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REVIEW

From the Deep Sea to Everywhere: Environmental Antigens for *i*NKT Cells

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Abstract Invariant natural killer T (*i*NKT) cells are a unique subset of innate T cells that share features with innate NK cells and adaptive memory T cells. The first *i*NKT cell antigen described was found 1993 in a marine sponge and it took over 10 years for other, bacterial antigens to be described. Given the paucity of known bacterial *i*NKT cell antigens, it appeared as if *i*NKT cells play a very specialist role in the protection against few, rare and unusual pathogenic bacteria. However, in the last few years several publications painted a very different picture, suggesting that antigens for *i*NKT cells are found almost ubiquitous in the environment. These environmental *i*NKT cell antigens can shape the distribution, phenotype and function of *i*NKT cells. Here, these recent findings will be reviewed and their implications for the field will be outlined.

Keywords Innate T cells · Invariant natural killer T (*i*NKT) cells · Environmental antigens · Mucosal immunology

Abbreviations

αGalCer	α-Galactosylceramide
EoE	Eosinophilic esophagitis
HDE	House dust extract
MHC	Major histocompatibility complex
TCR	T cell receptor
Th	T helper type
Vα14*i*	Invariant Vα14–Jα18 TCR rearrangement
Vα24*i*	Invariant Vα24–Jα18 TCR rearrangement

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Introduction

Invariant natural killer T (*i*NKT) cells are innate T cells that share features with innate NK cells and adaptive memory T cells (Bendelac et al. 2007; Brennan et al. 2013; Kronenberg 2005; Rossjohn et al. 2012; Salio et al. 2014; Wingender and Kronenberg 2008). They are characterized by three main features: first, *i*NKT cells utilize a semi-invariant T cell receptor (TCR) composed of a canonical Vα14–Jα18 TCR rearrangement (Vα14*i*) in mice and an orthologous Vα24–Jα18 TCR chain (Vα24*i*) in humans. This invariant TCRα—chain pairs with a limited set of TCRβ—chains, largely Vβ8, Vβ7 and Vβ2 in mice and Vβ11 in humans, although these have highly diverse rearrangements to Jβ segments. Second, *i*NKT cells are selected during development by CD1d, a non-polymorphic homolog of the MHC class I antigen-presenting molecules. Third, CD1d binds and presents glycolipids, especially glycosphingolipid structures, for recognition to *i*NKT cells. This recognition of glycolipids distinguishes *i*NKT cells from conventional T lymphocytes, which recognize peptide antigens. There is a surprising degree of interspecies cross-reactivity, with mouse Vα14*i* NKT cells recognizing human CD1d and vice versa (Brossay et al. 1998; Brossay and Kronenberg 1999). This conservation over 50 million years of evolution suggests an important function for these cells in the mammalian immune system.

Even as they differentiate in the thymus, *i*NKT cells begin to express a pattern of surface markers ($CD69^{+}$, $CD44^{high}$, $CD11a^{high}$, $CD62L^{low}$, $CD122^{+}$) typically associated with

activated or memory T cells (Engel and Kronenberg 2012). Additionally, *i*NKT cells constitutively express detectable mRNA for cytokines including interleukin (IL)-4 and interferon (IFN)-γ (Matsuda et al. 2003; Stetson et al. 2003), and these transcripts increase dramatically following activation. In line with their effector/memory phenotype, *i*NKT cells swiftly exert effector functions such as cytokine production and cytotoxicity (Wingender et al. 2010). Following TCR stimulation, they rapidly produce copious amounts of various cytokines including Th1 cytokines, like IFN-γ and tumor necrosis factor; Th2 cytokines, like IL-4 and IL-13; and Th17 cytokines (Bendelac et al. 2007; Brennan et al. 2013; Kronenberg 2005; Salio et al. 2014; Wingender and Kronenberg 2008). Furthermore, *i*NKT cell subsets specialized in T follicular helper-like functions (Chang et al. 2011a; King et al. 2011; Tonti et al. 2012) or IL-10 dependent regulatory functions (Lynch et al. 2015; Sag et al. 2014) have been described. This rapid production of cytokines is more similar to innate immune cells or to memory T cell response, as naïve conventional T cells would take days of stimulation to produce these cytokines. Due to their cytokine production, *i*NKT cells can have a pronounced effect on the immune system, impacting a dazzling variety of different immune reactions. They are involved in a range of chronic and acute inflammatory processes including responses to pathogens (Kinjo and Kronenberg 2009) and tumors (O'Konek et al. 2012), as well as in allergic (Berzins and Ritchie 2014; DeKruyff et al. 2014) and autoimmune responses (Simoni et al. 2013; Wingender and Kronenberg 2014).

