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Natural Killer T cell obsession with self-antigens

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Summary

Natural Killer T cells are distinct lymphocyte lineages that recognize lipid antigens presented by the non-classical MHC molecule CD1d. Two categories of NKT cells, type I and type II, have been described based on T-cell receptor expression and antigenic specificity. In both cases, the recognition of self-antigens by these cells plays an important role not only in their development but also in their regulation of a broad range of immune responses. Here we review recent advances in our understanding of how and when NKT cell autoreactivity manifests itself, how the NKT TCR engages self-antigens and the nature of these self-antigens.

Introduction

The term Natural Killer T (NKT) cells was framed about 20 years ago to define a population of mature T cells found within the mouse thymus, with the ability to secrete several effector cytokines in the absence of prior foreign antigen priming. These cells were identified phenotypically by the expression of several activation/memory surface markers and, in C57BL/6 mice, the NK1.1 marker, which is usually associated with the NK lineage. NKT cells also distinguish themselves by recognizing lipid antigens presented by the Major Histocompatibility Complex (MHC) class I-like molecule CD1d, in contrast with “conventional” T cells that are directed at peptide antigens presented by MHC molecules.

NKT cells are divided into type I and type II subsets [1]. The most prevalent NKT cells in mice are the type-I-NKT cells which express semi-invariant T cell receptors (TCRs) made of a canonical germline-encoded TCR α rearrangement between the variable (V) $\alpha 14$ and joining (J) $\alpha 18$ gene segments associated with a biased set of V β chains (V $\beta 8.2$, V $\beta 7$ and V $\beta 2$) with non-germline encoded diversity in their complementary determinant region (CDR) 3β . A similar population of type-I-NKT cells using the V $\alpha 24$ -J $\alpha 18$ and V $\beta 11$ TCR genes (paralogues of the V $\alpha 14$ and V $\beta 8$ mouse gene segments, respectively) are found in humans. Type-I-NKT cells are responsive to the marine sponge-derived antigen α -galactosylceramide (α GC) when presented by CD1d molecules. This contrast with type-II-

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NKT cells, which do not react with α GC and express a much more diverse TCR repertoire [2]. The antigenic specificity of type II cells is only starting to emerge [3], with some type II NKT cells being reactive to the self-antigens such as sulfatide [4,5] and β -glucosylceramide [6], but their functional and phenotypical characterization remains mostly unclear.

The development of CD1d tetramer loaded with α GC has allowed investigators to track and specifically study type-I-NKT cells based solely on their Ag-reactivity. This has revealed that, despite their conserved reactivity to α GC, α GC-CD1d tetramer+ cells are composed of several phenotypically and functionally different subpopulations [7]•[8].

Amongst the many unique characteristics of type-I-NKT cells, one stands out in particular. Namely, type-I-NKT cells tend to be autoreactive, meaning that they can respond to CD1d-expressing antigen presenting cells (APCs) in the absence of any external antigen. Therefore, in addition to recognizing several different microbe-derived and synthetic antigens [9], type-I-NKT cells also have a dual reactivity for self-antigen(s). This underlying autoreactivity has obvious implications for the role that type-I-NKT cells play in regulating immune responses, including cancer, autoimmunity, graft rejection and graft versus host disease but also, more recently, in controlling metabolism [10-13]••. Here, we review recent advances made in the identification of CD1d-restricted self-antigens, and the recognition thereof, by type-I-NKT cells.

Recent evidence of type-I-NKT cell autoreactivity *in vivo*

A transgenic mouse model expressing GFP under the control of the TCR-signaling induced transcription factor *Nr4a1* (Nur77) promoter as a means to measure the strength of TCR stimulus directly *in vivo*, demonstrated that type-I-NKT cells express high levels of GFP immediately after selection in the thymus and lose most GFP expression after maturation and export to the periphery [14]••. This suggests that NKT cells might not continually perceive autoreactive stimuli once they mature and migrate to the periphery. Alternatively, weak TCR stimulation through the recognition of self-antigen(s) might not lead to the full activation of type-I-NKT cells but may nevertheless have important consequences, such as epigenetic modifications at cytokine loci [15]. Yet, another model using an IFN- γ -inducible cytoplasmic protein as a reporter, showed, in unchallenged mice, intense expression in small foci with type-I-NKT cells present at the center of these foci [16]•. Altogether, these results suggest that type-I-NKT cells have the underlying capacity to sense CD1d-restricted self-antigen(s) and this autoreactivity can be unleashed when combined with inflammatory cytokines released by APCs, even in the context of infection where foreign glycolipid antigens are also present [8,17-19]•. Therefore, identification of the self-lipids that are involved in this autoreactivity, and determining how type-I-NKT TCRs recognize them, is key for our understanding of the biological relevance of these cells and for harnessing their therapeutic potential.

