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The Functions of Type I and Type II Natural Killer T (NKT) Cells in Inflammatory Bowel Diseases

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Abstract

CD1d-restricted natural killer T (NKT) cells are a distinct subset of T cells that rapidly produce an array of cytokines upon activation and play a critical role in regulating various immune responses. NKT cells are classified into two groups based on differences in T cell receptor (TCR) usage. Type I NKT cells have an invariant TCR α -chain and are readily detectable by α -galactosylceramide (α -GalCer)-loaded CD1d tetramers. Type II NKT cells have a more diverse TCR repertoire and cannot be directly identified. Both types of NKT cells as well as multiple CD1d-expressing cell types are present in the intestine and their interactions are likely to be modulated by pathogenic and commensal microbes, which in turn contribute to the intestinal immune responses in health and disease. Indeed, in several animal models of inflammatory bowel disease (IBD), Type I NKT cells have been shown to make both protective and pathogenic contributions to disease. In contrast, in human patients suffering from ulcerative colitis (UC), and a mouse model in which both CD1d expression and the frequency of Type II NKT cells are increased, Type II NKT cells appear to promote intestinal inflammation. In this review, we summarize present knowledge on the antigen recognition, activation and function of NKT cells with a particular focus on their role in IBD, and discuss factors that may influence the functional outcome of NKT cell responses in intestinal inflammation.

Keywords

NKT cells; CD1d; inflammatory bowel disease

CD1 and Natural Killer T (NKT) Cells

CD1 molecules present lipid antigens to T cells. The CD1 family consists of five proteins designated CD1a–e¹. A distinct lineage of T cells, the natural killer T (NKT) cells recognize lipid antigens presented by CD1d molecules. As the name implies, NKT cells share phenotypic and functional properties common to both conventional NK cells and T cells, including expression of NK1.1/CD161, Ly49 and NKG2 family members, and CD3 and the T cell receptor (TCR). In addition, NKT cells express activation markers such as CD44 and

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CD122 and as such are often described as having an effector or memory phenotype ². Consistent with this, stimulation of NKT cells elicits rapid effector functions. Investigations of the immunobiology of NKT cells, particularly over the past decade, have revealed a significant level of heterogeneity within this T cell lineage. The focus of this review is to summarize our current knowledge of the properties and functions of two major CD1d-restricted NKT cell subsets and their role in human and murine models of IBD. In addition, factors that influence the quality of NKT cell immune responses, as well as potential targets for manipulating NKT cells in intestinal immunity, are discussed.

Type I NKT Cells

CD1d-restricted NKT cells are divided into two subsets based upon differences in TCR characteristics (Table 1) ³. In mice, the most prominent CD1d-restricted NKT cells are Type I NKT cells which are defined by expression of TCR with limited diversity. Murine Type I NKT cells express an invariant V α 14-J α 18 TCR α chain paired with a limited set of TCR β chains, including V β 8.2, V β 7, and V β 2. Previously, these cells have also been referred to as 'classical' NKT cells, invariant NKT (iNKT) cells, and V α 14i NKT cells. In mice, Type I NKT cells either express CD4 or are double-negative (DN). Type I NKT cells are also present in humans and other primates. Similar to murine Type I NKT cells, human Type I NKT cells express an invariant V α 24-J α 18 TCR α chain paired predominantly with V β 11. Like murine Type I NKT cells, human Type I NKT cells are primarily CD4⁺ or DN. Human Type I NKT cells expressing the CD8 coreceptor (either the CD8 $\alpha\alpha$ homodimer or the CD8 $\alpha\beta$ heterodimer), although rare, have been described ².

All murine and human Type I NKT cells recognize the marine sponge-derived glycolipid α -galactosylceramide (α -GalCer), the prototypical Type I NKT cell ligand ^{4,5}. These cells can therefore be quantitated using α -GalCer/CD1d tetramers or multimers ⁶. The availability of α -GalCer/CD1d tetramers to identify and quantitate murine and human Type I NKT cells has facilitated the study of these cells, particularly in the human in which most studies are limited to the peripheral blood compartment.

