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Recognition of CD1d-restricted antigens by natural killer T cells

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Abstract

Natural killer T (NKT) cells are innate-like T cells that rapidly produce a variety of cytokines following T cell receptor (TCR) activation and can shape the immune response in many different settings. There are two main NKT cell subsets: type I NKT cells are typically characterized by the expression of a semi-invariant TCR, whereas the TCRs expressed by type II NKT cells are more diverse. This Review focuses on the defining features and emerging generalities regarding how NKT cells specifically recognize self, microbial and synthetic lipid-based antigens that are presented by CD1d. Such information is vitally important to better understand, and fully harness, the therapeutic potential of NKT cells.

T cells are usually viewed as being specific for peptide antigens that are presented on classical MHC molecules. However, many T cells actually respond to lipid-based antigens that are presented by the CD1 family of MHC-like molecules, which are typically expressed by professional antigen-presenting cells (APCs). The CD1 family is subdivided into at least three groups: group 1 comprises CD1a, CD1b and CD1c; group 2 comprises CD1d; and group 3 comprises CD1e[1,2]. The most extensively studied type of lipid-reactive T cell is the CD1d-restricted natural killer T (NKT) cell[3] (FIG. 1a).

NKT cells are a specialized T cell subset that is functionally distinct from MHC-restricted T cells[4]. For example, NKT cells can rapidly produce very large amounts of cytokines — including interferon- γ(IFNγ), interleukin-4 (IL-4), IL-10, IL-13, IL-17, IL-21 and tumour necrosis factor (TNF) — following stimulation, and they are able to either promote or suppress cell-mediated immunity without the need for clonal expansion[5,6]. Thus, NKT cells can enhance the immune response to a range of infectious organisms, and some types of cancer, but can also suppress autoimmune disease, allograft rejection and graft-versus-host disease[3]. Consequently, NKT cells represent a potentially important immunotherapeutic target with widespread clinical potential[7–9].

The interaction between the NKT cell antigen receptor — that is, the T cell receptor (TCR) expressed by NKT cells (referred to as the NKT TCR in this article) — and the antigen–CD1d complex represents a central event leading to NKT cell activation[10]. Since 2006,

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many studies on antigen recognition by NKT TCRs have helped us to begin to understand the factors that govern the antigenicity of a given ligand. Although CD1d is essentially monomorphic, it can bind to an array of lipid-based antigens, including synthetic, self and non-self antigens[11–13]. Uncovering the NKT cell stimulatory properties of these antigens, and the rules of engagement in the various NKT TCR–antigen–CD1d complexes, represents a key step in understanding NKT cell biology. This Review primarily focuses on how the various forms of type I NKT TCR function like 'pattern-recognition receptors' in engaging a variety of lipid-based antigens that exhibit diverse chemistries. We also highlight the contrasting 'snapshot' of type II NKT TCR-mediated antigen recognition, as well as the factors that govern the general principles of NKT cell-mediated antigen recognition and therapeutic implications.

NKT cell subsets

The αβ TCR is composed of an α-chain and a β-chain, with each chain being subdivided into a variable (V) domain and a constant (C) domain. In TCR α-chains, the V domains are encoded by V (*TRAV*) and joining (J; *TRAJ*) gene segments, whereas TCR β-chain V domains are encoded by V (*TRBV*), diversity (D; *TRBD*) and J (*TRBJ*) gene segments. Within the V domains, three complementarity-determining regions (CDRs) in each chain collectively form the antigen-binding site of the TCR. The CDR1 and CDR2 loops are encoded within the V gene segments, whereas the CDR3 loop is encoded at the junction of the rearranged V and J gene segments (for TCRα) or V, D and J gene segments (for TCRβ). The various permutations and combinations of V, D and J gene segments endow the CDR3 loops with the greatest diversity, which is further enhanced by random non-templated alterations at the V(D)J gene junctional boundaries (FIG. 1b).

Type I NKT cells

There are two main subsets of NKT cells, termed type I and type II NKT cells (FIG. 1). Type I NKT cells typically express an 'invariant' TCR α-chain (Vα24Jα18 (*TRAV10–TRAJ18*) in humans and the orthologous chain Vα14Jα18 (*Trav11–Traj18*) in mice), which is paired with a limited set of TCR β-chains (comprising Vβ11 (*TRBV25-1*) in humans and Vβ8.2 (*Trbv13-2*), Vβ7 (*Trbv1*) or Vβ2 (*Trbv29*) in mice)[4,14–16]. There is a high level of sequence identity in the CDR3α and CDR2β loops from human and mouse type I NKT TCRs[16], which underpins the evolutionarily conserved reciprocal cross-species reactivity of these cells between mice and humans[17–19]. The importance of the CDR3α loop for NKT cell function is highlighted by the loss of most type I NKT cells in TCR Jα18 gene-targeted mice[20], although studies on these mice may need to be revisited owing to a recent report that suggests that the diversity of the overall TCR repertoire is reduced in these mice[21]. As type I NKT cells typically express an invariant TCR α-chain, they are frequently referred to as 'invariant' NKT cells[4]. However, this is clearly a misnomer because NKT cells exhibit a high degree of natural variability in the CDR3β loop[16,22,23]. Thus, 'semi-invariant' is a more accurate, although still imperfect, description for type I NKT cells[4].

Another parameter that is typically used to define type I NKT cells is their ability to recognize α-galactosylceramide (αGalCer)[4]. αGalCer — a glyco-lipid originally derived from the marine sponge *Agelas mauritianus* — was identified because of its potent antitumour effects in mice[24]. αGalCer is an important experimental tool, as it is the main ligand used for functional studies of type I NKT cells because physiological antigens are not as potent or as well characterized. The most commonly used form of αGalCer (also known as KRN7000) comprises an α-linked galactose head group and a ceramide base (consisting of an 18-carbon phytosphingosine chain and a 26-carbon acyl chain) (FIG. 2). Subsequently, αGalCer was shown to bind to human and mouse CD1d and potently activate type I NKT cells[24–26].

Type II NKT cells

Type II NKT cells are CD1d-restricted T cells that lack the 'semi-invariant' TCR α-chain characteristic of type I NKT cells and do not recognize αGalCer[4,27]. Instead, type II NKT cells express a different and more diverse TCR repertoire than type I NKT cells. Although the antigen specificity of type II NKT cells is poorly understood, it includes antigens that are not generally considered to be agonists for type I NKT cells[28–30]. Currently, the most widely studied antigen for type II NKT cells is sulphatide, a sulphated glycolipid that is found abundantly in neuronal tissue and has been associated with the inhibition of experimental autoimmune encephalomyelitis by type II NKT cells[28]. Some biases in TCR chain usage have been described in type II NKT cells. For example, in mice, type II NKT cells appear to be enriched for particular TCR α-chain V segments (namely Vα3 and Vα8) and for Vβ8 (REF. 31). Furthermore, the TCRs of sulphatide-reactive type II NKT cells can have a more conserved CDR3β region than type I NKT TCRs[30]. Interestingly, a recent study of human sulphatide-reactive type II NKT cells suggested that this population includes some $V\delta1^+$ γδ T cells[32]. The diversity of type II NKT cells highlights the fact that a great deal needs to be learnt about CD1d-restricted antigen specificity and recognition by type II NKT TCRs.

Atypical NKT cells

Not all NKT cells fit neatly into the current type I–type II NKT cell classification system. Although such atypical NKT cells represent a minor population of the entire NKT cell pool, they can exhibit distinct specificities[33,34]. For example, one atypical NKT cell subset in humans consists of $Vα24^-Jα18^+Vβ11^+$ cells[33–35]. Another study examined αGalCer-reactive $Vα24^-$ NKT cell clones from healthy people and showed that, although many clones were enriched for Jα18 and Vβ11, others exhibited more diverse αβ TCR usage[36]. The recent characterization of $Vα10^+Jα50^+Vβ8^+$ NKT cells provides a clear example of NKT cells that fall within this grey zone[37]. These NKT cells can respond to αGalCer–CD1d, but they show a greater reactivity to α-glucosylceramide (αGlcCer) and preferentially recognize a mycobacterial lipid known as α-glucuronosyldiacylglycerol[37].