The Characterization of *i*NKT Cell Antigens

The first antigen for *i*NKT cells was described in 1995. It was originally purified from the deep sea sponge *Agelas Mauritanius* and was recognized due to its strong anti-tumor properties in mice (Morita et al. 1995). An active compound was subsequently synthesized and chemically optimized to obtain α-galactosylceramide (αGalCer, KRN7000) (Kawano et al. 1997). αGalCer contains an α-anomeric linkage of a galactose sugar to the ceramide lipid and this α-linkage is crucial for its stimulatory capacity (Kawano et al. 1997; Morita et al. 1995). In contrast, the overwhelming majority of mammalian glycolipids utilize a β-linkage of the sugar (Bendelac et al. 2007; Brennan et al. 2013; Kronenberg 2005; Rossjohn et al. 2012), although recently α-linked *i*NKT cell antigens of mammalian origin were also discovered (Kain et al. 2014). αGalCer binds to CD1d via its two lipid chains, exposing the α-linked sugar group for recognition by the invariant TCR. Due to its potent agonistic activity, αGalCer has been tested in clinical trials for the treatment of tumor patients (Fujii et al. 2013). Despite limiting success with free αGalCer itself, novel αGalCer analogs that are more potent for human *i*NKT cells, and different delivery routes, are under continuing development as therapeutic agents (Fujii et al. 2013; Singh et al. 2014).

Right from the beginning it seemed doubtful that evolution retained *i*NKT cells to protect mice and humans against marine sponges. Due to the important role of *i*NKT cells in anti-bacterial responses, especially in the early phases of infection, the prevalent hypothesis in the field was that *i*NKT cells recognize CD1d-bound glycolipid structures derived from pathogenic bacteria. Indeed, between 2005 and 2011 antigens for the majority of *i*NKT cells were described in several pathogenic bacteria.

The first bacterial antigens described that could stimulate the majority of *i*NKT cells in a CD1d-dependent manner was found in *Sphingobium* bacteria (Kinjo et al. 2005; Mattner et al. 2005; Sriram et al. 2005). *Sphingobium yanoikuyae* bacteria are aerobic, Gram-negative α-Proteobacteria that lack lipopolysaccharide (LPS) (Kinjo and Kronenberg 2009). Although not highly virulent, *Sphingobium* bacteria can cause infections, especially in immuno-compromised patients (Hsueh et al. 1998; Perola et al. 2002). Another α-Proteobacteria, *Ehrlichia muris*, a severe pathogen in mice and humans (Dumler 2005), could also elicit a CD1d-dependent cytokine response by *i*NKT cells (Mattner et al. 2005), although the antigenic glycolipid was not identified. *Borrelia burgdorferi*, the causative agent of Lyme disease, is a spirochete that also lacks LPS, and *i*NKT cells are activated in vivo after *B. burgdorferi* infection (Tupin et al. 2008). These activated *i*NKT cells play a role, depending on the strain background, in preventing arthritis (Tupin et al. 2008) or carditis (Olson et al. 2009). Major *B. burgdorferi* glycolipids have been purified (Ben-Menachem et al. 2003) and can be recognized by *i*NKT cells (Kinjo et al. 2006). Interestingly, this *i*NKT cell antigen is not a glycosphingolipid, but rather a monogalactosyl diacylglycerol (Kinjo et al. 2006). One of the most striking examples of a non-redundant immune function of *i*NKT cells is the response of mice to *Streptococcus pneumonia*. *Streptococcus pneumonia*, a Gram-negative extracellular microbe, is a leading cause of pneumonia and childhood disease with an estimated 1.6 million deaths annually worldwide. Mice deficient for *i*NKT cells are highly susceptible to infection with *S. pneumoniae*, with greatly increased mortality and four logs increased lung colony counts over wild type mice 3 days after infection (Kawakami et al. 2003). *Streptococcus pneumoniae* as well as group B Streptococcus have abundant diacylglycerol-based glycolipid antigens and activate *i*NKT cells in a CD1d- and TCR-dependent fashion to secrete cytokines within 6 h of infection (Girardi et al. 2011; Kinjo et al. 2011). Additionally, the presence of some unspecified

*i*NKT cell antigen in pathogenic bacteria has also been proposed for *Leishmania donovani* (Amprey et al. 2004; Karmakar et al. 2011) and *Chlamydia muridarum* (Jiang et al. 2012; Peng et al. 2012).

The Discovery of Environmental *i*NKT Cell Antigens

Given the scarcity of bacteria with known *i*NKT cell antigens, it appeared as if *i*NKT cells play a very specialist role in the protection against few, rare and unusual pathogenic bacteria. However, in the last few years several publications painted a completely different picture, indicating that antigens for *i*NKT cells are almost ubiquitous in the environment and that these have a major impact on the development and function of *i*NKT cells.

The first demonstration for this was our finding that the majority of sterile house dust extracts (HDEs) contained antigens to stimulate mouse Vα14*i* as well as human Vα24*i* NKT cells (Wingender et al. 2011). HDEs are simple aqueous preparations from house dust that provided a relative complete sampling of the environment without the need for a priori assumptions imposed by the nature of the purification (Horner 2010). HDEs displayed adjuvant-like properties in an allergen-induced mouse asthma model that were dependent on Vα14*i* NKT cells (Wingender et al. 2011). Interestingly, the simultaneous stimulation of *i*NKT cells in the lung via HDE and of conventional T cells via the allergen (ovalbumin) led to a mutual augmentation of the cytokine response of both *i*NKT and T cells, strongly exacerbating the airway inflammation (Wingender et al. 2011). The finding of *i*NKT cell antigens in house dust was completely novel and established that antigens for *i*NKT cells are nearly ubiquitous indoors. The antigenic component in the HDEs was not established as our data suggested a large variability. When different HDEs were tested with different Vα14*i* NKT hybridomas and Vα24*i* NKT cell lines, a large heterogeneity in the respective sensitivity of the cells was noticed (Wingender et al. 2011). This and preliminary enzymatic and chemical analysis suggested that different HDEs could contain several distinct *i*NKT cell antigens. However, experiments with dust mite extracts indicated that dust mites are likely not the source of the antigenic activity found in HDE (Wingender et al. 2011).