Type II NKT Cells

Other populations of CD1d-restricted NKT cells that respond to lipid antigens are broadly classified as Type II NKT cells. Type II NKT cells exhibit much more TCR sequence diversity compared to Type I NKT cells, more similar to conventional T cells. Functionally, Type II NKT cells do not respond to α -GalCer and therefore cannot be identified or quantitated using α -GalCer/CD1d tetramers. Such technical limitations have hampered efforts to study Type II NKT cells and, consequently, relatively less is known about the function of these cells in immunity compared to Type I NKT cells. Recent studies have shown that a fraction of Type II NKT cells respond to sulfatide ^{7,8}. However, sulfatide/CD1d tetramers have not been widely used to characterize type II NKT cells due to high background staining and poor stability of the sulfatide/CD1d tetramer complex. In the absence of reagents that can be used to specifically identify and quantitate Type II NKT cells, investigators have relied on the use of genetically deficient and/or transgenic mouse models. Indeed, genetically deficient mouse models have been instrumental in deciphering the biology of both Type I NKT cells and Type II NKT cells. CD1d-deficient (CD1d^{-/-}) mice lack both Type I and Type II NKT cells ⁹, while J α 18-deficient (J α 18^{-/-}) mice lack only Type I NKT cells ¹⁰. Combining the use of wild-type, CD1d^{-/-}, and J α 18^{-/-} mice, then, allows investigators to ascertain contributions of either Type I or Type II NKT cells to specific immune phenomena. However, a recent comparison of TCR diversity in wild-type, CD1d^{-/-}, and J α 18^{-/-} mice revealed significantly lower TCR diversity in J α 18^{-/-} mice ¹¹. The authors estimate that as much as 60% of the TCR α repertoire is absent in J α 18^{-/-} mice,

and suggest that this loss is a function of the mechanism used to target the *Traj18* gene which codes for J α 18. The defects in the T cell compartment of J α 18^{-/-} mice appear to extend beyond the lack of Type I NKT cells, and consequently, results obtained from studies utilizing these mice may require reevaluation¹¹.

The Development of NKT Cells

The developmental requirements of Type I NKT cells compared to conventional T cells have been the subject of intense investigation. Like conventional T cells, Type I NKT cell development is thymus-dependent. However, while the positive selection of conventional T cells is mediated by thymic epithelial cells, positive selection of Type I NKT cells is mediated by CD1d⁺ thymocytes¹². The distinction in positive selecting cell type between conventional T cells and Type I NKT cells may explain in part the unique phenotypic and functional properties of Type I NKT cells. Indeed, the requirement for hematopoietic cells during positive selection is a feature NKT cells share with other 'innate' T cells including H2-M3-restricted CD8⁺ T cells and MR1-restricted MAIT cells^{13,14}. Similar to conventional T cells, Type I NKT cells are subject to negative selection mediated by DC¹⁵.

Upon exiting the thymus, Type I NKT cells populate peripheral tissues. In mice, Type I NKT cells are found in significant numbers in the liver and spleen and to a lesser extent in lymph nodes and intestines. Adhesion molecules and chemokines regulate the migration of NKT cells to peripheral tissues. In particular, interactions between LFA-1 and ICAM-1 as well as recognition of CXCL16 by CXCR6-expressing NKT cells play crucial roles in NKT cell migration^{16,17}.

In addition to unique cellular requirements, a number of cell surface and intracellular molecules have been shown to be important for the selection, development, and function of Type I NKT cells. These include growth factors and their associated receptors, intracellular signaling molecules, transcription factors, and miRNAs. A detailed review of the molecular requirements for Type I NKT cell development is beyond the scope of this review, and readers are encouraged to visit recent reviews on this topic¹⁸.

Due to the limited availability of tools which can be used to study Type II NKT cells, much less is known of the cellular and molecular requirements for the development of Type II NKT cells compared to Type I NKT cells. The majority of what is known about Type II NKT cell development comes from studies of 24 α β mice, a TCR transgenic mouse line that expresses a Type II NKT cell-derived TCR (V α 3.2, V β 9)¹⁹. The selection and development of Type II NKT cells requires CD1d expression¹⁹. Similar to Type I NKT cells, 24 α β NKT cells are positively selected by CD1d-expressing hematopoietic cells (Liao *et al.*, unpublished data) and they also undergo enhanced negative selection when engaged by abundant self-antigens²⁰.