There may also be overlap between type I and type II NKT cell antigens. For example, the self antigen β-glucosylceramide (βGlcCer) is recognized by both subsets of NKT cells[27,38,39]. Thus, there seems to be a blurring of the boundaries regarding the classification, the antigen specificity and possibly the function of the various NKT cell subsets, and this overlap is likely to be determined, in part, by NKT TCR-mediated recognition. In order to properly understand the function and applications of NKT cells, we need to understand the TCR repertoire and associated antigen specificity of the different subsets of NKT cells.

Antigen presentation by CD1d

The first crystal structure of a CD1 family member, mouse CD1d, demonstrated that the CD1 family adopts an MHC class I-like fold with a hydrophobic antigen-binding cleft that has deep pockets ideally suited for binding lipid antigens[40]. Subsequently, the crystal structures of CD1a, CD1b, CD1c and CD1d have been determined with various lipid-based antigens bound[41–45]. In general, the CD1 binding cleft is characterized by two main pockets, the A′-pocket and the F′-pocket (FIG. 2a), which vary in shape and size between CD1 family members[46–48]. This variation, together with their distinct intracellular trafficking behaviour, enables each CD1 isoform to bind to a specific, albeit partly overlapping, repertoire of lipids[46,49].

The determination of the structure of human CD1d and mouse CD1d in complex with αGalCer (or a closely related analogue)[17,42,45] showed that the acyl chain of αGalCer is buried in the A′-pocket of CD1d and the phytosphingosine chain is buried in the F′-pocket.

By contrast, the α-galactosyl head group protrudes from the cleft, such that it is directly available to make contact with the NKT TCR (FIG. 2a). The surface-exposed head group explains why NKT cell agonists include a chemically distinct range of synthetic, foreign and self antigens[49,50] (FIG. 2b). These antigens can also vary in the nature of the linkage between the head group and the hydrophobic base region, and in the composition of their lipid tails[13] (FIG. 2b). Such antigenic diversity poses many important questions, which have not been addressed fully. For instance: what determines an optimal NKT cell agonist? How do NKT cells discriminate between self and non-self antigens? Do antigen-specific subsets of NKT cells exist? Understanding the molecular basis of NKT TCR–antigen–CD1d interactions is central to addressing these fundamental questions.

Antigen recognition by type I NKT cells

The parallel universe of type I NKT TCR binding

The fundamental principles underlying the NKT TCR– antigen–CD1d interaction were initially identified from the structure of the human type I Vα24–Vβ11 NKT TCR–αGalCer–CD1d complex[51,52] (FIG. 3a). The NKT TCR is relatively rigid when binding to αGalCer–CD1d[18,34,52], which exemplifies the innate-like characteristics of this interaction. Of interest, this type I NKT TCR ternary complex is remarkably different to all of the TCR–peptide–MHC class I structures that have been determined so far[53,54] (FIG. 3a, b). Specifically, the human type I NKT TCR adopts a tilted and parallel docking mode over the F′-pocket of CD1d. Highlighting the highly conserved reactivity of type I NKT cells, an almost identical interaction was observed between the mouse type I Vα14–Vβ8.2 NKT TCR and αGalCer–CD1d[55].

At the interface between the human type I Vα24–Vβ11 NKT TCR and αGalCer–CD1d, the CDR1α loop interacts with αGalCer, whereas the Jα18-encoded CDR3α loop contacts both CD1d and αGalCer[52,55] (FIG. 4a). The intimate interactions between the invariant TCR α-chain and the galactose head group of αGalCer provide a basis for understanding the potency of this antigen in stimulating type I NKT cells (FIG. 4a). By contrast, the TCR β-chain interactions are dominated by two residues (Tyr48 and Tyr50) within the CDR2β loop, which contacts residues above the F′-pocket of CD1d[52] (FIG. 4a). Although several residues within the CDR3α loop are important for binding, Leu99 seems to have a key role in modifying the CD1d interface. Specifically, this residue is inserted into the F′-pocket of CD1d, thereby forming a hydrophobic ‘roof’ that seems to be crucial for NKT TCR binding[55–58]. The energetic basis of the NKT TCR–antigen–CD1d interaction — as defined by residues that, when mutated, have a marked impact on the affinity of the interaction[55,59–62] — underscores the importance of the CDR2β and CDR3α loops in driving the antigen–CD1d interaction.

Modulation of TCR–CD1d interactions by the TCR α- and β-chains

All of the type I NKT TCR–antigen–CD1d structures elucidated so far have shown that the F′-pocket docking orientation is maintained, regardless of TCR α- and β-chain usage and the nature of the bound antigen[10,63–65] (FIG. 4; TABLE 1). This reveals that the type I NKT TCR functions like a pattern- recognition receptor — that is, an innate-like, germline-encoded receptor that interacts in a conserved manner with its ligands. For example, in mouse type I NKT cells, TCRs containing Vβ8.2, Vβ7 or Vβ2 adopt the same docking mode[55,62], despite sequence variation in the CDR2β loop[52,55,62]. Compensatory interactions are mediated by residues encoded within the respective Vβ gene segments of these NKT TCRs[55,62,66]. Furthermore, additional interactions are mediated by particular CDR3β loops that converge on a focal point within the a2 helix of CD1d[62,67–70] (FIG. 4). This suggests a

general mechanism pertaining to CD1d-dependent autoreactivity, as the CDR3β-mediated interaction does not involve direct contacts with the antigen itself.

Studies have also indicated how the interplay between the germline- and non-germline-encoded regions of the TCR β-chain could influence the binding of mouse NKT TCRs[66]. Although the TCR β-chain does not directly contact the glycolipid antigen, variations in TCR β-chain usage cause subtle structural modifications in the conformation of the TCR α-chain, which indirectly contribute to the preferential recognition of some antigens[55,62]. This feature may be important in the context of infection and autoreactivity. In addition, although the Jα50-encoded loop of the atypical Vα10 NKT TCR is markedly different to the Jα18-encoded loop of the Vα14 NKT TCR, the Jα50 and Jα18 loops interact with the same region of CD1d. This suggests that other atypical type I NKT TCRs may adopt similar F′-pocket docking modes[37], as has recently been demonstrated with the structure determination of a human Vα3.1Jα18 NKT TCR–αGalCer–CD1d complex[130]. Nevertheless, differences have been observed within the respective Vα10 and Vα14 NKT TCR–antigen–CD1d interfaces (FIG. 4), which provide insights into the molecular basis for the differing ligand specificities of the atypical $Vα10^+$ NKT cell subset. These findings highlight the importance of understanding the functional and structural role of atypical type I NKT cells in mice and humans.

Antigens recognized by the type I NKT TCR

It is clear that, within the confinement of the consensus type I NKT TCR–CD1d footprint[10] (FIG. 4), the type I NKT TCR binds to a diverse array of chemically distinct antigens. Here, we discuss how the NKT TCR achieves this feat of molecular recognition.

αGalCer and synthetic analogues

αGalCer can drive both T helper 1 (T_H1)-type and T_H2-type immune responses, thereby unpredictably causing either enhanced or suppressed immunity[5]. Thus, attention has centred on developing analogues of αGalCer that can bias the immune response in either direction[9,13]. These analogues have modifications in the head group, the acyl chain and/or the sphingosine chain[71–74]. The basis of how such modifications influence NKT TCR-mediated recognition has been established for a panel of αGalCer analogues[58,73,75–77]. The consensus type I NKT TCR–CD1d docking topology suggests that NKT cell function can be influenced, in part, by subtle alterations within the NKT TCR–antigen–CD1d interface[58]. As the effects of altered peptide ligands (APLs) on MHC-restricted immunity are associated with minor conformational shifts at the TCR–peptide–MHC interface[78], we suggest the term ‘altered glycolipid ligands’ (AGLs) to describe closely related analogues that promote distinct functional NKT cell outcomes[58].

