Invariant natural killer T cells: front line fighters in the war against pathogenic microbes

Catherine M. Crosby[1] and **Mitchell Kronenberg**[1,2]

[1]La Jolla Institute for Allergy and Immunology, La Jolla CA 92037

[2]Division of Biological Sciences, University of California San Diego, La Jolla CA 92037

Abstract

Invariant natural killer T (*i*NKT) cells constitute a unique subset of innate-like T cells that have been shown to have crucial roles in a variety of immune responses. *i*NKT cells are characterized by their expression of both NK cell markers and an invariant T cell receptor (TCR) α chain, which recognizes glycolipids presented by the MHC class I-like molecule CD1d. Despite having a limited antigen repertoire, the *i*NKT cell response can be very complex, and participate in both protective and harmful immune responses. The protective role of these cells against a variety of pathogens has been particularly well documented. Through the use of these pathogen models, our knowledge of the breadth of the *i*NKT cell response has been expanded. Specific *i*NKT cell antigens have been isolated from several different bacteria, from which *i*NKT cells are critical for protection in mouse models. These responses can be generated by direct, CD1d-mediated activation, or indirect, cytokine-mediated activation, or a combination of the two. This can lead to secretion of a variety of different Th1, Th2, or Th17 cytokines, which differentially impact the downstream immune response against these pathogens. This critical role is emphasized by the conservation of these cells between mice and humans, warranting further investigation into how *i*NKT cells participate in protective immune responses, with the ultimate goal of harnessing their potential for treatment.

Invariant natural killer T (*i*NKT) cells are a unique subset of innate-like T lymphocytes that have gained increasing interest over the last 20 years due to their potent immunostimulatory potential. While they were initially discovered in mice, it was found that the development and specificity of these cells is highly conserved throughout mammalian evolution, including humans (Koseki et al. 1990; Porcelli et al. 1993; Brossay et al. 1998). Thus, the many studies carried out in rodent model systems could have potential benefits for humankind. This has encouraged further studies to understand how *i*NKT cells are naturally activated, with the ultimate goal of harnessing their therapeutic potential.

*i*NKT cells are members of a class of natural lymphocytes that bridge the innate and adaptive immune system, and which can be actual participants in the innate immune response (Nagarajan and Kronenberg 2007). In contrast to mainstream T cell antigen receptor (TCR) $\alpha\beta$ CD4 and CD8 T lymphocytes, with diverse antigen receptor

Address correspondence to: Mitchell Kronenberg, La Jolla Institute for Allergy & Immunology, 9420 Athena Circle, La Jolla, CA 92037, Mitch@lji.org, Phone: 858-752-6540, Fax: 858-752-6990.

rearrangements, *i*NKT cells have an invariant TCRα chain. In mice, this is formed by an invariant Vα14-Jα18 rearrangement (*Trav11-Traj18*) (Dellabona et al. 1994; Lantz and Bendelac 1994), while humans have a homologous invariant V$_\alpha$24 rearrangement (*Trav10-Traj18*) (Porcelli et al. 1993; Dellabona et al. 1994). The invariant TCRα chain, combined with the use of relatively few Vβ segments, strongly limits the diversity of antigens recognized by these cells. In stark contrast to the mainstream CD4 and CD8 T cells that respond to peptides presented by major histocompatibility complex (MHC)-encoded antigen presenting molecules, the *i*NKT TCR recognizes glycolipids and phospholipids bound to class I-like CD1d molecules expressed by a variety of cell types, including antigen presenting cells (APCs) (Koseki et al. 1990; Porcelli et al. 1993; Brossay et al. 1998). When the TCR is engaged, *i*NKT cells are rapidly activated, and they secrete cytokines, including those typically secreted by Th1, Th2, and Th17 CD4$^+$ T cells.

Due in part to their antigen experienced phenotype, evident even in the thymus, it was originally believed *i*NKT cells only recognized self-antigens. This idea was also based on early data that showed *i*NKT cells were reactive to autologous splenocytes and to lipid extracts from tumor cells presented by CD1d, although specific antigens were not yet identified (Dellabona et al. 1994; Lantz and Bendelac 1994; Bendelac et al. 1995; Cardell et al. 1995; Gumperz et al. 2000). However, with the development of new tools and mouse models, the emphasis has since partially shifted because the response of *i*NKT cells to microbial antigens is well documented.

Despite their limited TCR repertoire, the activation and responses of *i*NKT cells are actually quite variable, depending on the stimuli, consistent with a potential role of *i*NKT cells in a variety of downstream immune responses. In fact, many functions have been attributed to *i*NKT cells, including immune regulation to prevent autoimmunity, tumor immune surveillance, and regulation of adipose tissue and the metabolic state. Additional roles include responses that cause asthma and some forms of autoimmunity, and exacerbation of sterile inflammatory responses (Godfrey and Kronenberg 2004; Wu and Kaer 2009; Santodomingo-Garzon and Swain 2011). In our view, however, the function of *i*NKT cells that has been most clearly and consistently demonstrated is the role of these cells in host defense from bacterial, fungal and viral infections. The few exceptional cases, in which *i*NKT cell responses were not protective from infections, were most likely instances of immune pathology in which excessive *i*NKT cell activity contributed to tissue damage. The role of *i*NKT cells as rapid responders, and as fighters against pathogenic microbes, is therefore the topic of this article. Characteristics of the investigated pathogens are described in Table 1. Several excellent reviews have appeared on this topic recently (Kinjo et al. 2013; Van Kaer et al. 2015; Zajonc and Girardi 2015), and in this review we will further describe how using these pathogen models has granted us a better understanding of the complexity and importance of the *i*NKT cell response.

Tools for studying *i*NKT cells

Intensive study of *i*NKT cells in the context of infections and other immune responses has been aided by the development of critical tools, including glycolipid antigens that activate

these cells, tetramers and antibodies with bright fluorescence that allow for their detection without prior enrichment, and mouse strains with a defect in generating *i*NKT cells.

αGalCer: a potent iNKT cell activator

When *i*NKT cells were initially discovered, specifically activating them was a challenge because their cognate antigens were unknown. The discovery of α-galactosylceramide (αGalCer) completely changed the way *i*NKT cells could be studied. αGalCer is a glycolipid that was originally extracted from the Okinawan marine sponge *Agelas mauritianus.* After its potent antitumor and immunostimulatory effects were revealed, Kobayashi *et al.* chemically synthesized a structurally and functionally similar compound for further investigations (Kobayashi et al. 1995). Subsequent *in vitro* studies using mouse cells found that αGalCer is presented by CD1d molecules on APCs, and it proved to be a high-binding, potent, and specific activator of *i*NKT cells (Kawano et al. 1997; Burdin et al. 1998). Notably, when bound to mouse CD1d, the αGalCer-CD1d complex interacted with the *i*NKT cell TCR with an affinity in the nM range (Naidenko et al. 1999; Benlagha et al. 2000; Cantu et al. 2003; Yu et al. 2005), which is stronger binding than most peptide antigens presented by MHC I or II molecules to CD8 or CD4 T cells.