In hindsight, the discovery of *i*NKT cell antigens in *Sphingobium* bacteria (Kinjo et al. 2005; Mattner et al. 2005; Sriram et al. 2005) hinted already to the presence of *i*NKT cell antigens in the environment. *Sphingobium* bacteria are distributed in soil, in association with plants, and in seawater (Laskin and White 1999; Neef et al. 1999; White et al. 1996). Therefore, they might be found in the marine sponges originally used to isolate the closely related αGalCer. Furthermore, there is evidence that *Sphingobium* species are a small, but normal constituent of the mouse and human intestine (Ivanov et al. 2009; Selmi et al. 2003). One member of this species, *Novosphingobium aromaticivorans,* has been linked to *i*NKT cell-dependent autoimmune responses against the bile duct in mice (Mattner et al. 2008) and humans (Olafsson et al. 2004; Selmi et al. 2003).

Environmental *i*NKT Cell Antigens in the Intestine

Indeed, the commensal microbiota appears to be an ample source for *i*NKT cell antigens. When we (Wei et al. 2010; Wingender et al. 2012) and others (Olszak et al. 2012) analyzed *i*NKT cells from germ-free animals, it was noted that *i*NKT cells were hypo-responsive and enriched in mucosal tissues. *i*NKT cells from spleen and liver of germ-free animals expressed lower levels of activation markers, produced lower amounts of cytokines following antigenic stimulation and were less cytotoxic active (Wingender et al. 2012). Importantly, intragastric exposure of germ-free mice with a bacterium that contains *i*NKT cell antigens (*S. yanoikuyae*) fully established phenotypic maturity of *i*NKT cells. In contrast, reconstitution with *E. coli*, which lack specific *i*NKT cell antigens, did not affect the phenotype of *i*NKT cells (Wingender et al. 2012).

Although the frequency of *i*NKT cells in germ-free animals was lower in spleen and liver compared to control mice (Olszak et al. 2012; Wei et al. 2010), it was increased in the small and large intestine (lamina propria lymphocytes and intraepithelial lymphocytes) (Wingender et al. 2012) and the colon (Olszak et al. 2012). Once this increased frequency of *i*NKT cells in the intestine was established early in life, it remained high and also the introduction of the normal microbiota at 5 weeks of age did not change the frequency in the mice (Olszak et al. 2012). Importantly, the increased frequency of *i*NKT cells in the intestine also had functional consequences for intestinal immune responses. Ozazolone-induced colitis is a mouse model of ulcerative colitis for which *i*NKT cells are known to be pathogenic due to their IL-13 production (Heller et al. 2002). Olszak et al. (2012) demonstrated that germ-free animals were more sensitive to Ozazolone-induced colitis. The authors interpreted these data in a way that the augmented disease was the consequence of an increased reactivity of individual *i*NKT cells in the intestine. This implied that exposure of germ-free *i*NKT cells to the bacterial microbiota after a critical early developmental stage not just lead to full functional maturation, but beyond that it actually induced a hyper-responsive state in the *i*NKT cells. However, no data on the cytokine response of

intestinal *i*NKT cells as a whole or on a single-cell level were provided by the authors (Olszak et al. 2012). In our hands, peripheral *i*NKT cells from germ-free animals were hypo-responsive and produced less cytokines after antigenic activation (Wingender et al. 2012). However, these hypo-responsive *i*NKT cells found in germ-free mice readily normalize their cytokine response when exposed to bacterial flora even in the adult stage, but this response was comparable and did not exceed the one observed to control animals (Wingender et al. 2012). Yet, this analysis was done only for the responses of hepatic and splenic *i*NKT cells and not for the intestine, where things could be different. Another point to consider is that the observation that mice without bacterial commensals in the first weeks of life have more *i*NKT cells in the mucosa does not in itself imply that *i*NKT cells are responding differently before and after this time cut-off. It is well established that once they migrated into the peripheral tissue, *i*NKT cells hardly migrate any longer and remain tissue resident (Lynch et al. 2015; Thomas et al. 2011). This would suggest that once *i*NKT cells migrate into the mucosa, they remain there irrespective of the presence of commensals. The idea that a particular sensitive phase early in development can shape the nature of *i*NKT cells seemed attractive to propose an *i*NKT cell specific hygiene-hypothesis. However, a more parsimonious explanation of the data (Olszak et al. 2012) would be that the increased amount of effector cells augmented the response. More *i*NKT cells were present in the mucosa of germ-free animals at the onset of the disease and therefore more cells could act as driver of the inflammatory response.

