Role of NKT Cells in the Digestive System.

III. Role of NKT cells in intestinal immunity

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Zeissig S, Kaser A, Dougan SK, Nieuwenhuis EE, Blumberg RS. Role of NKT Cells in the Digestive System. III. Role of NKT cells in intestinal immunity. Am J Physiol Gastrointest Liver Physiol 293: G1101–G1105, 2007. First published August 23, 2007; doi:10.1152/ajpgi.00342.2007.—Natural killer T (NKT) cells are a subset of T cells that express a semi-invariant T cell receptor (TCR) and recognize lipid antigens presented by the nonpolymorphic nonclassical MHC class I molecule CD1d. NKT cells recognize glycolipid antigens presented by CD1d, a nonpolymorphic nonclassical MHC class I molecule. In contrast to conventional T cells, most NKT cells express a semi-invariant TCR associated with different MHC molecules, NKT cells recognize glycolipid antigens presented by CD1d, a nonpolymorphic nonclassical MHC class I molecule. In contrast to conventional T cells, most NKT cells express a semi-invariant TCR composed of an invariant TCRα chain (Vα14-Jα18 in mice, Vα24-Jα18 in humans) paired with a restricted subset of TCRβ chains, including Vβ8.2, Vβ7, and Vβ2 in mice and Vβ11 in humans that display a wide variety of complementarity determining region 3 domains (9). NKT cells are known for their ability to produce a massive burst of cytokines early in an immune response. Ligation of the NKT TCR leads to rapid and copious secretion of the prototypic Th1 and Th2 cytokines interferon (IFN)-γ and interleukin (IL)-4 and, in some cases, granulocyte macrophage colony-stimulating factor, IL-10, IL-13, transforming growth factor (TGF)-β, and other cytokines and chemokines (12). NKT cells also mediate perforin-dependent and FasL-dependent cytotoxicity (12).

Definition of NKT Cells

The classification of NKT cells has been complicated by the fact that NKT cells were defined in many different ways, e.g., as CD3+ NK1.1+ cells, Vα14+ cells, or cells reactive to soluble CD1d tetramers loaded with the glycosphingolipid α-galactosylceramide (αGalCer). It soon became clear that the initial definition of NKT cells as cells coexpressing the CD3/TCR complex and NK1.1 was imprecise because (i) a significant subset of Vα14-NKT cells does not express NK1.1, (ii) many commonly used mouse strains including BALB/c, CBA, and NOD mice do not express NK1.1, (iii) NKT cells profoundly downregulate NK1.1 after activation, and (iv) conventional T cells can induce NK1.1 expression after activation (9). Therefore, a new classification of NKT cells was suggested based on the use of αGalCer/CD1d tetramers (9). Classical Vα14+ (Vα24+ in humans) invariant (i) NKT cells are highly reactive to αGalCer and were defined as type I NKT cells independent of their expression of NK1.1, whereas nonclassical, noninvariant, Vα14- αGalCer nonreactive NKT cells were defined as type II NKT cells, again independent of their expression of NK1.1. Both cell types are restricted by CD1d and are absent in β2-microglobulin (β2m)-deficient or CD1d-deficient mice, which distinguishes them from non-CD1d-restricted non-αGalCer-reactive but NK1.1+ cells. These NKT-like cells include conventional T cells, which have upregulated NK1.1 expression after activation, and invariant T cells, which are restricted by MHC class I molecules other than CD1d. The latter cells include mucosal-associated invariant T cells, which
express the canonical Vα19-Jα33 TCR and mainly localize in the gut mucosa (27).

**NKT Cells in the Intestine**

In contrast to conventional T cells, NKT cells respond within minutes after TCR ligation and do not depend on clonal expansion, differentiation, and migration to the target organ. This immediate immune response renders NKT cells a perfect first-line defense against microbial pathogens, and it is tempting to speculate that NKT cells might survey the intestinal mucosa for invasion of pathogens. Indeed, several reports indicated the presence of NKT cells in the intestine of humans and mice. However, because of different definitions of NKT cells and huge variations between different studies, the percentage of NKT cells in the intraepithelial compartment and the lamina propria is still unclear.