Recognition of αGalCer presented by dendritic cells (DCs) led *in vitro* to a robust and rapid, innate-like response, which included proliferation and cytotoxicity by Vα14 *i*NKT cells. This corresponded with an increase in IFN-γ and IL-4 cytokine production (Kawano et al. 1997; Burdin et al. 1998). *In vivo* mouse analyses of the αGalCer response revealed additional roles for *i*NKT cells, including rapid (within 3–24 hours) secretion of a variety of Th1, Th2, and Th17 cytokines; as well as activation of many cell types, including DCs, NK cells, B cells, and memory CD8^{+} and CD4^{+} T cells (Carnaud et al. 1999; Singh et al. 1999; Burdin et al. 1999). Despite this broad immune modulatory potential, it was notable that in some studies αGalCer tended to skew the immune response towards a Th2 phenotype (Singh et al. 1999; Burdin et al. 1999). Furthermore, upon re-exposure to αGalCer, the *i*NKT cell population acquired some properties typical of anergic T cells, including expression of PD-1 and an increased percentage of *i*NKT cells that produce IL-10 (Parekh et al. 2005; Sag et al. 2014). Altogether, these changes suggest a potential caveat regarding the use of this unnaturally high-binding antigen for inducing prolonged Th1 responses.

Regardless of its physiologic significance, because of its high affinity for the *i*NKT cell TCR when bound to CD1d, αGalCer, and antigens closely related to it, have proven very useful in the formation of CD1d tetramers, which can be used to precisely identify and purify *i*NKT cells in various situations. An antibody to the complementarity-determining region 3 (CDR3) of the invariant TCRα chain also can be used to define this population in humans, although despite the relatively high degree of CDR3α conservation, an antibody with a similar specificity is not available for studies in rodents. These tools have been critical in the studies described below.

While studies using αGalCer began to give answers as to what *i*NKT cells could do if they were pharmacologically activated, questions remained regarding how activation of *i*NKT cells by an exogenous antigen benefits the host. To begin to address this, αGalCer was utilized in the context of infections in mice. Administration of αGalCer prior to pathogen

challenge improved disease outcomes in several experimental infection models, including infections with rodent malaria parasites **Plasmodium yoelii** or **Plasmodium berghei**, or by the fungal pathogen **Cryptococcus neoformans** (Gonzalez-Aseguinolaza et al. 2000; Kawakami et al. 2001). Additionally, post-infection treatment of mice with αGalCer increased survival and promoted clearance of **Pseudomonas aeruginosa** (Nieuwenhuis et al. 2002) and **Mycobacterium tuberculosis** (Chackerian et al. 2002). These data suggest that *i*NKT cells could have protective roles if they were artificially stimulated, and that optimized glycolipid antigens could serve as vaccine adjuvants (Ko et al. 2005; Li et al. 2010; Carreño et al. 2014), but they did not fully explicate how *i*NKT cells were involved in protection, and under what circumstances they are naturally activated.

Mouse models of iNKT cell deficiency

While initial αGalCer studies showed the impact of activating *i*NKT cells, they didn't answer how the host is impacted by the absence of *i*NKT cells. To tackle this question, *i*NKT cell-deficient mouse models were developed, including *CD1d*$^{-/-}$ and Jα281KO (*Ja18*$^{-/-}$ *or Traj18*$^{-/-}$) mice. Reported strains of *CD1d*$^{-/-}$ mice lack either *Cd1d1* or both the *Cd1d1* and *Cd1d2* loci, and thus cannot efficiently positively select *i*NKT cells during thymus differentiation (Smiley et al. 1997; Chen et al. 1997). One caveat to using these mice is that in addition to lacking *i*NKT cells, sometimes called type 1 NKT cells, CD1d deficient mice also lack type 2 NKT cells. These are CD1d-dependent cells with a more diverse TCR repertoire, and which have different and more diverse specificities compared to *i*NKT cells. A similar caveat applies to experiments using *in vivo* treatment with blocking anti-CD1d mAbs.

Alternatively, *Ja18*$^{-/-}$ mice lack the *Traj18* gene segment and therefore cannot form the invariant TCRα chain necessary for *i*NKT development (Kawano et al. 1997). The original strain of these mice also had decreased rearrangement of all the Jα segments upstream of *Traj18* (Bedel et al. 2012). This is presumably because the neomycin resistance gene cassette, incorporated during construct generation, was not removed, and its transcription in the opposite orientation from the α chain genes interfered with rearrangement of the upstream Jα segments (Bedel et al. 2012). A new *Ja18*$^{-/-}$ mouse, with the neomycin resistance gene removed, and consequently a normal repertoire of Jα segment rearrangements, addressed this problem. This model strain has yet to be widely tested in different model systems (Chandra et al. 2015). Despite their various limitations, in tandem with pathogen challenges, all of these models have been highly useful for understanding what roles *i*NKT cells have in the immune response, and why they are so important for host survival. Of human relevance, at least one person deficient for CD1d has been described, and this individual had a disseminated varicella infection after receiving the varicella vaccine to prevent chicken pox (Leung et al. 2014). The vaccine contains a live, attenuated virus, further suggesting the importance of *i*NKT cells for human health, particularly in the context of infections.

*i*NKT cells help protect the host from infections

iNKT cell-mediated protection from pathogenic bacteria

An important step required for understanding the importance of *i*NKT cells in fighting infection has been to determine if the absence of these cells leads to host susceptibility. Infection with **Borrelia burgdorferi**, the spirochete that causes Lyme disease, was among the first pathogens with an antigen for *i*NKT cells that demonstrated that the absence of *i*NKT cells has detrimental effects. CD1d deficiency rendered otherwise disease-resistant mice susceptible to **B. burgdorferi**-induced arthritis (Kumar et al. 2000). Later studies in *Ja18*$^{-/-}$ mice confirmed *i*NKT cell deficiency resulted in greater and more persistent joint swelling than in wild type BALB/c mice (Tupin et al. 2008) and increased carditis on the C57BL/6 genetic background (Olson et al. 2009). This inflammation and disease correlated with systemic increases in the spirochetes, particularly in the liver, joints, and bladder in BALB/c mice (Lee et al. 2010). Altogether these findings suggest that *i*NKT cells play a role in controlling the disease burden in this infection, perhaps through increased IFN-γ secretion (Olson et al. 2009).

Likely the most striking impact of *i*NKT cell deficiency has been shown in the context of pulmonary **Streptococcus pneumoniae** infection. Whereas 75% of wild type mice survived pulmonary infection, 87.5% of *i*NKT cell-deficient *Ja18*$^{-/-}$ mice were dead by day 7 (Kawakami et al. 2003). This correlated with a dramatic increase in bacterial loads at this time point (Kawakami et al. 2003). Several other models of infection have demonstrated that *i*NKT cell deficiency led to increased bacterial loads, particularly **P. aeruginosa** (Nieuwenhuis et al. 2002; Hazlett et al. 2007), **M. tuberculosis** (Sada-Ovalle et al. 2008), and **Chlamydia pneumoniae** (Joyee et al. 2007).

Mechanisms for activating iNKT cells

The use of αGalCer demonstrated that the *i*NKT TCR recognizes lipids bound by CD1d molecules, and the cells thereby become activated. Use of αGalCer, however, was not informative as to whether direct antigen presentation is always both necessary and sufficient for *i*NKT cell activation in the context of bacterial infections.