Many head-group-modified AGLs are differentially recognized by NKT cells and thus have distinct effects on downstream immunity. Although no studies have examined the role of the 2′-OH of αGalCer, the distinct orientation of this moiety in α-mannosylceramide (a compound that has very little agonist activity[24]) suggests that the 2′-OH moiety is crucial for antigen recognition by type I NKT TCRs. AGLs with modifications at the 3′-OH and 4′-OH positions have fewer contacts with the NKT TCR than αGalCer does and thus are bound by the NKT TCR with lower affinity, resulting in decreased NKT cell activation[60,79]. Interestingly, αGlcCer is a more potent agonist than αGalCer for $Vα10^+Jα50^+$ NKT cells[37], highlighting how variations in NKT TCR α-chain usage influence antigen specificity. Further studies of the functional potential of these AGLs are warranted, especially regarding human NKT cells[80].

Several AGLs with substitutions or additions at the 6′-OH group promote T_H1-biased immune responses and provide superior protection against tumour growth in mice[73]. This is reminiscent of the prototypical T_H1-promoting AGL, α-C-GalCer, and its closely related derivatives[72,76]. Paradoxically, despite their superior anti-tumour and T_H1-inducing potential, such compounds are bound by the NKT TCR with a lower affinity than αGalCer owing to reduced interactions with the TCR[73,75]. The superior T_H1-type response induced by these (and other) ligands[77] is attributable to the enhanced *in vivo* [73,75]. Why do stability of the T_H1-promoting compounds these compounds lead to T_H1-biased responses? When NKT cells are activated, they immediately produce both IFNγ and IL-4. However, IL-4 production ceases within a few hours, whereas the production of IFNγ continues for 2–3 days and is mostly mediated by bystander natural killer (NK) cells following NKT cell activation[72,81]. This suggests that the lower affinity of the T_H1-promoting compounds results in lower IL-4 and IFNγ levels in the short term, but that the increased stability of these compounds ensures sustained stimulation of NKT cells and NK cells as well as prolonged IFNγ production, primarily by the NK cells.

Modifications of the acyl or sphingosine chains of αGalCer can also have an impact on type I NKT cell function[82,83]. For example, two such compounds — known as C20:2 αGalCer (which has an unsaturated acyl chain) and OCH (which has a truncated sphingosine chain) — promote T_H2-biased responses *in vivo*[13]. The C20:2 modification affects neither the affinity nor the mode of interaction with the NKT TCR[58]. This indicates that the T_H2-biased *in vivo* cytokine response is primarily due to the very efficient and rapid uptake of C20:2 αGalCer by CD1d-expressing APCs, and also due to the increased turnover of this ligand and less sustained NKT cell activation[83]. Thus, C20:2 αGalCer potently induces short-term NKT cell stimulation and IL-4 and IFNγ production, but the long-term activation of NKT cells and bystander NK cells is reduced, leading to decreased IFNγ production and an effective bias towards a T_H2-type response. A similar mechanism explains the T_H2-biased response mediated by OCH[58,81,83,84]. In this case, however, the truncated sphingosine chain also has an impact on the affinity and association kinetics of the NKT TCR for OCH, as it modulates the architecture of the F′-pocket of CD1d[58] (FIG. 5a).

Overall, the bias towards the production of T_H1- or T_H2-type cytokines seems to be mainly related to CD1d loading, downstream events, APC types, ligand stability and the pharmacological properties of the AGLs themselves, rather than to altered NKT TCR–antigen–CD1d affinity[58,83,85]. However, the affinity of the type I NKT TCR–antigen–CD1d interaction seems to be a good measure of the efficacy of an AGL, in terms of absolute levels of cytokines produced by the NKT cells[58]. Thus, the studies using αGalCer-based AGLs showcase the potential of rationally designing NKT cell agonists that may result in more targeted type I NKT cell-based therapeutic regimens[9]. Moreover, the work carried out using synthetic αGalCer antigens has provided valuable insights into the factors that shape the antigenicity of physiologically relevant ligands.

Microbial ligands

Microbial lipid antigens that activate type I NKT cells have been identified in a broad range of microorganisms (FIG. 2b). Examples include α-glucuronosylceramides and α-galacturonosyl-ceramides from *Sphingomonas* spp., α-galactosyldiacylglycerols (αGalDAGs) from *Borrelia burgdorferi*, α-glucosyldiacylglycerols (αGlcDAGs) from *Streptococcus pneumoniae* and group B *Streptococcus*, and phosphatidylinositol mannosides (PIMs) from *Mycobacterium tuberculosis*[37,86–92].

With the exception of the PIMs, which are phospho-lipids, these bacterial antigens possess a glycosyl head group that is conjugated, via an α-glycosidic linkage, to either a ceramide or a diacylglycerol (DAG) base. The α-glycosidic linkage defines a ‘microbial signature’, as

most mammalian glycolipids have β-glycosidic linkages and, as such, αGalCer is considered to be a mimetic of the microbial α-glycolipids[67,89]. Nonetheless, the structures of these microbial lipid antigens are quite diverse, which highlights the range of foreign antigens that can be recognized by the type I NKT TCR. This recognition is partly achieved by the ability of the NKT TCR to mould the various head groups into a position that facilitates binding[67,69], as well as by the diversity of the type I NKT TCR provided by the TCR β-chain[55,75]. As such, some microbial glycolipids seem to be recognized by only a subset of type I NKT cells[90–92].

Much like in the case of αGalCer analogues, natural variations within the lipid tails of these microbial ligands can dramatically affect their antigenicity, although it is unclear whether this is due to a differential ability to dock into CD1d or due to alterations in CD1d-mediated presentation of the polar head group[57,88,89,93]. In support of the latter scenario, modifications of the DAG chain can determine the orientation of the lipid tails within the A′-pocket and F′-pocket of CD1d, thereby affecting the positioning of the glycosyl head group[94]. Furthermore, some microbial ligands leave an 'open' F′-pocket in CD1d, which is reshaped (closed) following recognition by the NKT TCR, in a similar manner to NKT TCR-mediated recognition of the αGalCer analogue OCH[56] (FIG. 5a). Thus, there seems to be a subtle interplay between the head group and the lipid tails, which — together with type I NKT TCR specificity — ultimately determines the efficacy of the microbial lipid antigens[57]. Clearly, it will be important to establish a broader spectrum of microbial lipid antigens that can stimulate NKT cells and to address whether specific subsets within the NKT repertoire are more ideally tuned to interacting with defined microbial ligands.

Self antigens and NKT cell autoreactivity

Some NKT cells can respond to CD1d-expressing APCs in the absence of foreign agonist antigens[3]. Indeed, NKT cell-mediated recognition of CD1d-restricted self lipid antigens seems to be important for many immunopathological processes that involve NKT cells, including tumour rejection, autoimmunity, graft rejection and graft-versus-host disease[5]. Furthermore, when combined with pro-inflammatory cytokines, the reactivity of NKT cells to CD1d-restricted self lipids has an important role in NKT cell responses to infection[11,38,95,96]. Self antigens for NKT cells include phospholipid antigens and β-linked glycolipid antigens, and the relative importance of these molecules appears to be dependent on the cells involved and the presence of inflammatory signals.

