOX 1. As our understanding of NKT-cell biology increases, there seem to be as many new questions as there are answers. Many of the key issues that remain relate directly to the functional differences between different types of NKT cell, as outlined in this article. What is the significance of type I NKT-cell subsets defined as CD4$^+$ or CD4$^-$ (and CD8$^+$ in primates and rats)? Besides CD1d dependence, is there any functional overlap between type I and type II NKT cells? What is the significance of the expression of NK-cell-receptor family members that links type I NKT cells, type II NKT cells and NKT-like cell types? We suggest that the resolution of these issues will be of paramount importance to our understanding of how NKT cells and similar/related cells contribute to the immune response in the many different settings where they are implicated.

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Competing interests statement

The authors declare that they have no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
CD1d | CD4 | CD8 | CD95L | CD161 | GM-CSF | IFN-γ | IL-2 | IL-4 | IL-13 | Nkrp1c | TNF

FURTHER INFORMATION

Dale Godfrey: http://www.microbiol.unimelb.edu.au/micro-new
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