In support of the direct presentation model, in **P. aeruginosa** infection of wild type mice, blocking CD1d with an antibody significantly diminished bacterial clearance from the lungs (Nieuwenhuis et al. 2002). This result was similar to the diminished clearance observed after infection of *Cd1d*$^{-/-}$ mice. Comparable effects of anti-CD1d antibodies also were seen in mice infected with **S. typhimurium** or **Staphylococcus aureus**, which also showed an increase in disease susceptibility after CD1d blockage (Brigl and Brenner 2004). The use of Nur77GFP reporter mice, in which T lymphocytes express GFP after TCR stimulation, provided further proof of the importance of CD1d-mediated activation of the *i*NKT cell TCR. With this model, *i*NKT cells became GFP positive and produced cytokines in response to **S. pneumoniae** and **Sphingomonas paucimobilis**, microbes that have antigens that stimulate *i*NKT cells, but not in response to **mouse cytomegalovirus (MCMV)** or **Salmonella typhimurium** (Holzapfel et al. 2014). This agrees with data indicating that MCMV responses by *i*NKT cells do not require TCR engagement by either a self or foreign

antigen (Wesley et al. 2008; Tyznik et al. 2014), however, the result using the TCR reporter mice appears to contradict the earlier mentioned *S. typhimurium* data, where CD1d blockade negatively impacted *i*NKT cell activation (Brigl et al. 2003).

In many cases in which a protective response required antigen presentation to the *i*NKT TCR, it remained uncertain if the antigen was of pathogen or host origin. Several *i*NKT glycolipid antigens of bacterial origin have been identified. Among the first, Fisher *et al* identified a phosphatidylinositol mannoside from the **mycobacterial** cell wall, which induced *i*NKT cell IFN-γ release and cytotoxicity in a CD1d-dependent manner (Fischer et al. 2004). A later study with synthetic versions of these antigens did not confirm *i*NKT cell reactivity to these compounds (Kinjo et al. 2006). Since then, several other microbial glycolipid antigens have been isolated and tested, including *S. paucimobilis* glycosphingolipids, which activated mouse hybridomas to produce IL-2 and human *i*NKT cell lines to produce IL-4 and IFN-γ (Wu et al. 2005). The ability of these *Sphingomonas* glycosphingolipids to activate *i*NKT cells was further shown in mouse cell lines and *in vivo* studies (Kinjo et al. 2005; Mattner et al. 2005). A glycosphingolipid antigen for *i*NKT cells also has been found in *Bacteroides fragilis*, a commensal organism in mice (Wieland Brown et al. 2013). This antigen has immune modulatory properties, because when germ free mice are exposed to it early in life, they were more resistant to an IL-13-driven model of colitis (An et al. 2014). Sponges are associated with many bacterial species, and the finding of microbial antigens highly similar to αGalCer in *B. fragilis* is consistent with a microbial origin for αGalCer, and with the widely held view that the marine sponge-derived antigen actually originated from microbes that were associated with the sponge.

Diacylglycerol-containing glycolipids were found to be the primary *i*NKT antigens in *Borrelia burgdorferi* (Kinjo et al. 2006), *S. pneumoniae,* and **Group B streptococcus (*Streptococcus agalactiae*)** (Kinjo et al. 2011). Thus, these descriptions and others have shown that several bacteria contain *i*NKT cell antigens in their cell walls, and it is likely that these antigens are presented by CD1d in the course of natural infection. Additionally, it has been shown that *Helicobacter pylori* contains cholesteryl α-glucoside antigens that activate *i*NKT cells (Ito et al. 2013). These antigens are markedly different in structure, and moreover they are composite of microbial and host material, with the mammalian host providing the cholesterol moiety. The biochemical basis for the recognition of the cholesterol-containing antigens is much less well defined than it is for the glycosphingolipids and glycosylated diacylglycerol antigens.

Despite this evidence for the existence of microbial antigens, including biochemical and structural evidence that the *i*NKT cell TCR engages them when presented by CD1d (Kinjo et al. 2006; Zajonc et al. 2006; Wang et al. 2010; Li et al. 2010; Girardi et al. 2011), others have suggested the response to *S. typhimurium* and to many other bacteria involves the recognition of the elusive self-antigen(s) for *i*NKT cells; the structure of these self-antigens is still a source of controversy (Mattner et al. 2005; Mallevaey et al. 2011; Brennan et al. 2011). Self-antigen recognition was revealed most consistently in the context of IL-12 (Brigl et al. 2011; Brennan et al. 2011). If this were true, then in *S. typhimurium* infected mice, the TCR signal from the self-antigen must have been below the threshold for detection in the Nur77GFP reporter mice, because no TCR signal could be detected. Alternatively, it is

possible that the anti-CD1d blocking antibody treatment was effective because it elicited a cytokine response due to CD1d cross linking that was immune suppressive (Colgan et al. 1999; Brigl et al. 2003). Despite these unresolved issues, the data described above confirm that CD1d antigen presentation and TCR stimulation were necessary in a number of contexts for an *i*NKT cell response to some infectious bacteria, however, limited data exists on which APC must express CD1d and present the antigen.

Intravital imaging has granted the capability to directly look at interactions between APCs and *i*NKT cells in several tissues. **B. burgdorferi** clearance was shown to be due to direct CD1d-dependent interactions between *i*NKT cells and Kupffer cells in the liver sinusoids that have taken up **B. burgdorferi**. These interactions led to *i*NKT cell movement arrest and the formation of cell clusters in the liver sinusoids, an effect that was not seen in Kupffer cell-depleted or $Cd1d^{-/-}$ mice (Lee et al. 2010). Interestingly, injection of αGalCer also did not induce formation of *i*NKT-Kupffer cell clusters, further illustrating the limits of using αGalCer as a model for the responses of *i*NKT cells to infectious agents.

In addition to direct CD1d-mediated activation, many studies have also revealed the critical roles of cytokines in activating *i*NKT cells. Analyses of several bacterial infections in mice, including **S. typhimurium**, **S. aureus, S. paucimobilis**, and **S. pneumoniae**, revealed that IL-12 production by dendritic cells was also necessary for *i*NKT cell activation and production of IFN-γ (Brigl and Brenner 2004; Brigl et al. 2011; Kinjo et al. 2011). Notably, this requirement for IL-12 could be *i*NKT cell subset specific. Functional subsets of *i*NKT cells analogous to CD4$^+$ Th1, Th2 and Th17 cells have been described, and they differ for expression of cytokine receptors and their responses to cytokines. For example, in **Escherichia coli** and **S. aureus** infection, in addition to TCR stimulation, NKT17 cells required production of IL-1 and IL-23 by dendritic cells in order to secrete IL-17 and IL-22 (Doisne et al. 2011). In ocular **P. aeruginosa** infection, in contrast, IL-12p40 production by macrophages and Langerhans cells was required for activation and IFN-γ production by NKT1 cells (Hazlett et al. 2007). IL-12 therefore is not universally required by all *i*NKT cells in all infections for stimulating responses to relatively low affinity antigens. Mouse *i*NKT cells may even be activated purely by cytokines, in the absence of TCR engagement, and this has been documented most thoroughly during viral infection (Tyznik et al. 2008; Tyznik et al. 2014; Cocita et al. 2015). Activation of human *i*NKT cells, however, may be more dependent on a recent, if not a concomitant, TCR stimulation (Wang et al. 2012).

