New ways to turn on NKT cells

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Natural killer T (NKT) cells are CD1d-restricted, lipid antigen–reactive T cells with powerful immunoregulatory potential. The prototypic antigen for NKT cells is a marine sponge–derived glycolipid, α-galactosylceramide (α-GalCer), but this is not normally encountered in the mammalian environment. Thus, there is great interest in the identification of more physiological stimuli for NKT cells, and numerous studies have shown that NKT cells are capable of responding to a range of microbial lipid–based antigens. Two new studies expand our understanding of environmental NKT cell stimuli, with one showing that CD1d-restricted NKT cell antigens are present within common house dust extract (HDE), whereas the other shows that NKT cells can respond to innate stimuli irrespective of the presence of foreign microbial antigens. Collectively, these two investigations indicate that NKT cells are far more likely to encounter foreign antigens, or innate activating signals, than previously recognized, suggesting a more central role for these cells in the immune system.

NKT cells are lipid antigen (Ag)–reactive, CD1d-restricted T cells that express a limited array of αβ T cell receptors (TCRs). The most widely studied NKT cells are known as type 1 or classical NKT cells. They are present in mice and humans and are defined by their expression of an invariant TCR-α chain (Vα14-Jα18 in mice and Vα24-Jα18 in humans) paired with particular TCR-β chains (Vβ2, 7, or 8 in mice and Vβ11 in humans). Type 1 NKT cells are also characterized by their ability to recognize the prototypic CD1d-restricted glycosphingolipid Ag α-GalCer, which is a marine sponge–derived compound that has potent immunoregulatory potential. Type 2 NKT cells are distinct from type 1 NKT cells in that they express a more diverse αβ TCR repertoire; these cells recognize CD1d-restricted lipid Ags such as sulfatide, but they do not recognize α-GalCer (Godfrey et al., 2010b). Although type 2 NKT cells are likely to have a unique and important role in the immune system, the remainder of this article will focus on type 1 NKT cells.

The growing repertoire of NKT cell antigens

For many years, immunologists struggled to explain the existence of a highly conserved T cell lineage that appeared to be specific for Ags derived from such a seemingly innocuous source as marine sponges. However, it is now clear that NKT cells are activated by naturally occurring microbial Ags such as α-glucuronosylceramide and α-galacturonosylceramide from Sphingomonas spp. (Kinjo et al., 2005; Mattner et al., 2005; Sriman et al., 2005), α-galactosylsilylglucolipid from Borrelia burgdorferi (Kinjo et al., 2006), phosphatidylinositol-mannoisidase from Mycobacterium bovis BCG (Fischer et al., 2004), α-glucosyldiacetylglucolipid from Streptococcus pneumoniae (Kronenberg, M., personal communication), and a cholesteryl α-glucoside from Helicobacter pylori (Chang et al., 2011). Furthermore, NKT cells can also respond to self-lipid–based Ags, including β-linked glycosphingolipids β-galactosylceramide (β-GalCer), β-glucosylceramide (β-GlcCer), isoglobotrihexosylceramide (Gb3), and disialoganglioside, as well as self-phospholipid Ags such as phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine (Godfrey et al., 2010a; Venkataswamy and Porcelli, 2010). Thus, NKT cells may be busier than initially realized in dealing with a broad range of Ags from a variety of different sources, both exogenous and endogenous. There is also great interest in the use α-GalCer derivatives that have the potential to skew NKT cell–mediated responses toward Th1 or Th2 directions, which may lead to tailored NKT cell–based immunotherapy (Venkataswamy and Porcelli, 2010). Remarkably, the NKT TCR seems to recognize this diverse range of Ags by acting as a pattern recognition receptor in which the docking of NKT TCR–CD1d-Ag is conserved, regardless of the Vβ usage (Borg et al., 2007; Scott-Browne et al., 2007; Pellicci et al., 2009; Mallevaey et al., 2011).

Two papers in the latest issue of JEM provide critical new insight into the physiological factors that activate NKT cells. In one paper, Wingender et al. demonstrates that NKT cells respond to Ags present in environmentally ubiquitous HDE. In the second paper, Brügel et al. provides evidence that the NKT cell response to a diverse range of bacterial infections appears to occur in a microbial Ag-independent and an IL-12– and Toll-like receptor (TLR)–dependent manner, even when the infectious organisms produce NKT cell Ags (Fig. 1).