Interestingly, not only the microbiota per se, as shown in the germ-free animals, can affect *i*NKT cells, but also the composition of the microbiota. As we noticed that oral exposure of wild type mice with *S. yanoikuyae* could stimulate *i*NKT cells in the liver and spleen (Wei et al. 2010), we compared wild type mice derived from different vendors that were known to differ significantly in their gut flora (Ivanov et al. 2009). Indeed, *i*NKT cells isolated from these mice differed in their distribution in vivo, their phenotype and TCR Vβ7 frequency, and their antigen-specific cytokine response (Wingender et al. 2012). These differences were dependent on the environment and demonstrated that changes in the composition of the microbiota can significantly alter *i*NKT cell phenotype and function. Similarly, it was reported subsequently that some probiotic bacteria can alter the frequency of *i*NKT cells in the mouse liver and that lipids extracted from these probiotics could stimulate *i*NKT hybridomas (Liang et al. 2014).

Importantly, in recent years specific *i*NKT cell antigens were also characterized in two bacteria commonly found in the human intestine. (1) *Helicobacter pylori* is a gastric pathogen that infects an estimated 50 % of the world population and is linked to gastric ulcers and stomach cancer (Lin and Koskella 2015). About 25 % of *H. pylori's* lipids are cholesteryl α-glucosides (Hirai et al. 1995) that contain several related antigens for *i*NKT cells (Chang et al. 2011b; Ito et al. 2013). Importantly, in the mouse model, *i*NKT cells recognized *H. pylori* antigens in situ. Moreover, stomach infection of *i*NKT cell deficient animals led to five times higher amounts of recovered bacteria and to a lower cytokine response (Ito et al. 2013). (2) *Bacteroides fragilis* is a common intestinal symbiont present in basically all humans (Mazmanian and Kasper 2006). In 2013 it was reported that *B. fragilis* contains α-linked galactosylceramides reminiscent of αGalCer, that could stimulate *i*NKT cells in vitro (mouse, human) and in vivo (mouse) (Wieland Brown et al. 2013). Surprisingly, a subsequent study described that *B. fragilis* derived glycolipids could bind CD1d, but were not stimulatory (An et al. 2014).

Further Aspects of Environmental *i*NKT Cell Antigens

Interestingly, Olszak et al. (2012) also noted that the frequency of *i*NKT cells in germ-free animals was not only increased in the intestinal mucosa, but also in the lung. Similar to the intestine, the increased *i*NKT cell numbers in the lung of germ-free animals remain elevated during adult life and could thereby aggravate airway inflammation responses (Olszak et al. 2012). In the context of airway inflammations, it is of interest that patients with poorly controlled asthma have a higher bacterial load in the lung and some of the taxa present are known to bear *i*NKT cell antigens (e.g. *Sphingobium* spp) (Huang et al. 2011). Furthermore, it was reported that lipid extracts from cypress grains, a common pollen allergen involved in allergic asthma, could stimulate human T cell clones in a CD1d-dependent manner (Agea et al. 2005). However, only one of 27 T cell clones recovered by the authors was $V\alpha24^+$ $V\beta11^+$ (Agea et al. 2005). Given that some $V\alpha24^+$ $V\beta11^+$ are not *i*NKT cells (Exley et al. 2008; Montoya et al. 2007), it is unclear at this time whether *i*NKT cells can respond to pollen derived lipids. Another study implicated recognition of plant lipids by *i*NKT cells (Mirotti et al. 2013), but this recognition was not formally demonstrated.

Although the majority of environmental *i*NKT cell antigens described in recent years are derived from bacteria commensals, the latter studies suggested that *i*NKT cell antigens are not necessarily limited to bacteria. Indeed, some *i*NKT cell antigens have been detected in fungi, protozoa and in milk. *Aspergillus fumigatus* is an ubiquitous fungi, found for example in soil and buildings, that is

commonly associated with allergic sensitization and asthma in humans. *A. fumigatus* expresses asperamide B that could directly stimulate Vα14*i* and Vα24*i* NKT cells to produce the cytokines IFN-γ and IL-4 (Albacker et al. 2013; Godfrey et al. 2013). Furthermore, the intestinal protozoan parasite *Entamoeba histolytica* is the causative agent of human amoebiasis and it was suggested that purified lipopeptidophosphoglycans from its cell wall contain *i*NKT cell antigens (Lotter et al. 2009). Finally, human and cow milk was reported to contain lipids that could act as *i*NKT cell antigens, either sphingomyelin (cow) (Jyonouchi et al. 2011) or α-anomeric glucosylceramide (human, cow) (Brennan et al. 2014). Surprisingly, latter compound was also detected in bovine serum (Brennan et al. 2014) and could therefore be a potential contaminant in fetal calf serum, which is widely used for in vitro cultures. Interestingly, it has been suggested that milk lipids could also be the driving force in another *i*NKT cell dependent allergic inflammation (Jyonouchi et al. 2014). Eosinophilic esophagitis (EoE) is a chronic allergic inflammation of the esophagus (Wechsler and Bryce 2014), and mouse models demonstrated that *i*NKT cells and their Th2 cytokines are required for the disease (Dutt et al. 2015; Rajavelu et al. 2012; Rayapudi et al. 2014). In EoE patients, *i*NKT cell numbers and Th2 cytokines are increased in the esophagus (Jyonouchi et al. 2014; Lexmond et al. 2014; Rayapudi et al. 2014) and normalize following dietary removal of the allergen (Lexmond et al. 2014). Mucosal sensitization to various dietary antigens have been implicated in the etiology of EoE, and milk plays a major role (Wechsler and Bryce 2014). The discovery of *i*NKT cell antigens in milk (Brennan et al. 2014; Jyonouchi et al. 2011) suggests that such lipids could act as adjuvant to the allergen, a situation similar to HDE in asthma (Wingender et al. 2011).