Altogether these studies indicate that activation of *i*NKT cells during bacterial infections is a complex process. In some cases, antigen presentation by CD1d is necessary, and either self or foreign antigens are recognized. In other instances, only indirect or cytokine-dependent mechanisms are involved. Some data suggest that both direct TCR recognition and indirect, cytokine dependent mechanisms are required for protective immunity. These mechanisms of activation could have critical roles in determining the type of *i*NKT cell response and the downstream affects on other cell types.

iNKT cells activate the innate immune response

Perhaps the most critical part of the *i*NKT cell story to uncover is what steps occur after *i*NKT cell activation that lead to host protection. **P. aeruginosa** has been an interesting and

useful model, because in addition to increased bacterial loads and decreased survival, infected $Cd1d^{-/-}$ mice also had lower neutrophil numbers, and lower levels of macrophage inflammatory protein 2 (MIP-2) expression in the lungs after 24 hours (Nieuwenhuis et al. 2002). When *P. aeruginosa* infected BALB/c mice were treated with αGalCer, IFN-γ production was stimulated, which increased phagocytosis of *P. aeruginosa* by alveolar macrophages and local TNF-α production. Similar effects were seen in ocular infection, where IFN-γ production by *i*NKT cells was critical for control of bacterial growth and inflammation control (Hazlett et al. 2007). However, for ocular infection, neutrophil infiltration has a damaging affect, so at this site, opposite to the lungs, *i*NKT cell deficiency actually increased neutrophil recruitment (Hazlett et al. 2007). Together these findings suggest a potentially important role for *i*NKT cells in mediating neutrophil responses for protection. Notably, in the pulmonary model of *P. aeruginosa* infection, a protective role for *i*NKT cells was later shown to only be true in BALB/c mice, and not C57BL/6 mice (Benoit et al. 2015). This suggests different roles for the *i*NKT subsets, as BALB/c mice have more NKT2 cells, which might suppress neutrophil recruitment when activated, while C57BL/6 mice are more NKT1 subset biased.

The protective response to *S. pneumoniae* was also largely regulated by IFN-γ production, with evidence suggesting *i*NKT cells produced this cytokine (Kawakami et al. 2003). IFN-γ treatment or adoptive transfer of liver mononuclear cells to *i*NKT cell-deficient mice decreased bacterial loads, and increased neutrophil counts and MIP-2, a neutrophil chemotactic chemokine, and TNF-α production. While this suggests *i*NKT cells were producing the IFN-γ, in this particular study they could not consistently and directly detect IFN-γ production by *i*NKT cells (Nakamatsu et al. 2007).

In *C. pneumoniae* infection, protection was also dependent on IFN-γ and IL-12 production (Joyee et al. 2007). Interestingly, in this case *i*NKT cell deficiency increased IL-4 and IL-5 production, two cytokines which have been correlated with increased mortality (Joyee et al. 2007). This is in stark contrast to pulmonary *Chlamydia muridarum* infection, where the *i*NKT cell response was skewed towards producing IL-4 and IL-5, and was detrimental to survival (Bilenki et al. 2005). This is particularly interesting, as these two very similar pathogens both activated *i*NKT cells, yet activation led to two very different cytokine responses and disease outcomes.

The dark side of *i*NKT cell activity

While cytokine production and neutrophil recruitment are considered positive outcomes in the context of the above-mentioned infections, these responses did not always lead to protection. For example, *i*NKT cells were recruited and activated in a TCR-dependent manner in *Francisella tularensis* infection (Hill et al. 2015). Similar to other infections, $Cd1d^{-/-}$ mice infected with *F. tularensis* had lower levels of neutrophil recruitment and produced lower levels of MCP-1, TNF-α, and IFN-γ (Hill et al. 2015). However, in this case approximately 80% of $Cd1d^{-/-}$ mice survived challenge, compared to less than 20% of wild type mice, indicating the *i*NKT cell-dependent inflammatory response could actually be detrimental to the host following infection with *F. tularensis* (Hill et al. 2015). These investigators also monitored adaptive immune responses and iBALT formation, and the lack

of *i*NKT cells actually increased B and T cell lung infiltration, as well as the accumulation of dendritic cells, which together with lymphocytes formed the iBALT structures that correlated with protection (Hill et al. 2015). This suggests that there needs to be a balance of both innate and adaptive immune responses to protect fully from pathogens.

Is down regulation of CD1 an immune evasion mechanism?

A test of the importance of *i*NKT cells, or at least the importance of CD1d expression, has come from lessons we have learned from pathogens that have evolved ways to escape CD1d-mediated presentation. Several prominent examples derive from studies of viral infections, although as noted above, the *i*NKT cell response to some viruses may not be CD1d-dependent. In one report, CD1d levels were measured in both untreated and treated individuals infected with **human immunodeficiency virus (HIV)-1**. Untreated patients had significantly lower levels of CD1d expressed on CD14+ monocytes, and this correlated with an increase in viral load in these patients (Hage et al. 2005). The HIV nef protein enhanced CD1d internalization and inhibited its transport back to the cell surface, thereby preventing further activation of *i*NKT cells (Li and Xu 2008). HIV has also been found to directly and preferentially infect and kill *i*NKT cells (Li and Xu 2008).

Similarly, **Herpes simplex virus (HSV)** has evolved its own mechanism of rapid, efficient down regulation of CD1d surface expression. In this case, it does not appear that HSV increased endocytosis of CD1d, but instead it redistributed endocytosed CD1d to lysosomal membranes, and prevented newly synthesized CD1d from translocating to the extracellular membrane (Yuan et al. 2006; Raftery et al. 2006; Liu et al. 2013). The importance of CD1d presentation for *i*NKTcell functionality was reflected by impaired IFN-γ, IL-4, and IL-10 production by *i*NKT cells stimulated with infected DCs with reduced CD1d expression (Raftery et al. 2006).

Bacteria also have evolved what are likely immune evasion mechanisms based on CD1d down-regulation. In contrast to HIV-1 and HSV, ***Chlamydia trachomatis*** degrades CD1d in infected epithelium through its own chlamydial proteasome-like activity, which interacts with CD1d and causes its degradation (Kawana et al. 2006). By a completely different mechanism, ***Bacillus anthracis*** lethal toxin binds *i*NKT cells and disrupts their TCR signaling, causing them to become functionally anergic (Joshi et al. 2009).

Summary

There are a number of innate-like lymphocyte populations, but *i*NKT cells have been particularly well studied, in part because of excellent tools that allow for the precise identification and antigen specific activation of these lymphocytes. Because they rapidly secrete many types of cytokines, *i*NKT cells are truly multi-functional cells that play a role in a variety of immune and inflammatory responses. Their roles in the defense from infections, however, especially the responses to pathogenic and commensal bacteria, are particularly prominent. During infections *i*NKT cells can be activated by microbial glycolipids, by enhanced presentation by CD1d of self-antigens, and/or by cytokines such as IL-12 from myeloid cells. The activated *i*NKT cells then are able to stimulate a variety of

cell types, with the attraction of neutrophils to the infection site a key aspect of host protection in several contexts. The intense stimulation of the immune system following *i*NKT cell activation in mice has a downside, however, as it may lead to immune pathology. Despite this potential drawback, controlled activation of *i*NKT cells by glycolipids is currently being explored as a way to stimulate beneficial immune responses to vaccines or cancers. The concept underlying these efforts is that all humans have *i*NKT cells with the same specificity, and that glycolipids that stimulate *i*NKT cells could serve as adjuvants. While this is well established in mice, additional research will be required to determine if this is the case in humans.