Based on the initial definition of NKT cells as CD3⁺NK1.1⁺ or αβTCR⁺NK1.1⁺ cells, 0.3–11% of intraepithelial lymphocytes (IEL) and 7% of lamina propria lymphocytes (LPL) in mice were reported to be NKT cells with slightly higher levels in the large intestine compared with the small intestine (see Ref. 31 for an extensive review). In humans, reported values were even higher, with CD3⁺NK1.1⁺ cells constituting 18–58% of IEL and 9–58% of LPL (31). However, direct analysis of iNKT cells by detection of Vα14 in mice and Vα24 in humans or by staining with αGalCer-loaded CD1d tetramers revealed considerably lower numbers of iNKT cells ranging from 0–2% of IELs and LPLs in humans and mice (8, 14, 22, 31). Indeed, our laboratory consistently observes 1–2% iNKT cells in the murine IEL and LPL compartments (unpublished observation). It is therefore likely that the vast majority of CD3⁺ NK1.1⁺ cells in the lamina propria and the IEL compartment represents conventional T cells which have upregulated NK1.1 expression after activation consistent with the physiological inflammation of this compartment. Although the low number of NKT cells seems to argue against a physiological function of these cells in the intestine, there is growing evidence for a pivotal functional role of NKT cells in intestinal physiology and pathology.

**CD1d Expression in the Intestine**

CD1d is a nonpolymorphic nonclassical MHC class I molecule that presents lipid antigens to NKT cells. The CD1 family of proteins comprises five members in humans (CD1a-e), whereas mice only express two homologues of CD1d, CD1d1 and CD1d2. CD1d is synthesized in the endoplasmic reticulum where it associates with microsomal triglyceride transfer protein, which presumably assists in loading endogenous antigens into the CD1d groove before transport of CD1d to the cell surface (4, 6). After reaching the cell surface, CD1d extensively traffic through the endolysosomal system where it interacts with lysosomal lipid-editing proteins including saposins and GM2 activator protein, which can load exogenous antigens into the CD1d groove (32). Although nonpolymorphic, CD1d can bind a variety of antigens because of its narrow and deep hydrophobic antigen-binding groove. These include the marine sponge glycolipid αGalCer, certain α-linked bacterial glycosphingolipids, and bacterial-derived diacylglycerols (12). Although isoglobotrihexosylceramide (iGb3) was suggested to be the predominant endogenous antigen loaded on CD1d (33), recent studies have challenged this view, since iGb3 synthase-deficient mice have normal NKT cell numbers and unaltered NKT cell function despite lack of iGb3 expression (20). Moreover, iGb3 expression was shown to be limited to the dorsal root ganglion while it was not detectable in any other human or mouse tissue, including thymocytes and dendritic cells (DCs; see Ref. 26).

CD1d is expressed on a limited subset of cells, including thymocytes, professional antigen presenting cells, keratinocytes, and intestinal epithelial cells (IECs). In the intestine, DCs, macrophages, B cells and IECs express CD1d. Moreover, primary human IECs and mouse and human IEC lines can present CD1d-restricted glycolipid antigens to NKT cells (30). However, the relative contribution of these CD1d-expressing cell types in intestinal NKT cell activation in health and disease is unknown. Interestingly, IECs not only express the β2m-associated fully glycosylated 48-kDa form of CD1d that locates to the basolateral and apical cell membrane but also a minor species of a 37-kDa nonglycosylated isoform that is confined to the apical membrane and exists independently of β2m (2).

Intriguingly, intestinal CD1d expression is not only important for NKT cell activation but is also involved in an anti-inflammatory response by the IEC itself. Ligation of CD1d on IECs leads to retrograde signaling and production of bioactive IL-10, which can protect from IFN-γ-mediated intestinal barrier dysfunction (5). It is therefore likely that CD1d expression on the intestinal epithelium not only facilitates communication between IECs and NKT cells, but also essentially contributes to modulation of mucosal immune responses by the IEC itself. However, further studies are necessary to delineate the different functions of CD1d on the intestinal epithelium.

**Function of NKT Cells in the Intestine**

The first demonstration of CD1d function in the intestine occurred more than 10 years ago, when it was first shown that IECs can activate T cells in a CD1d-restricted manner and that oligoclonal T cells with a biased TCR repertoire (including Vα14) reside in the intestinal intraepithelial compartment and are capable of inducing CD1d-restricted cytotoxicity (19). These findings suggested close communication between NKT cells and CD1d-expressing cells in the intestinal mucosa, and their potential regulation of bacterial colonization and invasion, and induction of oral tolerance. However, although many studies have investigated the function of NKT cells in intestinal diseases, our knowledge about the physiological and pathogenic role of NKT cells in the intestine is still limited.

**Regulation of the Intestinal Microbiota by NKT Cells**

Although neither Jα18⁻/⁻ mice which lack iNKT cells, nor CD1d⁻/⁻ mice, which lack invariant and nonvariant NKT cells, are more susceptible to spontaneous infections, NKT cells have been shown to be involved in the immune response against a variety of bacteria, fungi, viruses, and parasites, including mucosal infections with Salmonella typhimurium, Listeria monocytogenes, and Toxoplasma gondii (for an extensive review, see Ref. 28).