Activation of NKT Cells by Endogenous and Microbial Lipid Antigens

Multiple mechanisms for NKT cell activation have been described²¹. Direct activation of NKT cells is analogous to conventional T cell activation and involves the recognition by the NKT TCR of foreign or microbial lipid antigens presented in the context of CD1d. A number of sphingolipids, and diacylglycerols that stimulate Type I NKT cells have been isolated from a range of pathogenic Gram-negative bacteria such as *Borrelia* and *Sphingomonas*, as well as the pathogenic Gram-positive *Streptococci*²²⁻²⁴. Whether Type I NKT cells recognize lipid antigens derived from nonpathogenic bacteria, such as commensal microbiota, is currently unknown, although recent reports have begun to decipher the nature of interactions between NKT cells and symbiotic bacteria^{17,25}. Microbial lipids that activate Type II NKT cells have not been described.

NKT cells can also be alerted to the presence of microbial pathogens when endogenous lipid antigens are presented by CD1d-expressing APCs that have been activated by pathogen-associated molecular patterns (PAMPs) in a Toll-like receptor (TLR)-dependent manner²⁶. Stimulation of DC with the TLR4 ligand lipopolysaccharide (LPS) resulted in the production of IL-12, and recognition of endogenous ligand/CD1d complexes coupled with IL-12 signaling elicited NKT cell effector function^{27,28}. Similarly, stimulation of DC with the TLR7/9 ligands resulted in the production of type I IFNs, and these signals combined with endogenous ligand/CD1d complexes lead to NKT cell activation²⁹. Finally, the combination of the proinflammatory cytokines IL-12 and IL-18 are sufficient to activate NKT cells in a manner independent of CD1d antigen presentation²¹. It is possible that all three mechanisms could be involved in the activation of NKT cells in the intestine during normal as well as inflammatory conditions. Importantly, NKT cell activation in these various settings is not equivalent. For example, while the potent agonist α -GalCer bound to CD1d elicited the production of both Th1 and Th2 cytokines, mechanisms involving the recognition of endogenous ligand/CD1d complexes and cytokine costimulation typically elicit polarized Th1 type cytokine production profiles by NKT cells³⁰.

Several different types of lipids such as phospholipids, β -glucosylceramides, isoglobotrihexosylceramide, and ether bonded phospholipids^{31–34} have been shown to be endogenous ligands for Type I NKT cells. Compared to Type I NKT cells, much less is known about the types of lipids recognized by Type II NKT cells. Studies with NKT cell hybridomas have suggested that the autoreactivity of Type I and Type II NKT cells is likely induced by distinct endogenous ligands as recycling of CD1d to the lysosomal compartments is required for Type I NKT cell autoreactivity, but not for type II NKT cells^{35,36}. Indeed, sulfatide and lysophosphatidylethanolamine have been shown to activate some Type II NKT cells but have no effect on Type I NKT cells^{7,37}. However, some Type II NKT cell hybridomas have recently been shown to be activated by β -glucosylceramide, a potent Type I NKT cell self antigen³⁸, suggesting some overlap in reactivity among Type I and Type II NKT cells may exist. Two groups have recently reported the first structures of Type II NKT cell TCR in complex with either CD1d-lysosulfatide³⁹ or CD1d-sulfatide⁴⁰ ligands. These studies reveal important distinctions in the recognition of CD1d-lipid antigen complexes between Type I and Type II NKT cell TCR. Whereas the TCR α chain of the Type I NKT cell TCR is the primary contributor to recognition of CD1d-lipid antigen complexes, both the TCR α and TCR β chains of the Type II NKT cell TCR, including all six complementarity-determining regions (CDR), contribute equally to CD1d-lipid antigen recognition. The unique modes of antigen recognition combined with distinct TCR repertoires may allow Type I and Type II NKT cells to assume different functional roles in various immunological settings.