Acknowledgments

References

AlonsoDeVelasco E, Verheul AF, Verhoef J, Snippe H. Streptococcus pneumoniae: virulence factors, pathogenesis, and vaccines. Microbiol Rev. 1995; 59:591–603. [PubMed: 8531887]

An D, Oh SF, Olszak T, et al. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. Cell. 2014; 156:123–133. DOI: 10.1016/j.cell.2013.11.042 [PubMed: 24439373]

Bai L, Deng S, Reboulet R, et al. Natural killer T (NKT)-B-cell interactions promote prolonged antibody responses and long-term memory to pneumococcal capsular polysaccharides. Proc Natl Acad Sci USA. 2013; 110:16097–16102. DOI: 10.1073/pnas.1303218110 [PubMed: 24043771]

Bedel R, Matsuda JL, Brigl M, et al. Lower TCR repertoire diversity in Traj18-deficient mice. Nat Immunol. 2012; 13:705–706. DOI: 10.1038/ni.2347 [PubMed: 22814339]

Bendelac A, Lantz O, Quimby ME, et al. CD1 recognition by mouse NK1+ T lymphocytes. Science (New York, NY. 1995; 268:863–865.

Benlagha K, Weiss A, Beavis A, et al. In vivo identification of glycolipid antigen-specific T cells using fluorescent CD1d tetramers. The Journal of experimental medicine. 2000; 191:1895–1903. [PubMed: 10839805]

Benoit P, Sigounas VY, Thompson JL, et al. The role of CD1d-restricted NKT cells in the clearance of Pseudomonas aeruginosa from the lung is dependent on the host genetic background. Infect Immun. 2015; 83:2557–2565. DOI: 10.1128/IAI.00015-15 [PubMed: 25870224]

Berntman E, Rolf J, Johansson C, et al. The role of CD1d-restricted NK T lymphocytes in the immune response to oral infection with Salmonella typhimurium. European journal of immunology. 2005; 35:2100–2109. DOI: 10.1002/eji.200425846 [PubMed: 15940666]

Bilenki L, Wang S, Yang J, et al. NK T Cell Activation Promotes Chlamydia trachomatis Infection In Vivo. J Immunol. 2005; 175:3197–3206. DOI: 10.4049/jimmunol.175.5.3197 [PubMed: 16116210]

Brennan PJ, Tatituri RVV, Brigl M, et al. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. Nat Immunol. 2011; 12:1202–1211. DOI: 10.1038/ni.2143 [PubMed: 22037601]

Brigl M, Brenner MB. CD1: Antigen Presentation and T Cell Function. Annu Rev Immunol. 2004; 22:817–890. DOI: 10.1146/annurev.immunol.22.012703.104608 [PubMed: 15032598]

Brigl M, Bry L, Kent SC, et al. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. Nat Immunol. 2003; 4:1230–1237. DOI: 10.1038/ni1002 [PubMed: 14578883]

Brigl M, Tatituri RVV, Watts GFM, et al. Innate and cytokine-driven signals, rather than microbial antigens, dominate in natural killer T cell activation during microbial infection. Journal of Experimental Medicine. 2011; 208:1163–1177. DOI: 10.1084/jem.20102555 [PubMed: 21555485]

Brossay L, Chioda M, Burdin N, et al. CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. The Journal of experimental medicine. 1998; 188:1521–1528. [PubMed: 9782129]

Burdin N, Brossay L, Koezuka Y, et al. Selective ability of mouse CD1 to present glycolipids: alpha-galactosylceramide specifically stimulates V alpha 14+ NK T lymphocytes. J Immunol. 1998; 161:3271–3281. [PubMed: 9759842]

Burdin N, Brossay L, Kronenberg M. Immunization with α-galactosylceramide polarizes CD1-reactive NK T cells towards Th2 cytokine synthesis. European journal of immunology. 1999; 29:2014–2025. DOI: 10.1002/(SICI)1521-4141(199906)29:06<2014::AID-IMMU2014>3.0.CO;2-G [PubMed: 10382765]

Cantu C, Benlagha K, Savage PB, et al. The paradox of immune molecular recognition of alpha-galactosylceramide: low affinity, low specificity for CD1d, high affinity for alpha beta TCRs. J Immunol. 2003; 170:4673–4682. [PubMed: 12707346]

Cardell S, Tangri S, Chan S, et al. CD1-restricted CD4+ T cells in major histocompatibility complex class II-deficient mice. The Journal of experimental medicine. 1995; 182:993–1004. [PubMed: 7561702]

Carnaud C, Lee D, Donnars O, et al. Cutting Edge: Cross-Talk Between Cells of the Innate Immune System: NKT Cells Rapidly Activate NK Cells. 1999

Carreño LJ, Kharkwal SS, Porcelli SA. Optimizing NKT cell ligands as vaccine adjuvants. Immunotherapy. 2014; 6:309–320. DOI: 10.2217/imt.13.175 [PubMed: 24762075]

Chackerian A, Alt J, Perera V, Behar SM. Activation of NKT cells protects mice from tuberculosis. Infect Immun. 2002; 70:6302–6309. [PubMed: 12379709]

Chandra S, Zhao M, Budelsky A, et al. A new mouse strain for the analysis of invariant NKT cell function. Nat Immunol. 2015; doi: 10.1038/ni.3203

Chen Y-H, Chiu NM, Mandal M, et al. Impaired NK1+ T Cell Development and Early IL-4 Production in CD1-Deficient Mice. Immunity. 1997; 6:459–467. DOI: 10.1016/S1074-7613(00)80289-7 [PubMed: 9133425]

Cocita C, Guiton R, Bessou G, Chasson L. PLOS Pathogens: Natural Killer Cell Sensing of Infected Cells Compensates for MyD88 Deficiency but Not IFN-I Activity in Resistance to Mouse Cytomegalovirus. 2015

Colgan SP, Hershberg RM, Furuta GT, Blumberg RS. Ligation of intestinal epithelial CD1d induces bioactive IL-10: Critical role of the cytoplasmic tail in autocrine signaling. Proc Natl Acad Sci USA. 1999; 96:13938–13943. DOI: 10.1073/pnas.96.24.13938 [PubMed: 10570177]

Cooper AM. Cell mediated immune responses in Tuberculosis. Annu Rev Immunol. 2009; 27:393.doi: 10.1146/annurev.immunol.021908.132703 [PubMed: 19302046]

Dellabona P, Padovan E, Casorati G, et al. An invariant V alpha 24-J alpha Q/V beta 11 T cell receptor is expressed in all individuals by clonally expanded CD4-8- T cells. The Journal of experimental medicine. 1994; 180:1171–1176. [PubMed: 8064234]

Doisne JM, Soulard V, Becourt C, et al. Cutting Edge: Crucial Role of IL-1 and IL-23 in the Innate IL-17 Response of Peripheral Lymph Node NK1.1- Invariant NKT Cells to Bacteria. J Immunol. 2011; 186:662–666. DOI: 10.4049/jimmunol.1002725 [PubMed: 21169541]

Fernandes-Alnemri T, Yu J-W, Juliana C, et al. The AIM2 inflammasome is critical for innate immunity to Francisella tularensis. Nat Immunol. 2010; 11:385–393. DOI: 10.1038/ni.1859 [PubMed: 20351693]

Fischer K, Scotet E, Niemeyer M, et al. Mycobacterial phosphatidylinositol mannoside is a natural antigen for CD1d-restricted T cells. Proc Natl Acad Sci USA. 2004; 101:10685–10690. DOI: 10.1073/pnas.0403787101 [PubMed: 15243159]