Outlook

Recent data indicate that environmental *i*NKT cell antigens are important in shaping the *i*NKT cell pool, its distribution and its potential to produce cytokines. Furthermore, the commensal microbiota appears as a major source for *i*NKT cell antigens. Appreciation of the strong environmental influence on *i*NKT cells is important to understand their function and role; and might explain some of the differences observed between mice and humans.

*i*NKT cells from human umbilical cord blood display an activated/memory like phenotype, already express cytokine mRNA and can produce cytokines rapidly after activation (Chan et al. 2013; de Lalla et al. 2008; Harner et al. 2011). However, whether these responses are indeed weaker than those observed by adult *i*NKT cells, as expected from the germ free mouse data, is not entirely clear (Chan et al. 2013; D'Andrea et al. 2000; de Lalla et al. 2008; Eger et al. 2006; Hagihara et al. 2002; Harner et al. 2011; Ueda et al. 2003; van der Vliet et al. 2000). Beyond the umbilical cord, little is known in regard to the function of intestinal *i*NKT cells in human neonates in particular. In neonates of 16–23 weeks, *i*NKT cells were more frequent in the small intestine than in the spleen or liver (Loh et al. 2014). Furthermore, intestinal *i*NKT cells were phenotypically more mature than cells in spleen or liver, and appeared more similar to *i*NKT cells from adult peripheral blood mononuclear cells (PBMCs) (Loh et al. 2014). Importantly, more neonatal *i*NKT cells in the intestine produced cytokines following stimulation than splenic or hepatic *i*NKT cells, and their response approached levels observed in adult PBMC *i*NKT cells (Loh et al. 2014). Altogether, these data appear on first sight to be in disagreement with the germ-free mouse data where *i*NKT cells were hyporesponsive. However, although the human fetus resides in a sterile environment, it is exposed to bacterial products (Mshvildadze et al. 2010; Rautava et al. 2012; Satokari et al. 2009). In line with this, we noticed that the hyporesponsive phenotype of *i*NKT cells from germ-free mice was less pronounced for mice obtained from one of the three independent germ-free facilities we worked with (unpublished result). This also suggests that gut derived substances, presumably bacterial products, can influence *i*NKT cells even in a sterile environment.

In summary, it became clear in recent years that antigens for *i*NKT cells are not limited to a few bacteria, but can be found almost ubiquitous in the environment. Exposure of *i*NKT cells at the mucosal surfaces to such specific antigens can shape the distribution, phenotype and function of *i*NKT cells. Although, this interaction has so far only been described for the gastro-intestinal tract and the lung, it seems likely that similar interactions might occur also in the skin. For example, bacteria phylogenetically related to *Bacteroides* and *Streptococcus* can be found in the vaginal area; and *B. fragilis* can cause bacterial vaginosis (Kenyon and Osbak 2014; Witkin and Ledger 2012). Furthermore, the effects of environmental antigens also suggest that the interaction between mucosal *i*NKT cells and the commensal microbiota is generally benign and mutualistic. Indeed, it has been shown that *i*NKT cells influence the composition of the intestinal microbiota (Nieuwenhuis et al. 2009). The appreciation of such a mutualistic interaction also suggests a role for *i*NKT cells in tissue homeostasis in mucosal tissues, supporting the health of the tissue and acting as an earlier sensor for tissue damage and bacterial translocation.

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References

Agea E, Russano A, Bistoni O et al (2005) Human CD1-restricted T cell recognition of lipids from pollens. J Exp Med 202:295–308

Albacker LA, Chaudhary V, Chang Y-J et al (2013) Invariant natural killer T cells recognize a fungal glycosphingolipid that can induce airway hyperreactivity. Nat Med 19:1297–1304

Amprey JL, Im JS, Turco SJ et al (2004) A subset of liver NK T cells is activated during Leishmania donovani infection by CD1d-bound lipophosphoglycan. J Exp Med 200:895–904

An D, Oh SF, Olszak T et al (2014) Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer t cells. Cell 156:123–133

Bendelac A, Savage PB, Teyton L (2007) The biology of NKT cells. Annu Rev Immunol 25:297–336

Ben-Menachem G, Kubler-Kielb J, Coxon B et al (2003) A newly discovered cholesteryl galactoside from Borrelia burgdorferi. Proc Natl Acad Sci USA 100:7913–7918

Berzins SP, Ritchie DS (2014) Natural killer T cells: drivers or passengers in preventing human disease? Nat Rev Immunol 14:640–646

Brennan PJ, Brigl M, Brenner MB (2013) Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. Nat Rev 13:101–117

Brennan PJ, Tatituri RVV, Heiss C et al (2014) Activation of iNKT cells by a distinct constituent of the endogenous glucosylceramide fraction. Proc Natl Acad Sci USA 111:13433–13438