Thymic selection by self antigens

Self-antigen recognition by NKT cells is likely to be imprinted in the thymus, where the recognition of CD1d-restricted self antigens is crucial for the intrathymic selection and survival of these cells[97], similarly to the positive selection of T cells by self-peptide–MHC complexes[98]. Thus, the identification of the self lipid antigens that are involved in intrathymic NKT cell selection is a central goal. One candidate self lipid — isoglobotrihexosylceramide (iGb3) — is a moderate agonist for type I NKT cells[99], although it remains controversial whether iGb3 is involved in NKT cell selection in mice or humans[100–103]. More recently, Facciotti *et al.* provided evidence that two peroxisome-generated, ether-bonded phospholipid antigens — plasmalogen lysophosphatidylethanolamine (pLPE) and ether lysophosphatidic acid (eLPA) — have a role in NKT cell development[104]. pLPE was capable of strongly activating type I NKT cells, and mice deficient in these peroxisomal lipids had impaired NKT cell development. It is quite possible that NKT cells are selected by a range of lipid-based antigens, including glycolipids and phospholipids, and that a level of redundancy exists in this process. Understanding the precise metabolic pathways involved in the generation of such self antigens will be central for determining whether different self antigens have unique roles in

NKT cell biology, as well as for addressing how NKT TCRs recognize these structurally distinct self antigens.

Self-glycolipid recognition

Most mammalian glycolipids possess a β-linked glycolipid head group that protrudes from CD1d, in contrast to the 'flattened' conformation of the α-linked ligands[105,106] (FIGS 3a, 5b). The study of NKT TCRs in complex with β-galactosylceramide (βGalCer) and iGb3 has provided insights into how type I NKT TCRs can recognize such distinct structural landscapes. Specifically, the NKT TCRs flatten the β-linked glycolipid head groups into a conformation that mirrors that of the α-linked antigens[67,69] (FIG. 5b). This flattening of self antigens is reminiscent of the 'bulldozer-like' effect observed in some MHC-restricted T cell responses[107–109] and reveals that, for the recognition of β-linked ligands, type I NKT TCRs operate through induced-fit molecular mimicry (FIG. 4a). In comparison to the NKT TCR-mediated recognition of α-linked antigens, the interactions between type I NKT TCRs and β-linked antigen–CD1d complexes are of considerably lower affinity[67,69]. This weak reactivity may be important for selecting an appropriate NKT TCR repertoire during development, so as to avoid overt NKT cell-mediated autoimmunity while ensuring the generation of an NKT cell repertoire that is poised for activation by foreign antigens.

Self-phospholipid recognition

Phospholipids represent a major component of biological membranes and thus are highly abundant but also extremely heterogeneous. Phospholipid self antigens for type I NKT cells include phosphatidylethanolamine, phosphatidylinositol[95,104,110] and pLPE[104]. The structure of an engineered autoreactive type I NKT TCR–phosphatidylinositol–CD1d complex was shown to be very similar to that of type I NKT TCR–glycolipid–CD1d complexes[68]. The main difference was in the positioning of the phosphatidylinositol head group, which was orientated away from the NKT TCR–CD1d interface. Recently, the structure of a human type I NKT TCR–lysophosphatidylcholine–CD1d complex was elucidated, showing that the phosphatidylcholine head group shifts conformation markedly following the ligation of the type I NKT TCR[70]. This shift enables the formation of the consensus type I NKT TCR–CD1d docking topology, although there seems to be a slight 'wobble' in the overall docking topology in relation to other type I NKT TCR–CD1d complexes.

Mechanisms of self-antigen recognition

The reactivity of type I NKT cells to self antigens is strongly influenced by the TCR β-chain, which can affect the fine specificity of the NKT TCR[55,61,62,66,68,111]. Diversity within the CDR3β loop can engender CD1d-mediated autoreactivity by facilitating the direct interaction of this loop with CD1d[68,111] (FIG. 4a), thereby reducing the specific energetic contribution required from the self antigens for interaction with the NKT TCR. Moreover, residues within the TCR β-chain can have an impact on the conformation of the invariant TCR α-chain, thereby indirectly modulating NKT TCR–antigen contacts[55,70]. These effects may explain the variability in the extent to which NKT TCRs can engage self antigens. Accordingly, multiple self antigens are recognized in a similar manner by these autoreactive type I NKT TCRs. Nevertheless, some CD1d-bound self glycolipids, such as the disialoganglioside GD3, can apparently 'thwart' the binding of the type I NKT TCR[68], presumably through steric hindrance or charge repulsion. As CD1d molecules at the surface of APCs are likely to be loaded with a variety of self antigens with differing chemistries[112], type I NKT cell autoreactivity caused by CDR3β loop diversity may be attributable to a shift in the balance between permissive and non-permissive self antigens.

Is autoreactivity limited to a subset of type I NKT TCRs with appropriate 'autoreactive' CDR3β loops? Given that all NKT cells are positively selected in the thymus, it is likely that they are all capable of interacting with self-antigen–CD1d complexes to some extent and that some self antigens may directly contribute to autoreactivity, regardless of CDR3β–CD1d interactions. These self antigens need not be strong agonists, and there may be heterogeneous lipid antigen species that support these processes in different settings, potentially with a differential role for type I and type II NKT cells. For example, the low-affinity self antigens βGlcCer and LPE are upregulated during bacterial and hepatitis B virus infections, respectively, resulting in NKT cell activation owing to the increased avidity of NKT TCR–antigen–CD1d binding combined with the presence of pro-inflammatory cytokines that co-stimulate these cells[38,95]. This illustrates two important concepts: first, that NKT cells are indirectly sensitized to microbial danger signals via the modulation of self lipid antigens presented by CD1d; and, second, that different self antigens may be important in different settings.

The factors that govern the antigenicity of specific ligands for type I and type II NKT cells are currently unclear, although the closely related nature of these antigens suggests that antigen density and CD1d-loading efficiency may be important factors in NKT cell auto-reactivity[70,95,104,113–116]. Overall, NKT cell autoreactivity remains a poorly understood area of NKT cell biology, and — considering that it is likely to be central for NKT cell responses in health and disease — it represents a key area for further investigation.

Type II NKT TCR-mediated recognition

Our understanding of the specificity and function of type II NKT cells is limited, and this is largely due to a lack of reagents to directly study this NKT cell subset. Do type I and type II NKT cells represent functionally similar cells with distinct antigen specificities or do they also mediate different immunological functions following activation? In support of the latter possibility, type I NKT cells are usually associated with the promotion of tumour rejection in mice, whereas type II NKT cells seem to suppress tumour rejection in mice and can antagonize the functions of type I NKT cells[117]. In a more recent study, type I and type II NKT cells seemed to work together in protecting against hepatitis B virus infection in mice[95]. Central to our understanding of the role of type II NKT cells in immunity is the establishment of their antigen specificity and the way in which their TCRs recognize these CD1d-presented antigens.

A key question is whether type II NKT TCRs adopt a different docking strategy from type I NKT TCRs. Two recent studies have provided the first snapshot of type II NKT TCR-mediated recognition, by determining the ternary structure of a type II NKT TCR (Vα1Jα26–Vβ16Jβ2.1) in complex with sulphatide–CD1d[118] or lysosulphatide–CD1d[119]. Despite the difference in these forms of sulphatide, the docking modes of the type II NKT TCR were remarkably similar to each other. The type II NKT TCR docked orthogonally above the A′-pocket of CD1d, and the complex was thus distinct from all type I NKT TCR complexes[10] (FIGS 3,4). Unlike the recognition of β-linked glycolipids by the type I NKT TCR[67], the type II NKT TCR does not 'flatten' the sulphatide head group during ligation, although the positioning of the sulphatide head groups in the two type II ternary complexes was slightly different, suggesting a degree of flexibility in type II NKT TCR-mediated recognition. Moreover, the CDR3 loops of this TCR dominate the interaction, with the CDR3α and CDR3β loops interacting primarily with CD1d and sulphatide, respectively[118] (FIG. 4b). These findings will fuel speculation as to whether all type II NKT TCRs dock over the A′-pocket, or whether the diverse type II NKT cell repertoire results in a myriad of distinct docking modes. Consistent with the latter possibility, mutagenesis studies of TCRs from other type II NKT cell clones have suggested that these TCRs have distinct docking

footprints[25,29,118–120]. These initial studies suggest that type II NKT TCR-mediated antigen recognition is fundamentally distinct from type I NKT TCR-mediated recognition.