Girardi E, Yu ED, Li Y, et al. Unique interplay between sugar and lipid in determining the antigenic potency of bacterial antigens for NKT cells. PLoS Biol. 2011; 9:e1001189.doi: 10.1371/journal.pbio.1001189 [PubMed: 22069376]

Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. The Journal of clinical investigation. 2004; 114:1379–1388. DOI: 10.1172/JCI23594 [PubMed: 15545985]

Gonzalez-Aseguinolaza G, de Oliveira C, Tomaska M, et al. alpha -galactosylceramide-activated Valpha 14 natural killer T cells mediate protection against murine malaria. Proc Natl Acad Sci USA. 2000; 97:8461–8466. [PubMed: 10900007]

Gumperz JE, Roy C, Makowska A, et al. Murine CD1d-restricted T cell recognition of cellular lipids. Immunity. 2000; 12:211–221. [PubMed: 10714687]

Hage CA, Kohli LL, Cho S, et al. Human immunodeficiency virus gp120 downregulates CD1d cell surface expression. Immunol Lett. 2005; 98:131–135. DOI: 10.1016/j.imlet.2004.10.025 [PubMed: 15790518]

Hazlett LD, Li Q, Liu J, et al. NKT cells are critical to initiate an inflammatory response after Pseudomonas aeruginosa ocular infection in susceptible mice. J Immunol. 2007; 179:1138–1146. [PubMed: 17617607]

Hill TM, Gilchuk P, Cicek BB, et al. Border Patrol Gone Awry: Lung NKT Cell Activation by Francisella tularensis Exacerbates Tularemia-Like Disease. PLoS Pathog. 2015; 11:e1004975.doi: 10.1371/journal.ppat.1004975 [PubMed: 26068662]

Holzapfel KL, Tyznik AJ, Kronenberg M, Hogquist KA. Antigen-dependent versus -independent activation of invariant NKT cells during infection. J Immunol. 2014; 192:5490–5498. DOI: 10.4049/jimmunol.1400722 [PubMed: 24813205]

Hsueh PR, Teng LJ, Yang PC, et al. Nosocomial Infections Caused by Sphingomonas paucimobilis: Clinical Features and Microbiological Characteristics. Clin Infect Dis. 1998; 26:676–681. DOI: 10.1086/514595 [PubMed: 9524843]

Ito Y, Vela JL, Matsumura F, et al. Helicobacter pylori Cholesteryl α-Glucosides Contribute to Its Pathogenicity and Immune Response by Natural Killer T Cells. PLoS ONE. 2013; 8:e78191.doi: 10.1371/journal.pone.0078191 [PubMed: 24312443]

Jiang J, Karimi O, Ouburg S, et al. Interruption of CXCL13-CXCR5 Axis Increases Upper Genital Tract Pathology and Activation of NKT Cells following Chlamydial Genital Infection. PLoS ONE. 2012; 7:e47487.doi: 10.1371/journal.pone.0047487 [PubMed: 23189125]

Joshi SK, Lang GA, Larabee JL, et al. Bacillus anthracis lethal toxin disrupts TCR signaling in CD1d-restricted NKT cells leading to functional anergy. PLoS Pathog. 2009; 5:e1000588.doi: 10.1371/journal.ppat.1000588 [PubMed: 19779559]

Joyee AG, Qiu H, Wang S, et al. Distinct NKT cell subsets are induced by different Chlamydia species leading to differential adaptive immunity and host resistance to the infections. J Immunol. 2007; 178:1048–1058. [PubMed: 17202368]

Kawakami K, Kinjo Y, Yara S, et al. Activation of Valpha14(+) natural killer T cells by alpha-galactosylceramide results in development of Th1 response and local host resistance in mice infected with Cryptococcus neoformans. Infect Immun. 2001; 69:213–220. DOI: 10.1128/IAI.69.1.213-220.2001 [PubMed: 11119508]

Kawakami K, Yamamoto N, Kinjo Y, et al. Critical role of Valpha14+ natural killer T cells in the innate phase of host protection against Streptococcus pneumoniae infection. European journal of immunology. 2003; 33:3322–3330. DOI: 10.1002/eji.200324254 [PubMed: 14635040]

Kawana K, Quayle AJ, Ficarra M, et al. CD1d Degradation in Chlamydia trachomatis-infected Epithelial Cells Is the Result of Both Cellular and Chlamydial Proteasomal Activity. The Journal of biological chemistry. 2006; 282:7368–7375. DOI: 10.1074/jbc.M610754200

Kawano T, Cui J, Koezuka Y, et al. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science (New York, NY. 1997; 278:1626–1629. DOI: 10.1126/science.278.5343.1626

Kelly-Quintos C, Cavacini LA, Posner MR, et al. Characterization of the opsonic and protective activity against Staphylococcus aureus of fully human monoclonal antibodies specific for the bacterial surface polysaccharide poly-N-acetylglucosamine. Infect Immun. 2006; 74:2742–2750. DOI: 10.1128/IAI.74.5.2742-2750.2006 [PubMed: 16622211]

Kinjo Y, Illarionov P, Vela JL, et al. Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. Nat Immunol. 2011; 12:966–974. DOI: 10.1038/ni.2096 [PubMed: 21892173]

Kinjo Y, Kitano N, Kronenberg M. The role of invariant natural killer T cells in microbial immunity. J Infect Chemother. 2013; 19:560–570. DOI: 10.1007/s10156-013-0638-1 [PubMed: 23846426]

Kinjo Y, Tupin E, Wu D, et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol. 2006; 7:978–986. DOI: 10.1038/ni1380 [PubMed: 16921381]

Kinjo Y, Wu D, Kim G, et al. Recognition of bacterial glycosphingolipids by natural killer T cells. Nature. 2005; 434:520–525. DOI: 10.1038/nature03407 [PubMed: 15791257]

Kirby AC, Yrlid U, Wick MJ. The innate immune response differs in primary and secondary Salmonella infection. J Immunol. 2002; 169:4450–4459. [PubMed: 12370380]

Ko SY, Ko HJ, Chang WS, et al. -Galactosylceramide Can Act As a Nasal Vaccine Adjuvant Inducing Protective Immune Responses against Viral Infection and Tumor. J Immunol. 2005; 175:3309–3317. DOI: 10.4049/jimmunol.175.5.3309 [PubMed: 16116223]

Kobayashi E, Motoki K, Uchida T, et al. KRN7000, a novel immunomodulator, and its antitumor activities. Oncol Res. 1995; 7:529–534. [PubMed: 8866665]

Koh AY, Priebe GP, Ray C, et al. Inescapable Need for Neutrophils as Mediators of Cellular Innate Immunity to Acute Pseudomonas aeruginosa Pneumonia. Infect Immun. 2009; 77:5300–5310. DOI: 10.1128/IAI.00501-09 [PubMed: 19805527]

Koseki H, Imai K, Nakayama F, et al. Homogenous junctional sequence of the V14+ T-cell antigen receptor alpha chain expanded in unprimed mice. Proc Natl Acad Sci USA. 1990; 87:5248–5252. DOI: 10.1073/pnas.87.14.5248 [PubMed: 2371269]

Kumar H, Belperron A, Barthold SW, Bockenstedt LK. Cutting Edge: CD1d Deficiency Impairs Murine Host Defense Against the Spirochete, Borrelia burgdorferi. J Immunol. 2000; 165:4797–4801. DOI: 10.4049/jimmunol.165.9.4797 [PubMed: 11046002]