Effector Functions of NKT Cells

Upon activation, Type I NKT cells produce a cocktail of soluble and membrane-bound effector molecules. Traditionally, Type I NKT cells have been defined by their unique ability to simultaneously produce both IFN- γ and IL-4 which influence the generation of Th1 and Th2 immune response, respectively. In addition to IFN- γ and IL-4, Type I NKT cells produce other Th1 cytokines such as TNF- α and other Th2 cytokines including IL-5 and IL-13. Type I NKT cells synthesize growth factors such as IL-2 and GM-CSF, pro-inflammatory cytokines such as IL-6, and anti-inflammatory cytokines such as IL-10 and TGF- β . In addition to cytokines, Type I NKT cells synthesize inflammatory chemokines such as RANTES, MIP-1 α , and MIP-1 β ⁴¹. Finally, Type I NKT cells produce soluble cytolytic proteins including perforin, granzymes, and in humans, granulysin, as well as cell surface molecules involved in cytotoxicity such as FasL^{41,42}. Type I NKT cells can be further subdivided into sub-subsets based upon differences in the expression of CD4 and

NK1.1. These studies lead to the identification of a CD43⁺NK1.1⁺ population of Type I NKT cells capable of producing IL-17, a proinflammatory cytokine which regulates immune responses in mucosal settings^{43,44}. This particular population of Type I NKT cells, then, may play a role in the development of IBD.

Studies of 24αβ NKT cells indicated that Type II NKT cells can secrete both pro-inflammatory (e.g. IFN-γ, TNF-α, IL-17A and IL-6) and anti-inflammatory cytokines (e.g. IL-4 and IL-10) (^{19,20} and unpublished results). In addition, Con A-activated 24αβ T cells lyse target cells in a CD1d-dependent manner⁴⁵. Thus, Type II NKT cells appear to share some immune functions with Type I NKT cells.

Because of their ability to produce an array of effector molecules, Type I NKT cells have the potential to regulate the function of essentially every type of hematopoietic cell. Type I NKT cells regulate the function of APCs such as DC, macrophages, and B cells and other lymphocytes including NK cells and multiple T cell subsets⁴⁶. Interestingly, Type II NKT cells stimulated with sulfatide can regulate the function of Type I NKT cells. This cross regulation among NKT cell subsets is an evolving paradigm⁴⁷. However, the notion that an imbalance in NKT cell subsets can predispose hosts to diseases is supported by a number of murine models of autoimmunity and antitumor and antimicrobial immunity⁴⁸.

CD1d Expression and NKT cell Populations in the Intestinal Mucosa

Human CD1d is expressed on most thymocytes, peripheral B cells, monocytes, and intestinal epithelial cells (IEC)^{49,50}. The expression of human CD1d on IEC is predominantly localized to the apical and lateral regions of the small and large intestinal epithelia⁵⁰, placing it in areas accessible for interaction with intraepithelial lymphocytes (IEL). Murine CD1d is expressed on cells of multiple hematopoietic lineages⁵¹. Conflicting data have been reported regarding the expression of CD1d on mouse IEC as most monoclonal antibodies that recognize mouse CD1d do not recognize the β₂-microglobulin-free form of CD1d, the predominant form of CD1d expressed on IEC⁵². However, Type I NKT cells are able to respond to α-GalCer presented by mouse and human IEC⁵³, suggesting that the low amounts of native CD1d expressed on IEC are sufficient to mediate Type I NKT cell activation. A recent study has shown that CD1d expression can be detected in mouse and rat Paneth cells⁵⁴, supporting a role for NKT cells in regulating the function of these cells to maintain intestinal homeostasis.

Contradictory data on the expression of CD1d in IBD patients have been reported. Page *et al.* reported that the expression of CD1d is higher in the affected ileum and cecum of IBD patients⁵⁵. However, a more recent report suggested that, in contrast to surface epithelium in the normal colon, epithelial cells derived from IBD patients do not express CD1d⁵⁶. Neither study addressed CD1d expression on other cell types, which could contribute to the activation of NKT cells and the modulation of intestinal immune responses. Nevertheless, they highlight the important association of CD1d expression levels with IBD pathogenesis. The conflicting findings of CD1d expression in IBD patients may reflect the heterogeneous nature of the disease and/or the effect of different treatments received by IBD patients, as CD1d expression can be unregulated by cytokines (such as IFN-γ, TNF-α, and IFN-β) and bacterial products^{57,58}.