NKT cell recognition: implications for therapy

Great progress has been made since the discovery that αGalCer is an antigen for type I NKT cells[24]. Despite the very high affinity of the interaction with this antigen and its obvious therapeutic potential as an NKT cell agonist[9], the physiological antigens for NKT cells are self lipid molecules that are recognized with much lower affinity[38]. For type I NKT cells, the antigen must be presented by CD1d in such a way that the polar head group can either contribute to the interaction or be flattened in a manner that does not destabilize the conserved interaction that defines all type I NKT TCR–antigen–CD1d complexes. Moreover, in the context of β-linked ligand recognition, we have shown that the antigenicity of a ligand is attributable to compensatory interactions between CD1d and the ligand itself that occur after NKT TCR ligation[67] (FIG. 5b). Collectively, ligand antigenicity is determined by multiple factors, including the composition of the polar head group and lipid tails, ligand flexibility, and the ability of the ligand to be stabilized by either the NKT TCR or CD1d. The same variables may influence antigen recognition by type II NKT cells, with the important distinction that the greater TCR diversity within this population may facilitate a greater diversity in antigen recognition and TCR docking modes. The observation that type II NKT cells do not recognize αGalCer, despite its potency for activating type I NKT cells, highlights the fact that these two lineages are not redundant, although some antigens may be capable of activating both type I and type II NKT cells[27,38].

Given that NKT cells can be activated by self antigens, the question that arises is how NKT cells normally avoid overt autoreactivity. It seems that, in steady-state conditions, the amount of signalling induced by self lipid antigens is insufficient to trigger NKT cell activation. However, modulation of the biosynthesis or degradation of self lipids, combined with increased co-stimulatory signalling, enables NKT cells to act as an early warning system for infections[38,95] (FIG. 6). Similar mechanisms may explain NKT cell responses to tumours and in auto-immune diseases. Nonetheless, only some self antigens are capable of promoting NKT cell activation under these circumstances[38,95,110], suggesting that low-affinity interactions with the NKT TCR are important factors in this process. Lastly, if NKT cell activation can be driven by the modulation of self-antigen expression, what is the significance of high-affinity, α-linked foreign antigens such as αGalCer in NKT cell biology? The difference in TCR signal strength is likely to result in distinct NKT cell functional programmes and, as such, have a differential impact on downstream effector responses. It is also possible that, for NKT cell activation, a small number of high-affinity antigens might be complemented by more abundant low-affinity self antigens, much like what is seen with peptide–MHC reactivity in conventional T cells[121].

NKT cells can have both beneficial and deleterious roles in the immune system. NKT cells can enhance immunity to infection and some forms of cancer, and they are capable of protecting against graft-versus-host disease, allograft rejection and some types of autoimmunity[5]. In other settings, however, NKT cells can be activated in response to lipid allergens found in house dust extract[12] or pollen[122] and trigger allergic responses and airway hyperreactivity[123]. Similarly, the activation of NKT cells by circulating lipid antigens that are associated with atherosclerosis contributes to cardiovascular disease[124], and NKT cell activation can also exacerbate some forms of autoimmune disease and prevent the efficient clearance of tumour cells in some models of cancer[5,117]. Thus, although the therapeutic potential of NKT cells is broad, the influence of these cells in different disease settings is complicated and unpredictable. Nevertheless, despite these challenges, recent clinical trials involving combination therapy using both αGalCer-pulsed autologous DCs and *in vitro*-

expanded autologous NKT cells resulted in an increase in NKT cell frequency and improved anti-tumour immunity in a cohort of patients suffering from head and neck squamous cell carcinoma[125,126]. Such studies highlight the fact that it is vital that we gain a better understanding of the functions of the different types of NKT cells, as well as of the influence of different classes of antigens and other microenvironmental factors on these cells, in order to safely harness their therapeutic potential.

Future directions

There are fundamental differences between the recognition of lipid-based antigens by the αβ TCRs of type I NKT cells and the interaction of peptide-based antigens with the TCRs of conventional T cells. Our understanding of lipid antigen recognition by type I NKT TCRs has progressed markedly in recent years and has provided valuable insights into the factors that govern antigenicity and the functional impact of different types of antigen. This in turn helps us to understand the natural antigenic targets of NKT cells and informs the development of superior and more tailored therapeutic reagents to harness NKT cell activity. There are many outstanding questions, including whether minor subsets of antigen-specific NKT cells can undergo clonal expansion and develop into memory-like populations. Moreover, what determines the threshold of NKT cell self-tolerance versus self-reactivity? Do NKT cells with distinct antigen specificities, such as type I and type II NKT cells, accordingly exhibit distinct functions? Addressing these questions is not only crucial for our understanding of NKT cell biology, but also central to the development of effective therapeutics.

Notably, CD1d-restricted NKT cells represent only a small subset of lipid-reactive T cells in humans[1]. There are also T cells that recognize lipids presented by group 1 CD1 molecules, as well as other subsets of innate-like T cells, such as MR1-restricted mucosa-associated invariant T cells (MAIT cells) — which have recently been shown to recognize microbial vitamin B metabolites[131] — and γδ T cells. These T cell subsets collectively represent 10% or more of the peripheral blood mono-nuclear cells in humans and an even higher proportion of the total leukocyte population found in tissues such as the gut and liver. Considering this, it is clear that there remains an enormous amount to learn about this important T cell arm of the immune system[127–129].

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Glossary

γδ T cells	T cells express either a T cell receptor (TCR) composed of α- and β-subunits (αβ TCR) or a TCR composed of γ- and δ-subunits (γδ TCR). The majority (more than 90%) of human T cells express αβ TCRs, which mainly recognize antigenic peptides bound to conventional MHC class I or II molecules. T cells that express γδ TCRs are less abundant, and the ligands for these receptors are less well characterized
Altered peptide ligands (APLs)	Peptides that are analogues of an original antigenic peptide. They commonly have amino acid substitutions at residues that make contact with the T cell receptor (TCR). TCR engagement by these APLs

	usually leads to partial or incomplete T cell activation. Some APLs (antagonists) can specifically antagonize and inhibit T cell activation by the wild-type antigenic peptide
Peroxisome	An indispensable cytoplasmic organelle that has essential roles in antioxidant defence, cholesterol and bile-acid synthesis, eicosanoid metabolism and the β- and ω-oxidation of long-chain and very-long-chain fatty acids
Mucosa-associated invariant T cells (MAIT cells)	A population of innate-like lymphocytes. MAIT cells express an evolutionarily conserved invariant T cell receptor and are selected by the MHC class I-related molecule MR1. They are abundant in human blood, in the intestinal mucosa and in mesenteric lymph nodes, and they can produce interferon-γ in response to various bacterial infections

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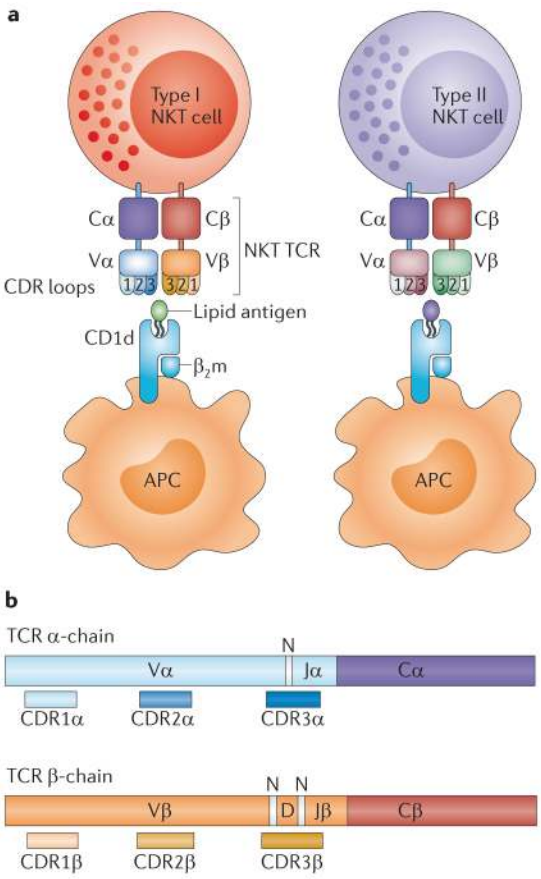

Figure 1. NKT cells

a | The figure shows a schematic representation of type I and type II natural killer T (NKT) cells. These two subsets use different variable (V) region gene segments in the α- and β-chains of their T cell receptors (TCRs), and they recognize different CD1d-restricted antigens. **b** | The αβ TCR is composed of two chains, with the V domains containing the complementarity-determining region (CDR) loops. The CDR3 loops are encoded by multiple gene segments and also contain non-templated (N) regions, which add further diversity to the TCR repertoire. The colour coding is the same as that used for the type I NKT TCR in part **a**. β_2m, β_2-microglobulin; APC, antigen-presenting cell; C, constant; D, diversity; J, joining.