Lantz O, Bendelac A. An invariant T cell receptor alpha chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4-8- T cells in mice and humans. The Journal of experimental medicine. 1994; 180:1097–1106. [PubMed: 7520467]

Lee W-Y, Moriarty TJ, Wong CHY, et al. An intravascular immune response to Borrelia burgdorferi involves Kupffer cells and iNKT cells. Nat Immunol. 2010; 11:295–302. DOI: 10.1038/ni.1855 [PubMed: 20228796]

Leung J, Siegel S, Jones JF, et al. Fatal varicella due to the vaccine-strain varicella-zoster virus. Hum Vaccin Immunother. 2014; 10:146–149. DOI: 10.4161/hv.26200 [PubMed: 23982221]

Li D, Xu X-N. NKT cells in HIV-1 infection. Cell Res. 2008; 18:817–822. DOI: 10.1038/cr.2008.85 [PubMed: 18645582]

Li Y, Girardi E, Wang J, et al. The Vα14 invariant natural killer T cell TCR forces microbial glycolipids and CD1d into a conserved binding mode. Journal of Experimental Medicine. 2010; 207:2383–2393. DOI: 10.1084/jem.20101335 [PubMed: 20921281]

Liu J, Glosson NL, Du W, et al. A Thr/Ser dual residue motif in the cytoplasmic tail of human CD1d is important for the down-regulation of antigen presentation following a herpes simplex virus 1 infection. Immunology. 2013; 140:191–201. DOI: 10.1111/imm.12127 [PubMed: 23710894]

Mallevaey T, Clarke AJ, Scott-Browne JP, et al. A molecular basis for NKT cell recognition of CD1d-self-antigen. Immunity. 2011; 34:315–326. DOI: 10.1016/j.immuni.2011.01.013 [PubMed: 21376640]

Mattner J, DeBord KL, Ismail N, et al. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature. 2005; 434:525–529. DOI: 10.1038/nature03408 [PubMed: 15791258]

Nagarajan NA, Kronenberg M. Invariant NKT Cells Amplify the Innate Immune Response to Lipopolysaccharide. 2007

Naidenko OV, Maher JK, Ernst WA, et al. Binding and antigen presentation of ceramide-containing glycolipids by soluble mouse and human CD1d molecules. The Journal of experimental medicine. 1999; 190:1069–1080. [PubMed: 10523605]

Nakamatsu M, Yamamoto N, Hatta M, et al. Role of interferon-gamma in Valpha14+ natural killer T cell-mediated host defense against Streptococcus pneumoniae infection in murine lungs. Microbes Infect. 2007; 9:364–374. DOI: 10.1016/j.micinf.2006.12.003 [PubMed: 17314060]

Nieuwenhuis EES, Matsumoto T, Exley M, et al. CD1d-dependent macrophage-mediated clearance of Pseudomonas aeruginosa from lung. Nat Med. 2002; 8:588–593. DOI: 10.1038/nm0602-588 [PubMed: 12042809]

Olson CM, Bates TC, Izadi H, et al. Local production of IFN-gamma by invariant NKT cells modulates acute Lyme carditis. J Immunol. 2009; 182:3728–3734. DOI: 10.4049/jimmunol. 0804111 [PubMed: 19265151]

Oosting M, Buffen K, van der Meer JWM, et al. Innate immunity networks during infection with Borrelia burgdorferi. Crit Rev Microbiol. 2016; 42:233–244. DOI: 10.3109/1040841X. 2014.929563 [PubMed: 24963691]

Parekh VV, Wilson MT, Olivares-Villagómez D, et al. Glycolipid antigen induces long-term natural killer T cell anergy in mice. The Journal of clinical investigation. 2005; 115:2572–2583. DOI: 10.1172/JCI24762 [PubMed: 16138194]

Porcelli S, Yockey CE, Brenner MB, Balk SB. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. Journal of Experimental Medicine. 1993; 178:1–16. DOI: 10.1084/jem.178.1.1 [PubMed: 8391057]

Raftery MJ, Winau F, Kaufmann SHE, et al. CD1 Antigen Presentation by Human Dendritic Cells as a Target for Herpes Simplex Virus Immune Evasion. J Immunol. 2006; 177:6207–6214. DOI: 10.4049/jimmunol.177.9.6207 [PubMed: 17056550]

Rothchild AC, Jayaraman P, Nunes-Alves C, Behar SM. iNKT cell production of GM-CSF controls Mycobacterium tuberculosis. PLoS Pathog. 2014; 10:e1003805.doi: 10.1371/journal.ppat.1003805 [PubMed: 24391492]

Sada-Ovalle I, Chiba A, Gonzales A, et al. Innate Invariant NKT Cells Recognize Mycobacterium tuberculosis– Infected Macrophages, Produce Interferon-γ, and Kill Intracellular Bacteria. PLoS Pathog. 2008; 4:e1000239.doi: 10.1371/journal.ppat.1000239 [PubMed: 19079582]

Sag D, Krause P, Hedrick CC, et al. IL-10-producing NKT10 cells are a distinct regulatory invariant NKT cell subset. The Journal of clinical investigation. 2014; 124:3725–3740. DOI: 10.1172/JCI72308 [PubMed: 25061873]

Santodomingo-Garzon T, Swain MG. Role of NKT cells in autoimmune liver disease. Autoimmunity Reviews. 2011; 10:793–800. DOI: 10.1016/j.autrev.2011.06.003 [PubMed: 21740985]

Sharma SK, Mitra DK, Balamurugan A, et al. Cytokine Polarization in Miliary and Pleural Tuberculosis. Journal of Clinical Immunology. 2002; 22:345–352. DOI: 10.1023/A: 1020604331886 [PubMed: 12462334]

Singh N, Hong S, Scherer DC, et al. Cutting Edge: Activation of NK T Cells by CD1d and α-Galactosylceramide Directs Conventional T Cells to the Acquisition of a Th2 Phenotype. 1999

Smiley ST, Kaplan MH, Grusby MJ. Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. Science (New York, NY. 1997; 275:977–979.

Sudhanshu Shekhar AGJXY. Dynamics of NKT-Cell Responses to Chlamydial Infection. Front Immunol. 2015; doi: 10.3389/fimmu.2015.00233

Tang Y-W, Meng S, Li H, et al. PCR Enhances acid-fast bacillus stain-based rapid detection of Mycobacterium tuberculosis. J Clin Microbiol. 2004; 42:1849–1850. [PubMed: 15071068]

Tupin E, Benhnia MR-E-I, Kinjo Y, et al. NKT cells prevent chronic joint inflammation after infection with Borrelia burgdorferi. Proc Natl Acad Sci USA. 2008; 105:19863–19868. DOI: 10.1073/pnas. 0810519105 [PubMed: 19060201]

Tyznik AJ, Tupin E, Nagarajan NA, et al. Cutting Edge: The Mechanism of Invariant NKT Cell Responses to Viral Danger Signals. J Immunol. 2008; 181:4452–4456. DOI: 10.4049/jimmunol. 181.7.4452 [PubMed: 18802047]

Tyznik AJ, Verma S, Wang Q, et al. Distinct requirements for activation of NKT and NK cells during viral infection. J Immunol. 2014; 192:3676–3685. DOI: 10.4049/jimmunol.1300837 [PubMed: 24634489]