Type I NKT cells are present in the small and large intestine in both mice and humans. However, it is difficult to estimate the proportion of Type II NKT cells in the intestine since a substantial proportion of NK1.1⁺ T cells (or CD161⁺ T cells) are not CD1d-restricted, likely representing activated conventional T cells⁵⁹. Several earlier studies showed that CD3⁺NK1.1⁺ cells compose about 4–10% of IELs and 7% of lamina propria lymphocytes (LPLs) in the mouse intestine^{60,61}. However, a significant proportion of these CD3⁺NK1.1⁺

cells are $\gamma\delta$ T cells. Using CD1d/ α -GalCer tetramer staining, a recent study showed that the frequency of Type I NKT cells varied in different sites in the intestine with the highest frequency detected in the LPL compartment of the small intestine (~0.6%). The frequency of Type I NKT cells in IELs is substantially lower (<0.1%)⁶². In humans, approximately 18–60% of IEL and 9–58% of LPL are CD3⁺ CD161⁺ cells^{60,61}. However, analysis of NKT cells by CD1d/ α -GalCer tetramer staining reveals only up to 1% of IEL and LPL are Type I NKT cells⁵⁹.

NKT Cells in the Pathogenesis of Human and Animal Models of IBD

The two most common types of IBD are Crohn's disease (CD) and ulcerative colitis (UC), which differ in their predominant cytokine profile and affect distinct portions of the intestine. CD is a Th1-dominated immune response with a phenotype of transmural inflammation throughout the whole intestine, including the small and large intestines⁶³. UC is an atypical Th2-mediated disease in which the inflammation is restricted to the mucosal layer of the colon⁶⁴.

Several clinical studies have reported a significant reduction in the proportion of Type I NKT cells in the peripheral blood and intestine of both human CD and UC patients^{65,66}. In contrast, a study by Fuss *et al.* has shown that a substantial proportion of Type II NKT cells accumulate in the lamina propria of patients with UC⁵⁹. Upon stimulation with cells expressing high levels of surface CD1d, these NKT cells produced IL-13, which may contribute to the Th2-oriented immune responses seen in UC patients. Although this study highlights the importance of CD1d expression level as well as the role of Type II NKT cells in the pathogenesis of IBD, it is not clear whether Type II NKT cells are directly involved in the induction of disease.

In several animal models of IBD, NKT cells have been shown to play either a protective or a pathogenic role, depending on the nature of inflammatory stimuli and possibly the lipid antigens recognized (Table 2).

Protective roles for NKT cells have been reported in three mouse colitis models characterized by Th1 immune responses. Dextran sodium sulfate (DSS) is a chemical compound commonly used to induce acute and chronic colitis in mice. In this model, improper macrophage activation, altered intestinal microbiota, and direct toxic effects on the intestinal epithelial cells result in inflammatory ulceration of the colonic mucosa⁶⁷. A protective role of Type I NKT cells has been shown using the DSS-induced colitis model. Wild-type mice treated with α -GalCer were shown to have less severe body weight loss and an overall improvement of colitis than mice receiving a control lipid α -ManCer⁶⁸. In addition, repeated stimulation of Type I NKT cells with α -GalCer altered the Th1/Th2 balance by reducing the Th1 response and improved the disease score in DSS-colitis⁶⁹. Consistent with the protective Th2 response in this model, administration of OCH, an α -GalCer analog with a truncated lipid tail, attenuated colonic inflammation by polarizing NKT cells to a Th2 type of cytokine production profile⁷⁰. These results demonstrate the protective role of activated Type I NKT cells in the DSS-induced colitis model.

In an adoptive transfer model of T cell-induced colitis, SCID mice receiving naïve CD62L⁺CD4⁺ T cells along with DX5⁺ NKT cells have less severe disease⁷¹. In addition, blocking CD1d in the presence of DX5⁺NKT cells results in more severe body weight loss. However, because DX5 is a cell surface marker expressed by both Type I and Type II NKT cells, it is currently unclear which NKT cell subset contributes to the protective role in this model system.