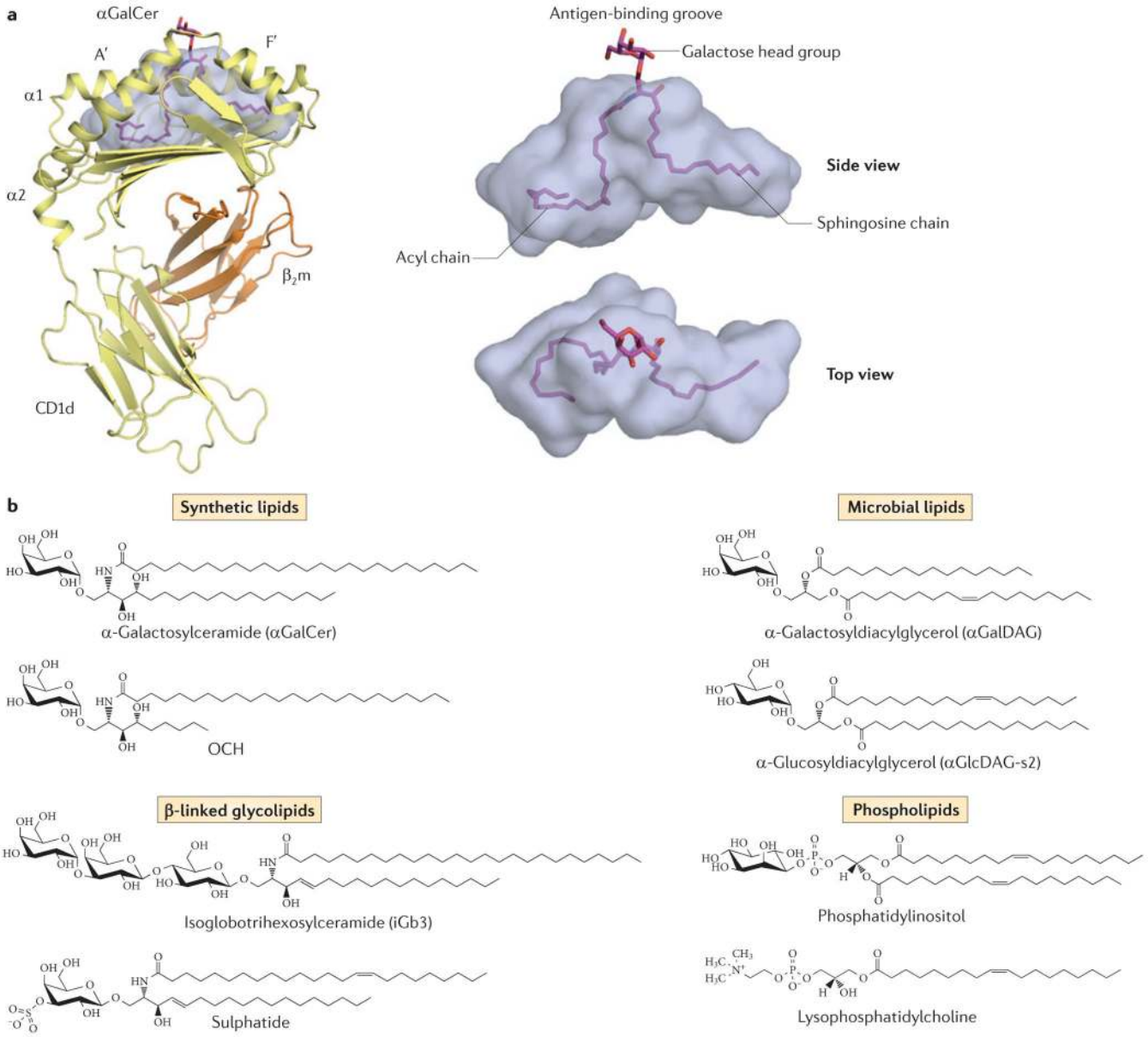

Figure 2. CD1d-mediated antigen presentation

a | The figure shows the structure of human CD1d bound to α-galactosylceramide (αGalCer) (PDB code 1ZT4). αGalCer is positioned within the CD1d antigen-binding groove, which is characterized by two main pockets: the A′-pocket and the F′-pocket. The galactose head group is surface exposed, whereas the lipid tails are buried within the cavity. **b** | The figure shows the chemical structures of various lipid antigens that bind to CD1d. These include examples of synthetic lipids (αGalCer and OCH), microbial lipids (α-galactosyldiacylglycerol from *Borrelia burgdorferi* and α-glucosyldiacylcerol from *Streptococcus pneumoniae*), β-linked glycolipids (isoglobotrihexosylceramide and sulphatide) and phospholipids (phosphatidylinositol and lysophosphatidylcholine). β_2m, β_2-microglobulin.

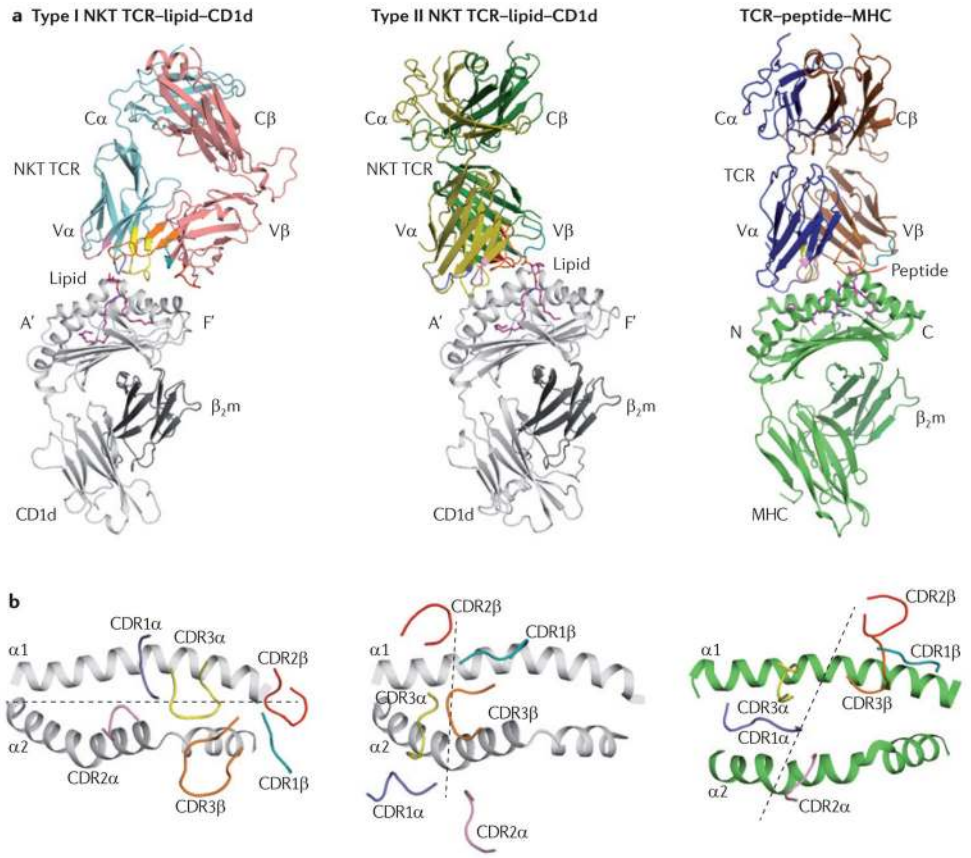

Figure 3. Structural comparison between NKT TCR–lipid–CD1d complexes and the conventional TCR–peptide–MHC complex