Van Kaer L, Parekh VV, Wu L. The Response of CD1d-Restricted Invariant NKT Cells to Microbial Pathogens and Their Products. Front Immunol. 2015; 6:226.doi: 10.3389/fimmu.2015.00226 [PubMed: 26029211]

Wang J, Li Y, Kinjo Y, et al. Lipid binding orientation within CD1d affects recognition of Borrelia burgorferi antigens by NKT cells. Proc Natl Acad Sci USA. 2010; 107:1535–1540. DOI: 10.1073/pnas.0909479107 [PubMed: 20080535]

Wang X, Bishop KA, Hegde S, et al. Human invariant natural killer T cells acquire transient innate responsiveness via histone H4 acetylation induced by weak TCR stimulation. Journal of Experimental Medicine. 2012; 209:987–1000. DOI: 10.1084/jem.20111024 [PubMed: 22508835]

Wesley JD, Tessmer MS, Chaukos D, Brossay L. NK Cell–Like Behavior of Vα14i NK T Cells during MCMV Infection. PLoS Pathog. 2008; 4:e1000106.doi: 10.1371/journal.ppat.1000106 [PubMed: 18636102]

Wieland Brown LC, Penaranda C, Kashyap PC, et al. Production of α-galactosylceramide by a prominent member of the human gut microbiota. PLoS Biol. 2013; 11:e1001610.doi: 10.1371/journal.pbio.1001610 [PubMed: 23874157]

Wu D, Xing GW, Poles MA, et al. Bacterial glycolipids and analogs as antigens for CD1d-restricted NKT cells. Proc Natl Acad Sci USA. 2005; 102:1351–1356. DOI: 10.1073/pnas.0408696102 [PubMed: 15665086]

Wu L, Kaer L. Natural Killer T Cells and Autoimmune Disease. CMM. 2009; 9:4–14. DOI: 10.2174/156652409787314534

Yu KOA, Im JS, Molano A, et al. Modulation of CD1d-restricted NKT cell responses by using N-acyl variants of -galactosylceramides. Proc Natl Acad Sci USA. 2005; 102:3383–3388. DOI: 10.1073/pnas.0407488102 [PubMed: 15722411]

Yuan W, Dasgupta A, Cresswell P. Herpes simplex virus evades natural killer T cell recognition by suppressing CD1d recycling. Nat Immunol. 2006; 7:835–842. DOI: 10.1038/ni1364 [PubMed: 16845396]

Zajonc DM, Ainge GD, Painter GF, et al. Structural Characterization of Mycobacterial Phosphatidylinositol Mannoside Binding to Mouse CD1d. J Immunol. 2006; 177:4577–4583. DOI: 10.4049/jimmunol.177.7.4577 [PubMed: 16982895]

Zajonc DM, Girardi E. Recognition of Microbial Glycolipids by Natural Killer T Cells. Front Immunol. 2015; 6:400.doi: 10.3389/fimmu.2015.00400 [PubMed: 26300885]

Table 1

Characteristics of bacteria known to activate *i*NKT cells

BACTERIA SPECIES	GENERAL CHARACTERISTICS	PRIMARY INFECTION SITE	ASSOCIATED DISEASES	CITED PROTECTION MECHANISMS	*iNKT CELL ACTIVATION MECHANISMS	REFERENCES
Pseudomonas aeruginosa	Gram negative, aerobic, mobilized with flagella	Lungs, kidneys, eyes	Pneumonia, keratitis, urinary tract infections, bacteremia, inflammation, sepsis	Alveolar macrophage-secreted chemokines and cytokines, neutrophils, *i*NKT cells	CD1d-dependent, unknown antigen plus IL-12	(Nieuwenhuis et al. 2002; Koh et al. 2009; Benoit et al. 2015)
Mycobacterium tuberculosis	Impervious to gram staining, intracellular (mononuclear phagocytes), rod-shaped	Lungs	Tuberculosis	Th1-polarized T cell responses (IFN-γ), GM-CSF production, *i*NKT cells	CD1d-dependent, self antigen plus IL-12	(Sharma et al. 2002; Chackerian et al. 2002; Tang et al. 2004; Sada-Ovalle et al. 2008; Cooper 2009; Brigl et al., 2011; Rothchild et al. 2014)
Borrelia burgdorferi	Double membrane capsule, spirochete	Blood, liver, joints, nervous system, heart	Lyme disease	Th1 adaptive immune responses, *i*NKT cells	CD1d-dependent, foreign antigen	(Kumar et al. 2000; Kinjo et al. 2006; Tupin et al. 2008; Olson et al. 2009; Lee et al. 2010; Oosting et al. 2016)
Streptococcus pneumoniae	Gram positive, α-hemolytic, facultative anaerobic	Lungs	Pneumonia, meningitis	Alveolar macrophages, neutrophils, antibodies, *i*NKT cells	CD1d-dependent, foreign or self antigen plus IL-12	(AlonsoDeVelasco et al. 1995; Kawakami et al. 2003; Nakamatsu et al. 2007; Brigl et al. 2011; Kinjo et al. 2011; Bai et al. 2013; Holzapfel et al. 2014)
Chlamydia pneumoniae	Gram negative, obligate intracellular	Lungs	Pneumonia	CD8+ T cells, Th17/IL-17 with Th1, *i*NKT cells	Unknown antigen plus IL-12	(Joyee et al. 2007; Sudhanshu Shekhar 2015)
Chlamydia muridarum	Gram negative, obligate intracellular	Lungs, genital tract	Pharyngitis, bronchitis, pneumonitis	CD4+ T cells, *i*NKT cells	CD1d-dependent, foreign antigen	(Bilenki et al. 2005; Jiang et al. 2012; Sudhanshu Shekhar 2015)
Salmonella typhimurium	Gram negative, intracellular	Intestinal lumen, mesenteric lymph node, liver, spleen	Gastroenteritis	Macrophages, neutrophils, IFN-γ, IL-4, *i*NKT cells	CD1d-dependent, self antigen plus IL-12 or cytokine alone	(Kirby et al. 2002; Brigl and Brenner 2004; Berntman et al. 2005; Hazlett et al. 2007; Holzapfel et al. 2014)
Sphingomonas paucimobilis	Gram negative, aerobic	Highly variable	Nosocomial infections, bacteremia, pneumonia	None cited	CD1d-dependent, foreign or self antigen plus IL-12	(Hsueh et al. 1998; Wu et al. 2005; Kinjo et al. 2005; Mattner et al. 2005; Hazlett et al. 2007; Holzapfel et al. 2014)
Staphylococcus aureus	Gram positive, facultative anaerobic	Nose, respiratory tract, skin	Abscesses, bacteremia, sepsis, pneumonia, meningitis	Neutrophils, Antibodies, *i*NKT cells	CD1d-dependent, unknown antigen plus IL-12, IL-1, and IL-23	(Brigl and Brenner 2004; Kelly-Quintos et al. 2006; Doisne et al. 2011)
Francisella tularensis	Gram negative, facultative intracellular	Lungs	Pulmonary tularemia, sepsis	Macrophages, AIM2 inflammasome, neutrophils	CD1d-dependent, unknown antigen	(Fernandes-Alnemri et al. 2010; Hill et al. 2015)

*
Foreign or self-antigen means that there is evidence in different papers for one type of antigenic stimulus or the other. CD1d dependent, unknown antigen means there is evidence for a role for CD1d, but there is not published evidence for the involvement of a self or foreign antigen.