How does the type-I-NKT TCR recognize “self”?

Most microbe-derived type-I-NKT cell antigens share a common characteristic: a lipid backbone attached to a sugar moiety via an α -glycosidic linkage [20]. This α -linkage causes the sugar to lie flat along the CD1d antigen-binding groove. However, most mammalian glycolipids have β -glycosidic linkages, which cause the sugar head group to protrude perpendicular to the CD1d antigen-binding groove and it was unclear how the type-I-NKT TCR could recognize such chemically distinct Ags. Two recent studies [21]•• and [22]•• shed light on this conundrum. Instead of the type-I-NKT TCR “adapting” to the protruding perpendicular β -linked self-antigens, it flattens the self-antigens into an α -like conformation that mimics the recognition of foreign antigens. This form of induced-fit molecular mimicry necessitates the repositioning of the β -linked antigens, incurring a considerable energetic

penalty upon binding of the type-I-NKT TCR to the β -antigen-CD1d complex. These low affinity interactions for self-antigen(s) might be important for selecting an appropriate type-I-NKT TCR repertoire during development, retaining weak affinity for self-antigens but stronger, high affinity interactions with foreign, α -linked antigens.

Triggering a type-I-NKT autoreactive response

With a relatively low affinity for self-antigen, how do type-I-NKT cells achieve an activation state when faced with autoreactivity? Several possibilities exist.

First, under certain circumstances, the activation and natural functions of type-I-NKT cells *in vivo* appear to be CD1d and TCR independent [23,24]. Exposure to IL-12 and IL-18 [25] or IL-12 and type I-interferons [24] can be sufficient to activate type-I-NKT cells. The neurotransmitter noradrenaline was also recently shown to modulate the activation and behavior of type-I-NKT cells *in vivo* in a CD1d-independent manner [26]••.

Second, inflammatory cytokines, upregulation of CD1d expression levels, and/or of co-stimulatory molecules might help reach the activation threshold necessary to activate type-I-NKT cells. Diminishing the expression of ligands for inhibitory receptors (such as NK receptors) expressed by type-I-NKT cells may also modulate the autoreactivity threshold (see ref [27] for review).

Third, we [28]••, and others [29]••, recently showed that the type-I-NKT TCR autoreactivity can occur due to the presence of unique sequences within the hypervariable CDR3 β loop, which can profoundly influence type-I NKT TCR affinity for CD1d presenting self-antigens. For example, CDR3 β sequences encoding a hydrophobic motif promoted self-association of the type-I-NKT TCR with CD1d, although other CDR3 β sequences lacking this motif can achieve an analogous effect. Thus, type-I-NKT cell autoreactivity can arise from the direct interaction between CD1d and the type-I-NKT TCR resulting in the accommodation of a broad range of CD1d-restricted self-antigens in a consensus NKT TCR-CD1d-Ag docking mode that is underpinned by germline-encoded recognition of the CD1d-Ag complex [28,30-32]. The type of self-antigen is still important however, as demonstrated when several self-lipids, when presented by CD1d molecules, were found to “hinder” the binding of the highly autoreactive type-I-like NKT TCR [28]••. CD1d molecules at the surface of APCs are loaded with a variety of different self-antigens including some “bulky” or rigid ones [33-36]. Thus, we suggest that type-I-NKT autoreactivity may in part result from a shift in the balance of permissive versus hindering self-antigens that are presented by CD1d-restricted on the cell surface.