Brossay L, Kronenberg M (1999) Highly conserved antigen-presenting function of CD1d molecules. Immunogenetics 50:146–151

Brossay L, Chioda M, Burdin N et al (1998) CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. J Exp Med 188:1521–1528

Chan AC, Leeansyah E, Cochrane A et al (2013) Ex-vivo analysis of human natural killer T cells demonstrates heterogeneity between tissues and within established CD4(+) and CD4(−) subsets. Clin Exp Immunol 172:129–137

Chang P-P, Barral P, Fitch J et al (2011a) Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses. Nat Immunol 13:35–43

Chang Y-J, Kim HY, Albacker LA et al (2011b) Influenza infection in suckling mice expands an NKT cell subset that protects against airway hyperreactivity. J Clin Invest 121:57–69

D'Andrea A, Goux D, De Lalla C et al (2000) Neonatal invariant Valpha24 + NKT lymphocytes are activated memory cells. Eur J Immunol 30:1544–1550

de Lalla C, Festuccia N, Albrecht I et al (2008) Innate-like effector differentiation of human invariant NKT cells driven by IL-7. J Immunol 180:4415–4424

DeKruyff RH, Yu S, Kim HY et al (2014) Innate immunity in the lung regulates the development of asthma. Immunol Rev 260:235–248

Dumler JS (2005) Anaplasma and Ehrlichia infection. Ann N Y Acad Sci 1063:361–373

Dutt P, Shukla JS, Ventateshaiah SU et al (2015) Allergen-induced interleukin-18 promotes experimental eosinophilic oesophagitis in mice. Immunol Cell Biol 93:849–857

Eger KA, Sundrud MS, Motsinger AA et al (2006) Human natural killer T cells are heterogeneous in their capacity to reprogram their effector functions. PLoS One 1:e50

Engel I, Kronenberg M (2012) Making memory at birth: understanding the differentiation of natural killer T cells. Curr Opin Immunol 24:184–190

Exley MA, Hou R, Shaulov A et al (2008) Selective activation, expansion, and monitoring of human iNKT cells with a monoclonal antibody specific for the TCR α-chain CDR3 loop. Eur J Immunol 38:1756–1766

Fujii S, Shimizu K, Okamoto Y et al (2013) NKT cells as an ideal anti-tumor immunotherapeutic. Front Immunol 4:409

Girardi E, Yu ED, Li Y et al (2011) Unique interplay between sugar and lipid in determining the antigenic potency of bacterial antigens for NKT cells. PLoS Biol 9:e1001189

Godfrey DI, Pellicci DG, Rossjohn J (2013) NKT cells: the smoking gun in fungal-induced asthma? Nat Med 19:1210–1211

Hagihara M, Gansuvd B, Ueda Y et al (2002) Killing activity of human umbilical cord blood-derived TCRValpha24(+) NKT cells against normal and malignant hematological cells in vitro: a comparative study with NK cells or OKT3 activated T lymphocytes or with adult peripheral blood NKT cells. Cancer Immunol Immunother 51:1–8

Harner S, Noessner E, Nadas K et al (2011) Cord blood Valpha24-vbeta11 natural killer T cells display a Th2-chemokine receptor profile and cytokine responses. PLoS One 6:e15714

Heller F, Fuss IJ, Nieuwenhuis EE et al (2002) Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity 17:629–638

Hirai Y, Haque M, Yoshida T et al (1995) Unique cholesteryl glucosides in Helicobacter pylori: composition and structural analysis. J Bacteriol 177:5327–5333

Horner AA (2010) Regulation of aeroallergen immunity by the innate immune system: laboratory evidence for a new paradigm. J Innate Immun 2:107–113

Hsueh PR, Teng LJ, Yang PC et al (1998) Nosocomial infections caused by Sphingomonas paucimobilis: clinical features and microbiological characteristics. Clin Infect Dis 26:676–681

Huang YJ, Nelson CE, Brodie EL et al (2011) Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol 127(372–381):e1–e3

Ito Y, Vela JL, Matsumura F et al (2013) Helicobacter pylori cholesteryl α-glucosides contribute to its pathogenicity and immune response by natural killer T cells. PLoS One 8:e78191

Ivanov II, Atarashi K, Manel N et al (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139:485–498

Jiang J, Karimi O, Ouburg S et al (2012) Interruption of CXCL13-CXCR5 axis increases upper genital tract pathology and activation of NKT cells following Chlamydial genital infection. PLoS One 7:e47487

Jyonouchi S, Abraham V, Orange JS et al (2011) Invariant natural killer T cells from children with versus without food allergy exhibit differential responsiveness to milk-derived sphingomyelin. J Allergy Clin Immunol 128(102–109):e13

Jyonouchi S, Smith CL, Saretta F et al (2014) Invariant natural killer T cells in children with eosinophilic esophagitis. Clin Exp Allergy 44:58–68

Kain L, Webb B, Anderson BL et al (2014) The Identification of the endogenous ligands of natural killer t cells reveals the presence of mammalian a-linked glycosylceramides. Immunity 41:543–554