CD1d-restricted NKT cell antigens in common HDE

HDE contains immunoregulatory adjuvant–like factors that can either...
enhance or suppress Th2 immune responses and airway hyperreactivity, depending on the dosing regimen (Ng et al., 2006; Lam et al., 2008). This suggests that components of HDE may be important in the development or pathogenesis of asthma, but conversely, may also provide immunotherapeutic opportunities for this disease (Ng et al., 2006). Wingender et al. (2011) now show that Ags present within HDE activate NKT cells in a CD1d-restricted, TCR-dependent manner. Moreover, NKT cell activation is critical for the adjuvant activity of HDE, the effects of which were markedly diminished in TCR-\(\alpha\beta^{18^-/^-}\) NKT cell-deficient mice. The NKT cell response to HDE resulted in bystander NK cell activation, increased airway inflammation, and a stronger adaptive immune response to a model Ag (ovalbumin) that was co-administered with HDE. Furthermore, the adaptive immune response to ovalbumin resulted in a positive feedback loop that further enhanced the NKT cell response. Many studies have suggested a role for NKT cells in the development or pathogenesis of airway hyperreactivity and asthma (Umetsu and Dekruyff, 2010), and airway disease can be artificially induced by intranasal administration of \(\alpha\)-GalCer (Meyer et al., 2006). However, the physiological mechanism of NKT cell activation and the identity and role of lipid-based Ags in association with such diseases is unclear. One study demonstrated the presence of CD1d-restricted phospholipid Ags (phosphatidylethanolamine and phosphatidylcholine) derived from allergy-inducing plant pollens, although these appeared to favor type 2 NKT cell stimulation (Agea et al., 2005), whereas the response to HDE as documented by Wingender et al. (2011) involved type 1 NKT cells. Interestingly, despite the fact that at least half of NKT cells in naive mice are CD4\(^+\), the majority of the HDE-responsive NKT cells appeared to be CD4\(^-\), many of which produced IL-17 (Wingender et al., 2011). This is further evidence for the existence of functionally distinct NKT cell subsets, and may indicate an important role for IL-17-producing NKT cells in this model of airway hyperreactivity, which is reminiscent of some earlier studies (Michel et al., 2007; Pichavant et al., 2008; Umetsu and Dekruyff, 2010). The critical questions that arise from these studies are whether HDE-mediated NKT cell stimulation promotes asthma in humans, and what is the nature of the HDE Ags involved? Although NKT cells are present in the lungs of asthmatic humans, the extent to which they are involved in disease is unclear and controversial (Thomas et al., 2010; Umetsu and Dekruyff, 2010). Both mouse and human NKT cells responded to HDE samples, and there was a rough correlation between the response of mouse and human NKT cells to each fraction, although some samples preferentially stimulated human or mouse NKT cells (Wingender et al., 2011). Furthermore, the hierarchy of HDE sample potency varied between different NKT cell hybridomas expressing different NKT TCR-V\(\beta\) chains, suggesting the presence of more than one NKT stimulatory Ag within HDE and that NKT TCR-V\(\beta\) chains determine the affinity for these Ags. This is consistent with previous work on NKT cell activation: (1) TCR mediated and (2) cytokine mediated. NKT TCR-mediated recognition of foreign lipid-based Ags presented by CD1d that appears to be important for the adjuvant effects of HDE (A), but less so for the response to bacterial infection, whereas innate inflammatory cytokine-mediated stimulation of NKT cells appears to dominate the response to bacterial infection (B). In the context of bacterial infections, NKT cells may concurrently recognize self- (blue hexagons) or bacterial (orange hexagons) glycolipids, but regardless, the innate (IL-12– and TLR-mediated) stimuli appear to be critical for NKT cell activation.
showing how the Vβ repertoire shapes the responsiveness to lipid Ags (Mallevaey et al., 2009). Preliminary studies suggested that the Ag activity was not derived from house dust mites, and that it was not a glycosphingolipid (Wingender et al., 2011). It is possible that the HDE Ags are of bacterial origin, and further investigations into different HDE components are clearly warranted. Regardless of the nature of these Ags, their presence in the majority of HDE samples tested suggests that we are literally surrounded by CD1d-restricted NKT cell Ags with the potential to exacerbate asthma and allergic airway disease. Whether NKT cells are also responsible for HDE-mediated suppression of AHR (Ng et al., 2006; Lam et al., 2008) was not investigated in the study by Wingender et al. (2011), but will be important to determine as it may represent a novel means of immunotherapy.

**NKT cells respond to infection through innate stimuli, regardless of the presence of microbial CD1d-restricted Ag**

NKT cells play an important role in many different types of infection, including bacteria, viruses, parasites, and fungal pathogens, regardless of whether the infectious organisms carry known NKT cell Ags (Brigl and Brenner, 2010). Two pathways are thought to exist for NKT cell activation in response to infection: one where NKT cells are activated through direct recognition of microbial glycolipid Ags via their TCR (Kinjo et al., 2005; Mattner et al., 2005; Sriram et al., 2005; Kinjo et al., 2006) and another that occurs in the absence of known microbial Ags because of the ability of NKT cells to respond to innate or inflammatory stimuli, possibly in conjunction with self-glycolipid Ag recognition (Brigl et al., 2003; Mattner et al., 2005; Paget et al., 2007; Salio et al., 2007; Brigl and Brenner, 2010; Darmoise et al., 2010). Collectively, these data suggest that NKT cell recognition of bacterial Ags, such as GSL-1, is at most redundant for their antimicrobial function.