Fourth, a limited number of self-antigens may specifically mediate the autoreactivity of type-I-NKT cells (Figure 1). These antigen(s) might be under-represented in the steady state, but induced and presented by CD1d molecules at the surface of APCs following particular inflammatory stimuli [17-19].

Type-I-NKT cell autoreactivity: which antigen(s)?

Amongst the many potential self-antigen candidates, the β -linked isoglobotrihexosylceramide (iGb3) was shown to activate type-I-NKT cells in a cell-free system [37], making it a good candidate for type-I-NKT self-antigen, with a potential role for their development in the thymus. However, the scarcity of iGb3 in mouse thymus and APCs [38], the absence of type-I-NKT cell defect in mice lacking the iGb3 synthase [39], and the lack of functional iGb3 synthase in humans [40], collectively challenged the concept that iGb3 is a key self-antigen involved in the positive selection of type-I-NKT cells.

Nonetheless, iGb3 certainly is a low affinity self-antigen for mouse type-I-NKT cells and may therefore play a role in type-I-NKT cell autoreactivity under particular circumstances.

A panel of naturally occurring GSLs was recently screened for antigenic activity on type-I-NKT cells [41]•. Surprisingly, the precursor of most GSLs outside of the central nervous system, β -D-glucopyranosylceramide (β -GlcCer) potently activated mouse and human type-I-NKT cells in a CD1d-restricted manner. Interestingly, β -GlcCer synthesis is enhanced leading to accumulation of this glycolipid within APCs after LPS stimulation and *in vivo* after bacterial infection, and inhibition of this process reduced type-I-NKT cell autoreactivity [41]•. This suggests that the presentation of β -GlcCer by CD1d molecules might contribute to the stimulation of type-I-NKT cells in a multitude of pathological contexts in the absence of foreign lipid antigens. However, it should also be noted that the modulation of β -GlcCer levels in APCs can affect the expression of several co-stimulatory molecules and cytokines, including IL-12 [42]. How presentation of this glycolipid, an abundant ceramide in cells [43], is regulated to avert constant stimulation of type-I-NKT cells *in vivo* remains to be addressed.

To identify lipids that might be involved in the development of type-I-NKT cells, Facciotti *et al.* fractionated lipids from mouse thymocytes according to polarity and size [44]•. They identified the peroxisome-generated, ether-bonded plasmalogens, lysophosphatidylethanolamine (pLPE) and lysophosphatidic acid (eLPA), as self-lipids capable of stimulating freshly isolated mouse and human type-I-NKT cells when presented by CD1d molecules in an APC-free assay, but they did not find any agonistic activity for the GSL-containing fractions, presumably including β -GlcCer [44]•. These findings are in contrast with the study from Brennan and colleagues [41]• that showed substantial levels of β -GlcCer present in the mouse thymus and β -GlcCer-containing fractions purified from mouse thymus readily stimulating type-I-NKT cells [41]•. Yet, the results are in agreement with a previous study that showed that GSL biosynthesis defect in APCs does not disturb the autoreactivity of type-I-NKT cells [45]. Interestingly, only the ether-bonded versions of the lysophospholipids stimulated both thymic and peripheral type-I-NKT cells [33]•, while the common glycerophospholipids LPE and LPA, which contain a fatty acid rather than a fatty alcohol [46], did not [47]. In contrast, LPE, which is induced following hepatitis B viral infection, can be a potential self-antigen for some type-II-NKT cells [47]. Moreover, this study showed that type-I-NKT cells may also respond to self-phospholipid antigens in the context of hepatitis B infection, although their precise identity was not determined [47]. The specific role for the ether bond in the stimulation of type-I-NKT cells remains to be explored.

Other potentially relevant self-antigens for type-I-NKT cells include lysophosphatidylcholine (LPC) and lysosphingomyelin, which can be found at elevated levels during inflammatory responses [48]•[49]. These two self-antigens weakly stimulate some human type-I-NKT cells [48]• but apparently fail to trigger a response by mouse type-I-NKT cells [41,44,45]•. This raises the possibility that mouse and human self-antigen(s) for type-I-NKT cells might be distinct, due to differing trafficking requirements and sequence differences between mouse and human CD1d [50]. Further, differences in the fine specificity of human and mouse type-I-NKT cells for the recognition of α -GalCer analogues have been reported [51].