Rectal administration of the haptens TNBS and oxazolone in mice has been used to induce Th1 and Th2 immune responses resembling human CD and UC, respectively. These haptening reagents may cause intestinal inflammation by enhancing the immunogenicity of autologous or microbe-derived antigens in the intestine⁷². A protective role of NKT cells was shown using the TNBS-induced colitis model. Specifically, depletion of NKT cells accelerated the body weight loss induced by TNBS treatment⁷³. In addition, adoptive transfer of pre-activated NKT cells by *ex vivo* exposure to colitis-extracted protein (CEP) significantly alleviated TNBS-mediated colitis in the recipient mice⁷⁴. The immunoregulatory role of activated NKT cells in Th1-mediated IBD models is further supported by the finding that activation of NKT cells by administration of natural ligands β -glucosylceramide results in alleviation of TNBS-induced colitis by increasing intrahepatic NKT cells, decreasing STAT1 and STAT4 expression, and decreasing IFN- γ production⁷⁵.

In contrast to the protective role of NKT cells in the colitis models described above, a role for Type I NKT cells in the pathogenesis of IBD has been described in the oxazolone-induced colitis model in which Th2 cytokines induce pathology. Wild-type mice receiving oxazolone were shown to have more severe colitis and body weight loss than CD1d^{-/-} or Ja18^{-/-} mice⁷⁶. In addition, lamina propria mononuclear cells from oxazolone-treated mice produced more IL-13 in response to α -GalCer stimulation⁷⁶, indicating that Type I NKT cells promote pathogenesis primarily by producing Th2 cytokines. In addition, Epstein-Barr virus-induced gene 3 (EBI3)-deficient mice, which have a significantly reduced number of Type I NKT cells, are resistant to oxazolone-induced colitis⁷⁷, possibly due to impaired IL-4 production by residual Type I NKT cells in EBI3^{-/-} mice.

The role of NKT cells has also been evaluated in a microbial infection-induced model of IBD. Mice infected orally with the protozoan parasite *Toxoplasma gondii* (*T. gondii*) develop acute ileal inflammation mediated by a dysregulated immune response in the intestinal mucosa, which induces a Crohn's disease-like pathology with a hyperinflammatory Th1 cytokine profile. CD1d^{-/-} mice exhibited severe weight loss and ultimately succumbed to disease, whereas all wild-type mice survived infection⁷⁸. Subsequent experiments using Ja18^{-/-} mice demonstrated a protective role for Type I NKT cells in *T. gondii*-induced colitis. In contrast, another study reported that Ja18^{-/-} mice are more resistant to *T. gondii* infection-induced pathology compared to wild-type mice, suggesting a pathogenic role for Type I NKT cells in this IBD model⁷⁹. The reason for the discrepancy in results obtained from these reports is unclear. Nevertheless, together they highlight a role for NKT cells in oral *T. gondii* infection-induced intestinal inflammation.

Because of the difficulties of studying Type II NKT cells in human chronic intestinal diseases, we have recently used a transgenic mouse model (CD1dTg/24 $\alpha\beta$ Tg) that expresses high levels of CD1d and a TCR from an autoreactive type II NKT cell to demonstrate a direct role of dysregulated Type II NKT cells in the pathogenesis of IBD²⁰. In this model, high levels of IFN- γ and IL-17A were produced by Type II NKT cells in the colon of transgenic mice upon anti-CD3 stimulation, with minimal production of Th2-related cytokines. This polarized cytokine response likely contributes to the intestinal inflammation observed in this model. Interestingly, these results differ from reports of UC patients in which Type II NKT cells produce the Th2 cytokine IL-13⁵⁹. However, it is likely that Type II NKT cells can be skewed towards Th1, Th2 or Th17 responses depending on the strength of stimulatory signals and the type of antigen presented, similar to mechanisms that regulate Type I NKT cell activation^{70,80}. Taken together, these data suggest a complex role for NKT cells in IBD, which may be regulated by various factors in the local microenvironment, such as the level of CD1d expression, the nature of the antigens presented by CD1d, the type of stimulatory APC, the cytokine milieu, and the composition of intestinal microbiota (Figure 1).

Future Areas of Study

A number of studies have begun to decipher the significance of interactions between NKT cells and the intestinal microbiota. Colonization of the small intestine with both commensal and pathogenic bacteria is enhanced in CD1d-deficient mice compared to wild-type, and treatment of wild-type mice with α -GalCer, but not the control glycolipid antigen α -ManCer, inhibited intestinal colonization by bacteria, suggesting an active role for Type I NKT cells in regulating bacterial colonization of the intestine²⁵. Interestingly, intestinal Paneth cells from CD1d^{-/-} mice exhibit functional defects compared to wild-type mice. Therefore, Type I NKT cells may indirectly regulate colonization of the small intestine through a mechanism involving Paneth cell function²⁵. Whether Type II NKT cells are capable of similar functions is currently unknown.