a | The figure shows the docking mode of the T cell receptor (TCR) in a type I natural killer T (NKT) cell TCR–lipid–CD1d complex (left; PDB code 2PO6), a type II NKT TCR–lipid–CD1d complex (middle; PDB code 4EI5) and a TCR–peptide–MHC complex (right; PDB code 3SJV). The CD1d antigen-binding pockets are labelled A′ and F′, and the amino and carboxyl termini of the peptide are labelled N and C, respectively. **b** | The figure shows the view looking down into the antigen-binding groove of the three complexes showing the parallel docking mode in the type I NKT–lipid–CD1d complex (left), the orthogonal docking mode in the type II NKT–lipid–CD1d complex (middle) and the diagonal docking mode in the TCR–peptide–MHC complex (right). Dashed lines represent the docking mode. β_2m, β_2-microglobulin.

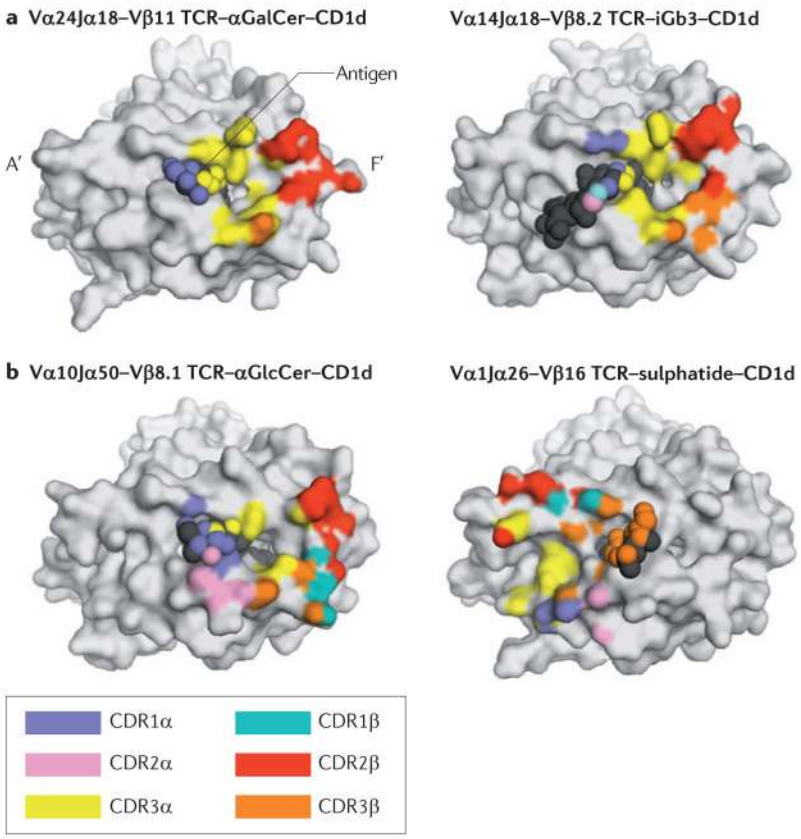

Figure 4. The footprint of contact made by NKT TCRs on the surface of CD1d. a | The image on the left shows the footprint of the human type I natural killer T (NKT) cell Vα24Jα18–Vβ11 T cell receptor (TCR) on the surface of human CD1d, which is presenting an α-linked glycolipid, α-galactosylceramide (αGalCer) (PDB code 2PO6). The image on the right shows the footprint of the mouse type I NKT cell Vα14Jα18–Vβ8.2 TCR on the surface of mouse CD1d, which is presenting a β-linked glycolipid, isoglobotrihexosyl-ceramide (iGb3) (PDB code 3SCM). **b** | On the left is the footprint of the type I NKT cell Vα10Jα50–Vβ8.1 TCR on the surface of mouse CD1d, which is presenting α-glucosylceramide (αGlcCer) (PDB code 3RUG). On the right is the footprint of the mouse type II NKT cell Vα1Jα26–Vβ16 TCR on the surface of mouse CD1d, which is presenting a β-linked self glycolipid, sulphatide (PDB code 4EI5).

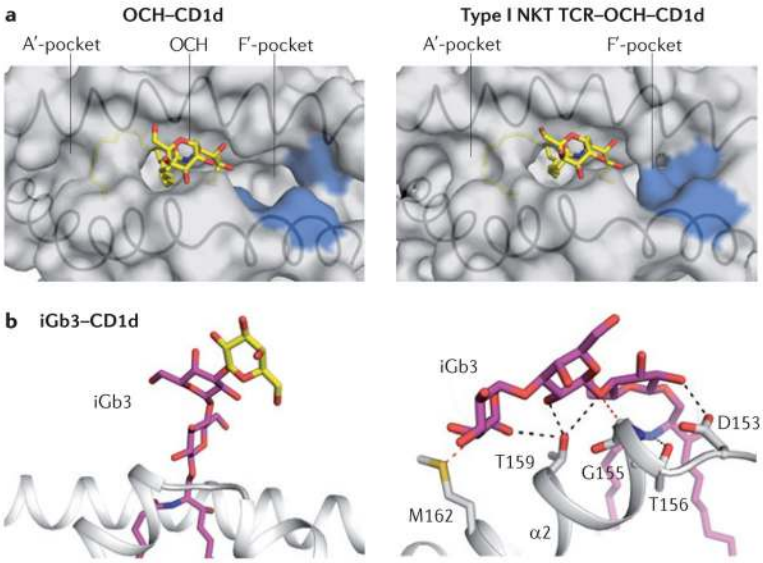

Figure 5. Changes in the conformation of the lipid or CD1d following binding to NKT TCRs

a | The image on the left shows a surface representation of the α-galactosylceramide analogue OCH presented by CD1d, showing the A′ and F′ pockets (PDB code 3G08). The image on the right shows the closing of the F′-pocket roof in the CD1d–OCH complex following binding to the T cell receptor (TCR) (PDB code 3ARB), which is caused by movement of the side chains of Leu84, Vα1149 and Leu150 in CD1d (these residues are shown in blue). **b** | On the left is the structure of the β-linked glycolipid isoglobotrihexosylceramide (iGb3) presented by CD1d (PDB code 2Q7Y). The terminal sugar for iGb3 is modelled and shown in yellow. The image on the right shows the extensive interactions between the three sugars of the iGb3 head group and the residues of the CD1d α2-helix within type I natural killer T (NKT) cell TCR–iGb3–CD1d complex (PDB code 3SCM).