Taken together, it seems likely that there is not simply one self-antigen for type-I-NKT cells but rather several different self-antigens with various structures might be capable of providing an agonistic signal when recognized by the type-I-NKT TCR.

Back to the future

As NKT cell functions are likely mediated through their recognition of “self”, developing a mouse model specifically lacking type-I-NKT cells (the Ja.18 knockout) was key to the study of type-I-NKT and type-II-NKT cells [52]. However, recent high throughput sequencing of TCR α rearrangements from CD4+CD8+ (DP) thymocytes of CD1d $^{-/-}$ and Ja.18 $^{-/-}$ mice [53]•• revealed that transcripts encoding Ja genes located upstream of the Ja.18 in the Ja locus were essentially absent from samples derived from Ja.18 $^{-/-}$ mice, indicating that the repertoire of other T lymphocyte populations is severely impaired in these mice.

Non-productive rearrangements were also affected in these mice, most likely due to the inadvertent effect that the PGK-neo^r cassette has on transcription and gene rearrangements [54]. Two independent colonies of Ja.18 $^{-/-}$ mice, maintained separately for over 10 years, were affected, suggesting a common effect within the original strain. However, a population of α GC/CD1d-reactive NKT cells with a TCR α chain using an upstream Ja gene segment (Va.10-Ja.50) was recently identified in Ja.18 $^{-/-}$ mice [55]•, implying that not all colonies might be affected or that some upstream Ja transcripts might still be expressed, albeit at much reduced levels, and can be utilized by the NKT cell repertoire.

Nonetheless, the consequences of this unanticipated finding are potentially far reaching, because they raise the question of whether some results that have been generated with Ja.18 $^{-/-}$ mice, might, at least in part, be attributed to the change in TCR repertoire diversity, rather than the absence of type-I NKT cells. Further, as many studies of type-II-NKT cells have relied on observed differences between Ja.18 $^{-/-}$ and CD1d $^{-/-}$ mice to indicate a presumed role for type-II-NKT cells, we suggest that the doubly deficient strain (Ja.18 $^{-/-}$ x CD1d $^{-/-}$) might serve as a more appropriate control.

Conclusions and future directions

While the past few years have provided us with a wealth of information as to how different self-lipids are recognized by the type-I-NKT TCR (reviewed in [20,56]), the next challenge will be to understand when, where and in which conditions the orchestration of the presentation of these various self-antigens by CD1d occurs and how type-I-NKT cells might differentially respond to them. It will also be important to address the mechanisms of type-II-NKT cell autoreactivity and how this differs from type-I-NKT cell autoreactivity. Recent insights into type II autoreactivity were provided by solving the crystal structure of a type II NKT TCR recognizing sulfatide/lysosulfatide [57,58]••, an abundant class of lipid-antigens in neuronal tissue. It remains to be seen whether other type II NKT cells exhibit differing patterns of autoreactivity. Finally, developing the appropriate reagents to directly detect autoreactive type-I and type-II NKT cells will ultimately allow the field to assess the autoreactive potential of all CD1d-restricted NKT subsets and their role in health and disease.

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Highlights

- NKT cells are inherently reactive to self-antigens presented by CD1d molecules
- Self-antigens are made to mimic foreign antigens upon recognition by type-I NKT TCRs
- There are multiple ways to trigger a type-I NKT response in vivo
- Several different self-antigens can stimulate type-I NKT cells
- The main mouse model for the study of type-I NKT cell autoreactivity is imperfect

Figure 1. NKT cell self-antigens

The chemical structure of the various self-antigens currently reported for NKT cells is shown. The type of NKT cells for which antigen reactivity has been shown is indicated by +, while absence of reactivity is indicated by -. A schematic of NKT TCR – CD1d interaction is depicted with the arrow indicating the presence of the self-antigen(s) within the groove of the CD1d molecule. iGb3, isoglobotrihexosylceramide; β -GlcCer, β -D-glucopyranosylceramide; pLPE, ether-bonded lysophosphatidylethanolamine; eLPA, ether-bonded lysophosphatidic acid; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; LSM, lysosphingomyelin.