

# Natural killer T cell recognition of lipid antigens

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## Abstract

Natural killer T cells recognize lipid antigens in the context of CD1 molecules. Recent publications show that this mode of recognition differs substantially from that of classic T-cell receptor peptide-major histocompatibility complex interaction.

## Introduction and context

The recognition of lipids and glycolipids by the immune system was exclusively focused on antibody production until Brenner's group [1] described the recognition of mycobacterial lipids by human  $\alpha\beta$  T cells restricted on CD1b. Until then, the common admission was that T cells were tolerized against the main lipid components of cells and lipid mediators; the possible recognition of foreign lipids by CD4 or CD8 T cells was not even considered. The admission that CD1 molecules [2] may bind lipid was not settled until the first structure of a CD1 molecule revealed an antigen-binding groove unsuited to peptide binding [3] but ideal for lipid interactions as it is lined with hydrophobic residues [4]. During the same period, it was discovered that natural killer T (NKT) cells were restricted on CD1 [5] and that a natural product extracted from marine sponge could stimulate NKT cells in a CD1-restricted manner [6]. This situation shifted the entire field of anti-lipid immunity from the recognition of lipid/CD1 complexes by classical  $\alpha\beta$  and  $\gamma\delta$  T cells toward the exclusive niche of NKT biology and semi-invariant T-cell recognition. This choice has proven very beneficial by concentrating the effort on a very simplified model system. Indeed, NKT cells are innate regulatory T cells essential for the initial coordination of immune responses. As such, they express a semi-invariant T-cell receptor (TCR), which certain consider a pattern recognition receptor [7,8], made of a unique  $\alpha$  chain conserved between species ( $V\alpha 14$  in mice and  $V\alpha 24$  in humans) and a limited set of  $V\beta$  chains (8.2, 7, and 2 in mice and 11 in humans). Serendipity

brought an unusual exogenous agonist of NKT, alpha-galactosylceramide ( $\alpha$ GalCer), to the field that allowed, in sequence, the development of CD1 tetramers capable of detecting NKT cells and the discovery of similar bacterial ligands capable of stimulating NKT during the course of infection. These developments were vital for the rapid advances in the field of cellular immunology of NKT cells as well as chemistry of NKT ligands and structural biology of CD1 and semi-invariant TCR. The search for endogenous ligands that drive thymic selection as well as normal innate stimulation of NKT cells during the course of immune responses has remained more elusive and brings forward some critical structural considerations that will be discussed in the concluding paragraph.

## Major recent advances

Our understanding of the recognition of CD1 lipid complexes by semi-invariant TCR has progressed rapidly over the past 2 years with the determination of CD1/ $\alpha$ GalCer structures [9,10],  $V\alpha 14$  and  $V\alpha 24$  TCR structures [11,12], and finally the uncovering of the  $V\alpha 24$ /CD1  $\alpha$ GalCer complex structure [8,13]. We need to keep in mind that most of our knowledge is focused on the recognition of  $\alpha$ GalCer and  $\alpha$ GalCer-like ligands, a family of exogenous antigens found only in *Sphingomonas*, *Ehrlichia*, and probably a very limited set of other bacteria [14,15].

On the CD1 side, the mode of binding of phospholipids and glycolipids is now well understood and appears to

be universal [16]. The head group of the lipid is accessible for TCR interaction, and its orientation is critically influenced by the chemical nature of its linkage to the acyl chains (glycerol versus ceramide and  $\alpha$  versus  $\beta$ ). The acyl chains of the lipid occupy the groove of the CD1 molecule and impose some discrete but noticeable conformational changes of the groove [10,17] that may influence T-cell recognition to some extent [18].