Intestinal microbes have been shown to affect the recruitment of Type I NKT cells to various peripheral tissues. Compared to mice with a conventional (specific pathogen-free) microflora, germ-free mice have reduced numbers of Type I NKT cells in peripheral tissues including the spleen and liver⁸¹. Interestingly, mice with a well-defined 'restricted' microflora exhibited even larger deficiencies in Type I NKT cells in peripheral tissues, suggesting the composition of the enteric microbiota has significant effects on NKT cells⁸¹. More recently it was reported that germ-free mice contain higher numbers of Type I NKT cells in mucosal tissues such as the colon and lungs compared to control (specific pathogen-free) mice¹⁷. The increased frequency of Type I NKT cells in the colon and lungs of germ-free mice resulted in more severe pathology in models of colitis and allergic asthma, respectively, compared to control mice. Colonization of adult germ-free mice with microbiota failed to reduce the number of Type I NKT cells in mucosal tissues. However, exposure of neonatal germ-free mice to microbes did reduce the number of Type I NKT cells to normal physiological numbers, and consequently, the severity of colitis and allergic asthma in these mice was significantly reduced¹⁷. Taken together, these results highlight the importance of the interactions between NKT cells and commensal microbes, and suggest that this crosstalk begins at birth.

In contrast to these studies of Type I NKT cells, relatively little is known of the significance of microbiota interactions with Type II NKT cells. The microbiota might play a role in contributing to the pathogenesis of IBD mediated by Type II NKT cells. Consistent with this, treatment of mice with broad-spectrum antibiotics attenuated colitis caused by Type II NKT cells, although further studies are needed to determine the underlying mechanism of these results²⁰. One likely possibility is that Type II NKT cells recognize commensal-derived lipid antigens (See Figure 1). Alternatively, commensal microbes might induce the expression and/or upregulation of an endogenous lipid antigen capable of being presented by CD1d and recognized by Type II NKT cells. Indeed, the identification of endogenous and exogenous lipid antigens recognized by Type II NKT cells is an area of study which deserves significant attention. Given the role of Type II NKT cells in the pathogenesis of IBD, limiting the availability of lipids recognized by these cells might modulate their function. Finally, the frequency of Type II NKT cells increases significantly in patients with multiple myeloma, suggesting this feature might be used as a clinical biomarker of disease⁸². Similar studies should be conducted in patients with CD and UC to determine the potential for changes in the frequency of Type II NKT cells to serve as a biomarker of IBD.

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Figure 1. Activated NKT cell can play both protective and pathogenic roles during intestinal inflammation

NKT cells are activated in the intestinal mucosa when lipid antigens from commensal or pathogenic bacteria or self-lipids are presented by APCs. The cytokine milieu, types of lipid antigens, APCs and microbiota determine whether activated NKT cells play a protective or pathogenic role in the intestine. Death of CD1d-expressing IECs or APCs due to the cytotoxic activity of NKT cells can lead to chronic intestinal inflammation, a mechanism by which NKT cells exert their pathogenic effect. The types of cytokines secreted by NKT cells can either augment or suppress ongoing inflammation depending on whether the immune response is Th1, Th2 or Th17-mediated. Lastly, NKT cells can enhance Paneth cell-mediated microbial clearance. Inadequate NKT cell regulation of Paneth cells can result in dysbiosis of intestinal microbiota which can contribute to the development of IBD.