In oral infections with Listeria monocytogenes, iNKT cells were shown to be early producers of IFN-γ and to induce IFN-γ secretion by NK cells. Transfer of iNKT cells in
RAG−/− mice decreased the systemic bacterial burden and prevented mortality, which is in accordance with another study reporting increased systemic bacterial burden after oral inoculation with *Listeria monocytogenes* in CD1d−/− mice (1, 21). However, these results were challenged by a recent study demonstrating decreased bacterial burden after oral inoculation with *Listeria monocytogenes* in Jα18−/− mice, suggesting that iNKT cells may normally exhibit a regulatory role during *Listeria* sp. infections (7).

Studies investigating the role of NKT cells in response to oral inoculation with *Toxoplasma gondii* cysts also revealed conflicting data. Although one study reported decreased survival in both CD1d−/− mice and Jα18−/− mice because of unopposed IFN-γ production by conventional CD4-positive T cells, another study showed protection of Jα18−/− mice from *Toxoplasma gondii*-related mortality because of decreased IFN-γ production (22, 25).

Finally, although *Salmonella typhimurium* infections are normally cleared in CD1d−/− mice, iNKT cells were shown to be activated and to contribute to IFN-γ secretion and skewing toward a Th1 cytokine profile (3, 15). Moreover, it was shown that, while lipopolysaccharide (LPS)-negative *Ehrlichia* and *Sphingomonas* spp. activate NKT cells by direct TCR-mediated recognition, LPS-positive *Salmonella typhimurium* are not directly recognized by NKT cells. Instead, LPS stimulates IL-12 production by DCs which, in combination with recognition of CD1d bearing an endogenous ligand, activates NKT cells (15). Together, these data indicate that, although NKT cells might not be sufficient for protection against microbial infection, they clearly influence the immune response against certain pathogens and modulate bacterial colonization. This is in accordance with recent data showing more rapid intestinal colonization with Gram-positive and Gram-negative bacteria in germ-free CD1d−/− mice compared with germ-free wild-type mice (18).

**Role of NKT Cells in Induction of Oral Tolerance**

Oral tolerance, the suppression of an immune response by prior oral administration of the antigen, is a major mechanism for induction of peripheral tolerance and prevents immune responses against food proteins and bacterial antigens. In a mouse model of oral tolerance, feeding with haptenized colonic proteins was shown to protect from 2,4,6-trinitrobenzenesulfonic acid (TNBS) colitis by induction of TGF-β and Th2 cytokine-producing mucosal T lymphocytes (16). Because depletion of NK1.1+ cells prevented induction of oral tolerance to colitis-extracted proteins and abrogated protection from TNBS colitis, NKT cells were suggested to play a key role in oral tolerance (13). This was supported by data showing that protection from TNBS colitis can be adoptively transferred by splenocytes from tolerized mice while transfer of NK1.1-depleted splenocytes does not lead to alleviation of disease. Moreover, while transfer of nontolerized NK1.1+ T cells led to aggravation of TNBS colitis, in vitro tolerized NK1.1+ cells ameliorated TNBS colitis, arguing for a pathogenic role of NKT cells in TNBS colitis that can be reversed by tolerization of these cells (13). However, as a major limitation of these studies, analysis was limited to NK1.1+ CD3+ cells, a significant proportion of which were likely to be activated conventional T cells. Indeed, it was shown that oral tolerance can be induced normally in Jα18−/− mice, which argues against a significant role of invariant NKT cells in induction of oral tolerance while leaving open the possibility of a contribution by noninvariant NKT cells (11).

**NKT Cells in IBD**

IBD are chronic inflammatory conditions affecting the small and/or large intestine. The two most common types of IBD, Crohn’s disease (CD) and ulcerative colitis (UC), share many clinical features, although they differ in their predominant cytokine profile and in their microscopic and macroscopic extension. Although the inflammatory process in UC is restricted to the superficial intestinal mucosal layers of the large intestine, CD represents a transmural inflammation affecting the small and/or large intestine. Moreover, LPLs in UC primarily secrete Th2 cytokines (IL-13, IL-4, IL-5), while Th1 cytokines (IFN-γ) are predominant in CD.

In mice, sensitization and intrarectal application of the haptenizing agents TNBS and 4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) were shown to induce Th1- and Th2-dominated immune responses resembling human CD and UC, respectively (10, 17). Although the exact pathogenesis in these mouse models of IBD is unknown, it is assumed that both chemicals induce intestinal inflammation by haptenization of autologous or microbial-derived proteins, thus rendering them immunogenic.