The structures of  $V_{\alpha 24}$  and  $V_{\alpha 14}$  TCRs alone were as expected for  $\alpha\beta$  TCR and only confirmed the high homology between human and murine receptors [11,12]. The architecture of the  $V_{\alpha 24}$  TCR/CD1- $\alpha$ GalCer revealed a dramatic parallel docking of TCR over CD1 at the very end of its binding groove, an arrangement never seen before; this footprint placed the complementarity-determining regions (CDRs) of the  $\alpha$  chain next to the protruding  $\alpha$ -linked galactose of the ligand [13]. One should not overreact about this apparently unusual docking or try to over-interpret it. Indeed, the more TCR/major histocompatibility complex (MHC) structures we have, the more variations we see in the overall mode of docking, with some extreme situations now described for MHC class II/TCR complexes. These great variations in docking batter, to some extent, the concept of genomic 'interaction codons' that was developed for V $\beta$ 8.2 TCR/peptide-MHC recognition [20] since the V $\beta$ 8.2 of NKT receptors does not respect that same recognition mode. However, the most important finding brought about by the elucidation of the first  $V_{\alpha 24}$  TCR/CD1- $\alpha$ GalCer and now  $V_{\alpha 14}$  TCR/CD1- $\alpha$ GalCer structures is the ideal placement of the  $\alpha$  chain next to the galactose of  $\alpha$ GalCer and the dominance of recognition by CDR1 and CDR3 $\alpha$  loops. Interestingly enough, these structures also revealed that the  $\beta$  chain is playing some accessory role in binding by revealing the participation of the CDR2 $\beta$  and CDR3 $\beta$  loops in the overall footprint. Given the sequence similarities between CDR2 $\beta$  of V $\beta$ 8.2 and V $\beta$ 7, the influence of single-residue mutagenesis was evaluated with respect to TCR/CD1 affinity. Whereas the higher affinity of the V $\beta$ 8.2 retaining TCR could not be improved, a single change in V $\beta$ 7 (S54 $\rightarrow$ A) was capable of increasing the affinity four-fold, indicating that the CDR2 $\beta$  of V $\beta$ 7 was suboptimal. However, it is too early to conclude that this affinity difference is sufficient to explain the preferential usage of V $\beta$ 8 over V $\beta$ 7 in the mouse NKT repertoire. Indeed, if one looks at the human system where V $\beta$ 11 is used almost exclusively, TCR/CD1- $\alpha$ GalCer has a much lower affinity than its murine counterpart, and it is likely that some other V $\beta$  could pair just as well with  $V_{\alpha 24}$  to achieve such a low-affinity interaction; but they do not. More importantly, measurements carried out with  $\alpha$ GalCer as the CD1-bound ligand might have no

relevance to the thymic situation and repertoire selection of NKT cells. Indeed, this process will be driven by CD1 bound to natural unknown ligands which might have no chemical relationship to  $\alpha$ GalCer whatsoever. It is still interesting to argue the opposite situation (that the CD1- $\alpha$ GalCer measurements are relevant) and propose that lipid recognition by semi-invariant TCR will obey the same set of rules for binding independently of the CD1-bound lipid. The fact that the hierarchy of V $\beta$  usage is identical for hybridomas stimulated with CD1 $\alpha$ -GalCer and CD1-iGb3 (CD1 isoglobotrihexosylceramide) may support the argument [21]. However, again, caution is required as different approaches have come to different conclusions and were arguing that the repertoire of CD1-iGb3-reactive cells was much more restricted than that of  $\alpha$ GalCer-restricted cells [22].

### Future directions

Have all of the questions regarding the semi-invariant TCR recognition of CD1 lipid complexes been answered? Of course not, even though it is clear that for the particular ligand that is  $\alpha$ GalCer, we have a very good understanding of the molecular events that led to its detection. However, two essential paradigms remain poorly understood and largely unexplored and will require attention and investment: (a) the recognition of CD1 lipid complexes by non-semi-invariant TCR and (b) the recognition of endogenous selecting and activating ligands. With respect to point (a), it is important to remember that the recognition of bacterial lipids in a CD1-restricted manner by 'normal'  $\alpha\beta$  and  $\gamma\delta$  T cells was the first established link between lipids and T cells [1]. Ever since, the recognition of exogenous lipids by T cells has not been studied as extensively. The main reasons for this situation are simple: lack of reagents (for example, tetramers or animal models [23]) and the inability to immunize efficiently against lipids. Consequently, we do not know how to efficiently expand these T-cell populations *in vivo*, and even if we did, we would be unable to follow them. The second challenge faced by researchers in the field is just as daunting: discovering endogenous ligands. As compelling as the argument is about iGb3 being one of the endogenous NKT ligands [24], it is quite obvious that other ligands remain to be discovered. However, what is not obvious is the approach amenable to their discovery. Genetic analysis has proven unfruitful, with the exception of iGb3, and biochemistry and direct isolation are mostly limited by the fact that we do not know what we are looking for (a classic chicken-egg problem in lipid chemistry). At this point, new screening methods must be explored or we will all have to hope that serendipity strikes again like it did in the discovery of  $\alpha$ GalCer.

## Abbreviations

$\alpha$ GalCer, alpha-galactosylceramide; CDR, complementarity-determining region; iGb3, isoglobotrihexosylceramide; MHC, major histocompatibility complex; NKT, natural killer T; TCR, T-cell receptor.

## Competing interests

The author declares that he has no competing interests.

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