This raises the important question of why NKT cells express TCRs that are highly conserved through evolution from mice to humans, with specificity for foreign glycolipid Ags—this must be of some benefit to the host. A simple explanation might be that the existence of multiple mechanisms for NKT cell stimulation in response to infection provides greater protection. For example, it is possible that Ag-specific NKT cell activation is important during stages of the antimicrobial immune response different than those tested in this study. Indeed, NKT cell activation by α-GalCer, the prototypic foreign NKT cell Ag, has a major impact on DC activation and the magnitude and quality of subsequent adaptive immune responses (Fuji et al., 2007; Hermans et al., 2007; Guillonneau et al., 2009) that were underway at the time of NKT cell activation (Cerundolo et al., 2009). Moreover, an enhanced adaptive immune response was observed in the HDE study by Wingender et al. (2011). Thus, Ag-specific NKT cell activation might be important for the development of adaptive immunity and/or immunological memory in infectious organisms, which was not assessed in the study by Brigl et al. (2011). It is clear that IL-12 does not promote the full spectrum of NKT cell functionality, such as production of IL-4 and possibly other important factors (Brigl et al., 2011). Determining the extent to which IL-12 and other Ag-independent inflammatory stimuli can mimic Ag-dependent NKT cell responses, and the downstream influence of these innate signals on adaptive immunity and memory, will be an important goal for future studies in the field.

**Future directions**

In addition to demonstrating that NKT cells are far more likely to encounter Ags or other stimulatory factors than previously appreciated, these new studies touch on some of the key issues in the NKT cell field. One study demonstrates how NKT cell activation can promote airway disease, thus having deleterious effects on the host (Wingender et al., 2011), whereas the other shows the beneficial effects of NKT cells via their importance in microbial immunity (Brigl et al., 2011). There are also published examples where NKT cells play an immunosuppressive rather than immunostimulatory role (Godfrey and Kronenberg, 2004). This raises the question of whether therapeutic NKT cell activation to boost antimicrobial or anticancer immunity might trigger undesirable side effects, and whether there are ways to safely and effectively harness the potent immunomodulatory potential of these cells? This is likely to depend on the type of Ags used, and the dose, timing, and context of
NKT cell activation. What are the key self-Ags that underpin the development and self-reactivity of NKT cells, and what is the relationship between self-NKT cell Ags and foreign (microbial/environmental) NKT cell Ags? Do innate signals produced in response to infection result in a different repertoire of self-Ags to those involved in NKT cell development and maintenance, and is this important for self-Ag-mediated NKT cell stimulation? What Ags are responsible for maintaining the evolutionarily conserved NKT TCR specificity? Presumably, these Ags have a critical role to play in NKT cell biology. Whereas microbial glycolipid Ags appear to be the most potent agonists for NKT cells, perhaps the ability to recognize self-glycolipid Ags such as β-GalCer, β-GlcCer, or iGb3 presented by CD1d is more important for these cells. It is critical that we develop a more thorough understanding of the antigenic targets, and the role of the NKT TCR and/or other factors in the maintenance and activation of these cells. What are the biological consequences of TCR variability within the broader NKT cell population, and do microbial and/or self-glycolipid Ag-reactive subsets of NKT cells exist? There is a growing body of evidence to suggest that this is the case, even within the classical type 1 NKT cell pool where TCR-β variations can influence Ag reactivity and auto-reactivity (Schümann et al., 2006; Mallevey et al., 2009, 2011; Pellicci et al., 2009; Matulis et al., 2010; Wei et al., 2006; Wu et al., 2011). Also, despite clear differences in α-GalCer reactivity that distinguishes type 1 and 2 NKT cells, do the functions and natural antigenic targets of these cells otherwise overlap?

Our understanding of NKT cell biology, and our ability to manipulate these cells as a form of therapy, is critically dependent on our understanding of the factors that regulate and activate these cells. The two studies appearing in this issue have shown us that we are surrounded by Ags and other stimuli that turn on NKT cells. Now we just need to determine the consequences of these findings and whether they can be used to our advantage.

A National Health and Medical Research Council (NHMRC) Principal Research Fellowship supported D.I. Godfrey. J. Rossjohn is supported by an Australian Research Council Federation Fellowship. D.I. Godfrey and J. Rossjohn are also supported by NHMRC program and project grants, the Australian Research Council, and the Cancer Council of Victoria. There are authors who have no conflicting financial interests.

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Author/s: Godfrey, DI; Rossjohn, J

Title: New ways to turn on NKT cells

Date: 2011-06-06

Citation: Godfrey, D. I. & Rossjohn, J. (2011). New ways to turn on NKT cells. JOURNAL OF EXPERIMENTAL MEDICINE, 208 (6), pp.1121-1125. https://doi.org/10.1084/jem.20110983.

Persistent Link: http://hdl.handle.net/11343/264484

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