Karmakar S, Paul J, De T (2011) Leishmania donovani glycosphingolipid facilitates antigen presentation by inducing relocation of CD1d into lipid rafts in infected macrophages. Eur J Immunol 41:1376–1387

Kawakami K, Yamamoto N, Kinjo Y et al (2003) Critical role of Valpha14 + natural killer T cells in the innate phase of host protection against Streptococcus pneumoniae infection. Eur J Immunol 33:3322–3330

Kawano T, Cui J, Koezuka Y et al (1997) CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science 278:1626–1629

Kenyon CR, Osbak K (2014) Recent progress in understanding the epidemiology of bacterial vaginosis. Curr Opin Obstet Gynecol 26:448–454

King IL, Fortier A, Tighe M et al (2011) Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner. Nat Immunol 13:44–50

Kinjo Y, Kronenberg M (2009) Detection of microbes by natural killer T cells. In: Schoenberger et al (eds) Crossroads between innate and adaptive immunity II. Springer, New York, pp 17–26

Kinjo Y, Wu D, Kim G et al (2005) Recognition of bacterial glycosphingolipids by natural killer T cells. Nature 434:520–525

Kinjo Y, Tupin E, Wu D et al (2006) Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol 7:978–986

Kinjo Y, Illarionov P, Vela JL et al (2011) Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. Nat Immunol 12:966–974

Kronenberg M (2005) Towards an understanding of NKT cell biology: progress and paradoxes. Annu Rev Immunol 23:877–900

Laskin AI, White DC (1999) Preface to special issue on Sphingomonas. J Ind Microbiol Biotechnol 23:231

Lexmond WS, Neves JF, Nurko S et al (2014) Involvement of the iNKT cell pathway is associated with early-onset eosinophilic esophagitis and response to allergen avoidance therapy. Am J Gastroenterol 109:646–657

Liang S, Webb T, Li Z (2014) Probiotic antigens stimulate hepatic natural killer T cells. Immunology 141:203–210

Lin D, Koskella B (2015) Friend and foe: factors influencing the movement of the bacterium Helicobacter pylori along the parasitism-mutualism continuum. Evol Appl 8:9–22

Loh L, Ivarsson MA, Michaelsson J et al (2014) Invariant natural killer T cells developing in the human fetus accumulate and mature in the small intestine. Mucosal Immunol 7:1233–1243

Lotter H, Gonzalez-Roldan N, Lindner B et al (2009) Natural killer T cells activated by a lipopeptidophosphoglycan from Entamoeba histolytica are critically important to control amebic liver abscess. PLoS Pathog 5:e1000434

Lynch L, Michelet X, Zhang S et al (2015) Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of Treg cells and macrophages in adipose tissue. Nat Immunol 16:85–95

Matsuda JL, Gapin L, Baron JL et al (2003) Mouse V alpha 14i natural killer T cells are resistant to cytokine polarization in vivo. Proc Natl Acad Sci USA 100:8395–8400

Mattner J, Debord KL, Ismail N et al (2005) Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature 434:525–529

Mattner J, Savage PB, Leung P et al (2008) Liver autoimmunity triggered by microbial activation of natural killer T cells. Cell Host Microbe 3:304–315

Mazmanian SK, Kasper DL (2006) The love–hate relationship between bacterial polysaccharides and the host immune system. Nat Rev Immunol 6:849–858

Mirotti L, Florsheim E, Rundqvist L et al (2013) Lipids are required for the development of Brazil nut allergy: the role of mouse and human iNKT cells. Allergy 68:74–83

Montoya CJ, Pollard D, Martinson J et al (2007) Characterization of human invariant natural killer T subsets in health and disease using a novel invariant natural killer T cell-clonotypic monoclonal antibody, 6B11. Immunology 122:1–14

Morita M, Motoki K, Akimoto K et al (1995) Structure-activity relationship of alpha-galactosylceramides against B16-bearing mice. J Med Chem 38:2176–2187

Mshvildadze M, Neu J, Shuster J et al (2010) Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. J Pediatr 156:20–25

Neef A, Witzenberger R, Kampfer P (1999) Detection of sphingomonads and in situ identification in activated sludge using 16S rRNA-targeted oligonucleotide probes. J Ind Microbiol Biotechnol 23:261–267

Nieuwenhuis EE, Matsumoto T, Lindenbergh D et al (2009) Cd1d-dependent regulation of bacterial colonization in the intestine of mice. J Clin Invest 119:1241–1250

O'Konek JJ, Berzofsky JA, Terabe M (2012) Immune regulation of tumor immunity by NKT cells. In: Terabe M, Berzofsky JA (eds) Natural killer T cells. Springer, New York, pp 55–70

Olafsson S, Gudjonsson H, Selmi C et al (2004) Antimitochondrial antibodies and reactivity to N. aromaticivorans proteins in icelandic patients with primary biliary cirrhosis and their relatives. Am J Gastroenterol 99:2143–2146

Olson CMJ, Bates TC, Izadi H et al (2009) Local production of IFN-gamma by invariant NKT cells modulates acute Lyme carditis. J Immunol 182:3728–3734

Olszak T, An D, Zeissig S et al (2012) Microbial exposure during early life has persistent effects on natural killer T cell function. Science 336:489–493