Recent work by Strober and colleagues (8) and Blumberg and colleagues (10) has revealed a central role for NKT cells in the pathogenesis of human UC and oxazolone colitis in mice. First, Heller et al. (10) demonstrated that administration of oxazolone leads to Th2-dominated intestinal inflammation with secretion of high amounts of IL-13, IL-4, and IL-5 by LPL, a condition that resembles human UC. In contrast to wild-type mice, CD1d−/− mice as well as wild-type mice injected with anti-CD1d antibodies were completely protected from oxazolone colitis. Although the authors did not directly investigate iNKT cells in the lamina propria of *αGalCer/CD1d*-tetramer stained, LPL of mice with oxazolone colitis secreted large amounts of IL-13 after stimulation with the iNKT ligand *αGalCer*. Moreover, Jα18−/− mice, which lack iNKT cells but not noninvariant NKT cells, and CD1d−/− mice, which lack all CD1d-restricted T cells, were protected from oxazolone colitis, indicating that iNKT cells but not noninvariant NKT cells are necessary for the development of oxazolone colitis.

In a second study performed by Fuss et al. (8), CD3+ NK1.1+ LPL of patients with UC were shown to secrete high amounts of IL-13 and IL-5. Because IL-13 production could be elicited by stimulation with CD1d-transfected B cells and could be blocked by anti-CD1d antibodies, these data indicated a pivotal role of NKT cells in the pathogenesis of human UC. However, LPL did not secrete IL-13 upon *αGalCer* stimulation, and analysis of LPL by *αGalCer/CD1d* tetramers and *Vα24/Vβ11* antibodies revealed an almost undetectable number of iNKT cells in the lamina propria. These data indicate that, although both oxazolone colitis in mice and UC in humans are partly mediated by NKT cells, the responsible subtype of NKT cell may differ between both diseases. While iNKT cells contribute to induction of oxazolone colitis, non-
invariant NKT cells may be more responsible for intestinal inflammation in human UC.

In contrast to Th2-mediated types of intestinal inflammation, the role of NKT cells in Th1 diseases is less obvious. Although the percentage of CD3+ NK1.1+ cells in the intestinal lamina propria is similarly high in CD compared with UC, these cells could not be stimulated in a CD1d-restricted manner and are therefore assumed to be conventional T cells rather than NKT cells (8). Moreover, investigation of TNBS colitis, a mouse model of Th1 inflammation, revealed conflicting data. Although one group showed no effect of NK1.1 depletion in TNBS colitis, another group demonstrated alleviation of colitis by NK1.1 depletion or transfer of NK1.1-depleted splenocytes (10, 24).

Finally, two studies investigated the role of NKT cells in dextran sulfate sodium (DSS) colitis showing that administration of αGalCer and OCH, a truncated analog of αGalCer known to preferentially induce Th2 polarization, alleviates colitis (23, 29). IEC-mediated presentation of αGalCer leading to NKT cell secretion of Th2 cytokines was suggested as a mechanism of disease amelioration arguing for a regulatory role of activated NKT cells in colitis (23).

Conclusion and Perspective

iNKT cells reside in the intestinal epithelium and the lamina propria in mice and humans (~0.5–2% of cells in these compartments) where they modulate the host response to microbes and likely play a role in peripheral tolerance. Studies on mouse models of IBD clearly demonstrate a pivotal role of iNKT cells in the pathogenesis of colitis. In contrast to mice, noninvariant NKT cells seem to contribute to the intestinal inflammation in human UC, whereas iNKT cells seem to be dispensable for the inflammatory process. These studies indicate a previously unrecognized role of noninvariant NKT cells in the intestine. Although there is still no method of direct detection of these cells and our knowledge of the abundance and function of noninvariant NKT cells is limited, future studies investigating intestinal NKT cell function in mice should systematically investigate the response of wild-type, Jo18−/−, and CD1d−/− mice to delineate the contributions of invariant and noninvariant NKT cells.

NKT cells are implicated in the pathogenesis of a variety of intestinal diseases, including microbial infections and IBD. However, the antigens responsible for CD1d-restricted activation of NKT cells in the intestine remain elusive. Is NKT cell activation in IBD induced by CD1d-restricted presentation of microbial antigens or the consequence of an uncontrolled response against endogenous antigens? What are the endogenous antigens presented by IECs and professional APCs in the lamina propria? Whether NKT cells directly recognize ligands derived from commensal flora or whether they become activated by CD1d bearing endogenous ligands is of key importance for future studies, which might have broad implications for IBD therapy.

REFERENCES

initiation of an inflammatory bowel response against *Toxoplasma gondii*. 