TABLE 1

Characteristics of CD1d-restricted NKT cells

	<i>Type I NKT cells</i>		<i>Type II NKT cells</i>
Feature	Mouse	Human	Mouse/Human
TCR α	V α 14-J α 18	V α 24-J α 18	Diverse, V α 3.2, V α 8, V α 1 bias
TCR β	V β 8.2, V β 7, V β 2	V β 11	Diverse, V β 8 and V β 3 bias
α -GalCer reactivity	Yes	Yes	No
Antigens recognized	α -GalCer analogs, glycosphingolipids, diacylglycerols, phospholipids	α -GalCer analogs, glycosphingolipids, diacylglycerols, phospholipids	Sulfatides, lysosulfatide, lysophospholipids
Detection	α -GalCer-loaded CD1d tetramers	α -GalCer-loaded CD1d tetramers, anti-V α 24/anti-V β 11 mAbs	Sulfatide-loaded CD1d tetramers, indirectly with a combination of CD1d-deficient and J α 18-deficient mice
Mature phenotype	NK1.1 ⁺	CD161 ⁺	NK1.1 ⁺ /CD161 ⁺
Subsets	CD4 ⁺ , DN, CD4 ⁻ /NK1.1 ⁻	CD4 ⁺ , DN, CD8 ⁺	CD4 ⁺ , DN, CD8 ⁺
Frequency in thymus	0.2–0.5%	0.001–0.01%	ND
Frequency in blood	0.2–0.5%	0.001–0.5%	ND
Frequency in spleen	0.5–1.0%	0.01–0.5%	ND
Frequency in liver	20–40%	~1%	In humans, more abundant than Type I NKT cells
Frequency in intestine	0.05– 1.0%, enriched in lamina propria	<1.0%	ND
Cytokine production	Th1, Th2, Th17 (CD4 ⁻ /NK1.1 ⁻ subset)	Th1, Th2, Th17	Th1, Th2, Th17

ND, not determined.

TABLE 2

The role of NKT cells in the murine model of IBD

Type I NKT cells	Type II NKT cells
Protective role	
Saubermann <i>et al</i> (2000) Mice treated with α -GalCer have less IBD in DSS- induced colitis.	Ronet <i>et al</i> (2005) $\text{Ja}\alpha 18^{-/-}$ mice have higher survival rate than WT and $\text{CD}1^{-/-}$ mice to <i>Toxoplasma gondii</i> induced ileitis.
Shibolet <i>et al</i> (2004) Adoptive transfer of ex vivo immune-programed NKT cells alleviates disease in TNBS-induced colitis.	Hornung <i>et al</i> (2006) ¹ SCID mice receiving transferred $\text{CD}62\text{L}^{+}\text{CD}4^{+}$ cells along with $\text{DX}5^{+}\text{NK}1.1^{+}$ cells ameliorates the disease.
Ueno <i>et al</i> (2005) Administration of OCH mitigates DSS-induced colitis.	
Smiley <i>et al</i> (2005) Lack of $\text{V}\alpha 14^{+}\text{T}$ cells increases susceptibility to chronic <i>Toxoplasma gondii</i> infection.	
Numata <i>et al</i> (2005) Repeated stimulation of NKT cells with α -GalCer reduces inflammation in DSS-induced colitis by altering Th1/Th2 response.	
Hornung <i>et al</i> (2006) ¹ SCID mice receiving transferred $\text{CD}62\text{L}^{+}\text{CD}4^{+}$ cells along with $\text{DX}5^{+}\text{NK}1.1^{+}$ cells ameliorates disease.	
Zigmond <i>et al</i> (2007) β -glucosylceramide alleviates TNBS-induced colitis by altering the plasticity of NKT cells.	
Nieuwenhuis <i>et al</i> (2009) Impaired NKT cell-mediated regulation of Paneth cells may result in entero-colitis by altering bacterial colonization.	
Pathogenic role	
Nieuwenhuis <i>et al</i> (2002) $\text{EBI}3^{-/-}$ mice have decreased NKT cells and are resistant to oxazolone-induced colitis.	Liao <i>et al</i> (2012) Type II TCR Tg T cells that develop in the context of high levels of CD1d are intrinsically pathogenic and contribute directly to the development of IBD.
Heller <i>et al</i> (2002) $\text{NK}1.1^{+}\text{J}\alpha 281^{+}\text{CD}4^{+}$ cells mediate the inflammation in oxazolone-induced colitis.	
Ronet <i>et al</i> (2005) WT mice have more severe <i>Toxoplasma gondii</i> induced ileitis than $\text{Ja}\alpha 18^{-/-}$ mice.	

¹ Both Type I and Type II NKT cells express DX5. Therefore, both NKT cell subsets may provide protection in this adoptive transfer model.