Peng Y, Zhao L, Shekhar S et al (2012) The glycolipid exoantigen derived from Chlamydia muridarum activates invariant natural killer T cells. Cell Mol Immunol 9:361–366

Perola O, Nousiainen T, Suomalainen S et al (2002) Recurrent Sphingomonas paucimobilis -bacteraemia associated with a multi-bacterial water-borne epidemic among neutropenic patients. J Hosp Infect 50:196–201

Rajavelu P, Rayapudi M, Moffitt M et al (2012) Significance of para-esophageal lymph nodes in food or aeroallergen-induced iNKT cell-mediated experimental eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol 302:G645–G654

Rautava S, Collado MC, Salminen S et al (2012) Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. Neonatology 102:178–184

Rayapudi M, Rajavelu P, Zhu X et al (2014) Invariant natural killer T-cell neutralization is a possible novel therapy for human eosinophilic esophagitis. Clin Transl Immunol 3:e9

Rossjohn J, Pellicci DG, Patel O et al (2012) Recognition of CD1d-restricted antigens by natural killer T cells. Nat Rev 12:845–857

Sag D, Krause P, Hedrick CC et al (2014) IL-10-producing NKT10 cells are a distinct regulatory invariant NKT cell subset. J Clin Invest 124:3725–3740

Salio M, Silk JD, Yvonne Jones E et al (2014) Biology of CD1- and MR1-restricted T cells. Annu Rev Immunol 32:323–366

Satokari R, Grönroos T, Laitinen K et al (2009) Bifidobacteriumand lactobacillusDNA in the human placenta. Lett Appl Microbiol 48:8–12

Selmi C, Balkwill DL, Invernizzi P et al (2003) Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. Hepatology 38:1250–1257

Simoni Y, Diana J, Ghazarian L et al (2013) Therapeutic manipulation of natural killer (NK) T cells in autoimmunity: are we close to reality? Clin Exp Immunol 171:8–19

Singh AK, Gaur P, Das SN (2014) Natural killer T cell anergy, co-stimulatory molecules and immunotherapeutic interventions. Hum Immunol 75:250–260

Sriram V, Du W, Gervay-Hague J et al (2005) Cell wall glycosphingolipids of Sphingomonas paucimobilis are CD1d-specific ligands for NKT cells. Eur J Immunol 35:1692–1701

Stetson DB, Mohrs M, Reinhardt RL et al (2003) Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. J Exp Med 198:1069–1076

Thomas SY, Scanlon ST, Griewank KG et al (2011) PLZF induces an intravascular surveillance program mediated by long-lived LFA-1-ICAM-1 interactions. J Exp Med 208:1179–1188

Tonti E, Fedeli M, Napolitano A et al (2012) Follicular helper NKT cells induce limited B cell responses and germinal center formation in the absence of CD4+ T cell help. J Immunol 188:3217–3222

Tupin E, Benhnia MR, Kinjo Y et al (2008) NKT cells prevent chronic joint inflammation after infection with Borrelia burgdorferi. Proc Natl Acad Sci USA 105:19863–19868

Ueda Y, Hagihara M, Gansuvd B et al (2003) The effects of alphaGalCer-induced TCRValpha24 Vbeta11(+) natural killer T cells on NK cell cytotoxicity in umbilical cord blood. Cancer Immunol Immunother 52:625–631

van der Vliet HJ, Nishi N, de Gruijl TD et al (2000) Human natural killer T cells acquire a memory-activated phenotype before birth. Blood 95:2440–2442

Wechsler JB, Bryce PJ (2014) Allergic mechanisms in eosinophilic esophagitis. Gastroenterol Clin North Am 43:281–296

Wei B, Wingender G, Fujiwara D et al (2010) Commensal microbiota and CD8+ T cells shape the formation of invariant NKT cells. J Immunol 184:1218–1226

White DC, Sutton SD, Ringelberg DB (1996) The genus Sphingomonas: physiology and ecology. Curr Opin Biotechnol 7:301–306

Wieland Brown LC, Penaranda C, Kashyap PC et al (2013) Production of α-galactosylceramide by a prominent member of the human gut microbiota. PLoS Biol 11:e1001610

Wingender G, Kronenberg M (2008) Role of NKT cells in the digestive system. IV. The role of canonical natural killer T cells in mucosal immunity and inflammation. Am J Physiol Gastrointest Liver Physiol 294:G1–G8

Wingender G, Kronenberg M (2014) The role of invariant natural killer T Cells in autoimmune diseases. In: Rose NR, Mackay IR (eds) The autoimmune diseases. Fifth Edition. Elsevier, pp 103–129

Wingender G, Krebs P, Beutler B et al (2010) Antigen-specific cytotoxicity by invariant NKT cells in vivo is CD95/CD178-dependent and is correlated with antigenic potency. J Immunol 185:2721–2729

Wingender G, Rogers P, Batzer G et al (2011) Invariant NKT cells are required for airway inflammation induced by environmental antigens. J Exp Med 208:1151–1162

Wingender G, Stepniak D, Krebs P et al (2012) Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. Gastroenterology 143:418–428

Witkin SS, Ledger WJ (2012) Complexities of the uniquely human vagina. Sci Transl Med 4:132fs11