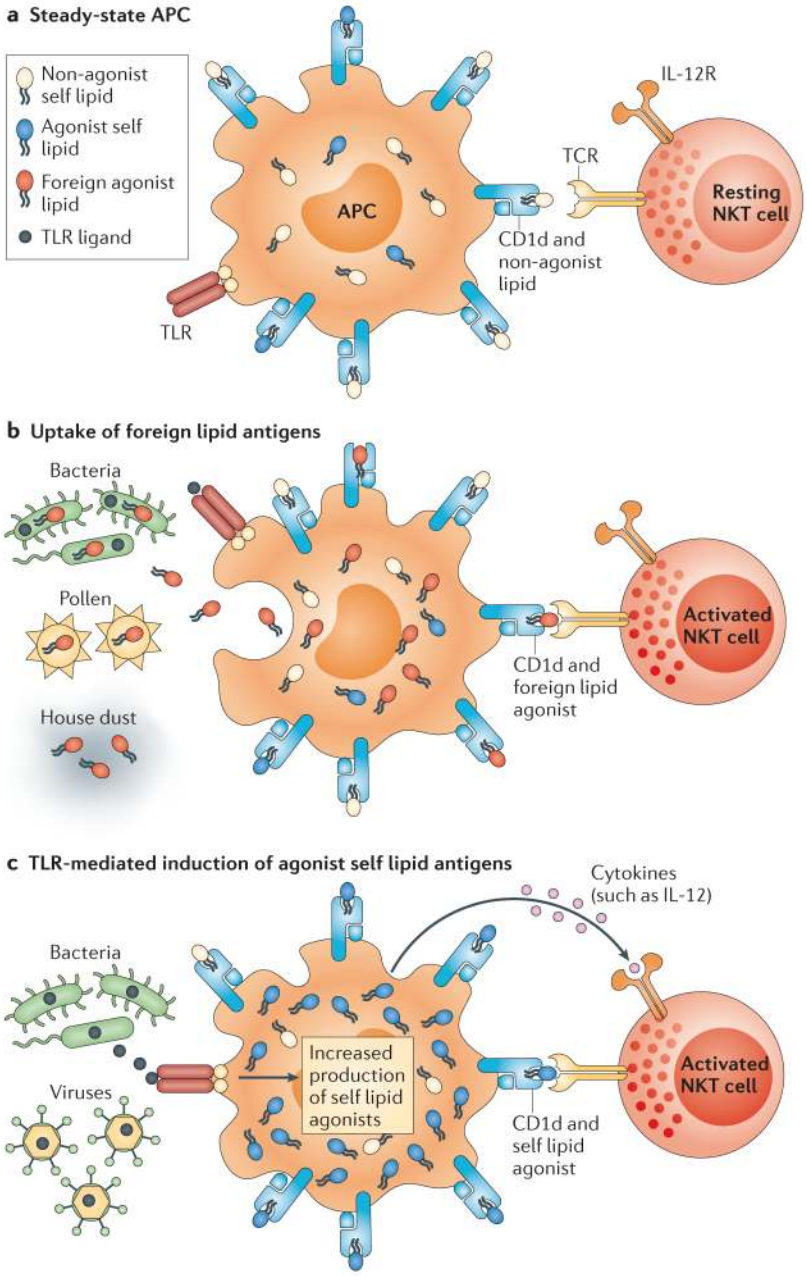

Figure 6. Modes of NKT cell activation

a | In the steady state, antigen-presenting cells (APCs) present non-agonist self lipids on CD1d molecules that do not promote the activation of the natural killer T (NKT) cell T cell receptor (TCR).**b** | APCs can present non-self lipids derived from bacteria or environmental allergens on CD1d molecules. The direct recognition of foreign lipid antigens from these sources can promote NKT cell activation. **c** | Activation of Toll-like receptors (TLRs) on APCs can induce the production of self lipid antigens (such as β-galactosylceramide) that can serve as agonists for the NKT TCR. Recognition of these CD1d-presented self lipid agonists, in conjunction with exposure to TLR-induced inflammatory cytokines, leads to NKT cell activation. IL-12, interleukin-12; IL-12R, IL-12 receptor.

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Table 1

Structures of NKT–TCR–lipid CD1d complexes

Origin	Species	Lipid antigen	TCR α-chain	TCR β-chain	CDR3β sequence	PDB code	Refs
Type I NKT cell complexes							
Synthetic lipids	Human	αGalCer	Vα24Jα18	Vβ11	CASSGLRDRGLYEQYF	2PO6	52
	Human	αGalCer	Vα24Jα18	Vβ11	CASSGLRDRGLYEQYF	3HUJ	55
	Human	αGalCer	Vα3.1Jα18	Vβ11	CASSENSGTGRIYEQYF	4EN3	130
	Mouse	αGalCer	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3HE6	55
	Mouse	αGalCer	Vα14Jα18	Vβ7	CASSSTGLDTQYF	3HE7	55
	Mouse	αGalCer	Vα14Jα18	Vβ2	CTSSADHWTNTGQLYF	3TO4	62
	Mouse	OCH	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3ARB	58
	Mouse	C20:2 αGalCer	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3ARF	58
	Mouse	3′,4″-deoxy-αGalCer	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3ARD	58
	Mouse	4′,4″-deoxy-αGalCer	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3ARE	58
	Mouse	αGlcCer	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3ARG	58
	Mouse	α-C-GalCer	Vα14Jα18	Vβ8.2	CASGDAGGNYAEQF	3TN0	75
	Mouse	NU-αGalCer	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3QUZ	73
	Mouse	α-C-GalCer	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3QUX	73
	Mouse	BnNH-GSL-1′	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3QUY	73
	Mouse	SMC124 (a plakoside A analogue)	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3TVM	77
	Mouse	HS44 (aminocyclitol)	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3RTQ	76
	Mouse	αGlcCer	Vα10 Jα50	Vβ8.1	CASRLGGYEQYF	3RUG	37
Microbial lipids	Mouse	GalA-GSL	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3O8X	56
	Mouse	αGalDAG	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3O9W	56
	Mouse	αGlcDAG-s2	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3TA3	57
Self lipids	Mouse	Phosphatidylinositol	Vα14Jα18	Vβ6–Vβ8.2 hybrid	CASGSLLDVREVF	3QI9	68
	Mouse	iGb3	Vα14Jα18	Vβ6–Vβ8.2 hybrid	CASGSLLDVREVF	3SCM	67
	Mouse	βGalCer	Vα14Jα18	Vβ6–Vβ8.2 hybrid	CASGSLLDVREVF	3SDA	67
	Mouse	Gb3	Vα14Jα18	Vβ6–Vβ8.2 hybrid	CASGSLLDVREVF	3SDC	67
	Mouse	βLacCer	Vα14Jα18	Vβ6–Vβ8.2 hybrid	CASGSLLDVREVF	3SDD	67

Origin	Species	Lipid antigen	TCR α-chain	TCR β-chain	CDR3β sequence	PDB code	Refs
	Mouse	iGb3	Vα14Jα18	Vβ8.2	CASGDEGYTQYF	3RZC	69
	Human	Lysophosphatidylcholine	Vα24Jα18	Vβ11	CASSEEGALKESVGTQYF	3TZV	70
	Human	βGalCer	Vα24Jα18	Vβ11	CASSEFGGTERTQETQYF	3SDX	67
Type II NKT cell complexes							
Self lipids	Mouse	Sulphatide	Vα1	Vβ16	CASSFWGAYAEQFF	4EI5	118
	Mouse	Lysosulphatide	Vα1	Vβ16	CASSFWGAYAEQFF	4ELM	119

3′,4″-deoxy-αGalCer, an analogue of αGalCer with the 3′-OH of the sugar head group and the 4′-OH of the sphingosine chain removed; 4′,4″-deoxy-αGalCer, an analogue of αGalCer with the 4′-OH of the sugar head group and the 4′-OH of the sphingosine chain removed; α-C-GalCer, α-C-galactosylceramide (a C-glycoside analogue of αGalCer); αGalCer, α-galactosylceramide; αGalDAG, α-galactosyldiacylglycerol; αGlcCer, α-glucosylceramide; αGlcDAG-s2, α-glucosyldiacylglycerol; βGalCer, β-galactosylceramide; βLacCer, β-lactosylceramide; BnNH-GSL-1′, an analogue of αGalCer with an aromatic moiety connected to the sugar head group via an amide linkage; GalA-GSL, α-galacturonosylceramide; Gb3, globotrihexosylceramide; HS44, an analogue of αGalCer with the sugar moiety replaced by a carba cyclitol ring with an amino linkage to the lipid tail; iGb3, isoglobotrihexosylceramide; NKT, natural killer T; NU-αGalCer, naphthylurea-αGalCer; OCH, an analogue of αGalCer with a shorter acyl chain and sphingosine chain; SMC124, an analogue of αGalCer with the acyl chain of αGalCer and the sphingoid base of plakoside A; TCR, T cell receptor.