Role of NKT cells in the digestive system.

IV. The role of canonical natural killer T cells in mucosal immunity and inflammation

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Wingender G, Kronenberg M. 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, 2008. First published October 18, 2007; doi:10.1152/ajpgi.00437.2007.—Lymphocytes that combine features of T cells and natural killer (NK) cells are named natural killer T (NKT) cells. The majority of NKT cells in mice bear highly conserved invariant V_{i}14 chains, and to date two populations of such canonical NKT cells are known in mice: those that express V_{i}α14 and those that express V_{α}7.2. Both populations are selected by nonpolymorphic major histocompatibility complex class I-like antigen-presenting molecules expressed by hematopoietic cells in the thymus: CD1d for V_{i}α14-expressing NKT cells and MR1 for those cells expressing V_{α}7.2. The more intensely studied V_{i}α14 NKT cells have been implicated in diverse immune reactions, including immune regulation and inflammation in the intestine; the V_{α}7.2 expressing cells are most frequently found in the lamina propria. In humans, populations of canonical NKT cells are found to be highly similar in terms of the expression of homologous, invariant T cell antigen receptor (TCR) α-chains, specificity, and function, although their frequency differs from those in the mouse. In this review, we will focus on the role of both of these canonical NKT cell populations in the mucosal tissues of the intestine.

innate immunity; colitis

TO BE OR NOT TO BE: THE MANY NAMES OF NKT CELLS

Natural killer T (NKT) cells are a unique subset of T lymphocytes found in mice, humans, and other mammals. Like natural killer (NK) cells, they exhibit features of innate immunity, but they also share properties with conventional T lymphocytes. They were originally defined by their coexpression of an αβ T cell antigen receptor (TCR) and NK cell receptors, especially NKP/1 (NKR-P1C) in certain mouse strains and CD161 (NKR-P1A) in humans. However, this classification is an oversimplification, as conventional T cells can express NK cell receptors, especially after activation. Moreover, the expression of NK cell receptors by NKT cells varies with their maturity and activation state and, in mice, with the genetic background. As knowledge of the properties of NKT cells has increased, these cells have been subdivided according to their TCR α-chain, the selecting antigen-presenting molecule, and the expression of various surface molecules (for reviews, see Refs. 30, 62, 68).

However, despite attempts, the lack of a generally accepted nomenclature has made the field confusing. Here, we propose a classification based on TCR usage and the antigen-presenting molecule recognized. Thereby, four populations of NKT cells can be distinguished (see Table 1). The first two NKT cell populations have a canonical TCR and are specific for a nonpolymorphic major histocompatibility complex (MHC) class I-like molecule. “Canonical” and “invariant” have been used synonymously, but historically the mouse V_{α}14 invariant (V_{α}14i) NKT cells, along with their human homologs that express a V_{α}24i TCR, have been termed invariant (iNKT). Therefore, we will use the expression “canonical (c)NKT cell” to include both the V_{α}14/V_{α}24i NKT cells and a second population that expresses a fixed V_{α}7.2i TCR in mice and a polymorphic V_{α}19i in humans, named here mucosal (m)NKT cells. A third group, termed variant (v)NKT cells, encompasses T lymphocytes reactive to CD1d but that do not have a restricted TCR usage. The fourth group is more heterogeneous because it combines all other T cells that express NK receptors. This group has been termed xNKT cells or IgNKT cells (when they express a TCR transgene). Not listed in Table 1 are T cells subsets reactive with other MHC class I-like or nonclassical class I molecules. Examples include T cells reactive with the human group I CD1 molecules, CD1a, -b, -c, which also present lipid-containing antigens, and T cells specific for the mouse H2-M3 molecule, which present peptides with a formylated amino terminus derived from bacteria. Although these cells may share some properties with the NKT cells described above, they generally do not express NK receptors. In this review, we focus on the two populations of cNKT cells, particularly on their role in mucosal immunity.

THE JANUS-LIKE CHARACTER OF NKT CELLS

The largest and best-studied fraction of NKT cells carries a canonical V_{α}14 to Jα18 TCR rearrangement (V_{α}14i) in mice and an orthogonal V_{α}24-Jα18 TCR chain (V_{α}24i) in humans. These are coexpressed with a limited set of Vβ-chains, predominantly Vβ8.2 in mice and Vβ11 in humans, although these have highly diverse rearrangements to Jβ-segments. V_{α}14i and V_{α}24i iNKT cells recognize glycolipid structures, presented by CD1d, a nonpolymorphic MHC class I homolog. There is a surprising degree of interspecies cross-reactivity, with mouse V_{α}14i NKT cells recognizing human CD1d and vice versa. The recently described trimolecular structure of a glycosphingolipid (GSL) bound to human CD1d, which is recognized by a V_{α}24i TCR (6), illustrates the importance of

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Table 1. Features of different NKT cell subsets

<table>
<thead>
<tr>
<th>NKT Cells (also known as iNKT cells)</th>
<th>mNKT cells (also known as vNKT cells)</th>
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<tr>
<td>TCR repertoire</td>
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<td>Invariant V(^{14})J(^{18})NKT</td>
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<td>BbGL, iGb3, Sulfatide</td>
<td>BbGL, iGb3, Sulfatide</td>
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<td>Diverse peptides</td>
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<td>Restriction</td>
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<td>TH1, TH2, IFN-(\gamma), IL-12, IL-17</td>
<td>TH1, TH2, IFN-(\gamma), IL-12, IL-17</td>
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<td>Positive selection</td>
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<td>Thymus, hematopoietic cell</td>
<td>Thymus, hematopoietic cell</td>
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<tr>
<td>DN, CD4, CD8a, CD44++, CD62L low</td>
<td>DN, CD4, CD8a, CD44++, CD62L low</td>
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<td>Coreceptors</td>
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<td>Prominent steady-state location</td>
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<td>Liver, spleen</td>
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\(\alpha\)-GalCer, \(\alpha\)-galactosylceramide; \(\alpha\)-ManCer, \(\alpha\)-mannosylceramide; mNKT, mucosal natural killer T cells.

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As noted above, there is inherent ambiguity in the term “NKT cell.” However, since the development of CD1d tetramers loaded with \(\alpha\)-galactosylceramide (\(\alpha\)GalCer), it has been possible to unequivocally identify the iNKT cell subpopulation (33). As summarized in Fig. 1, \(\alpha\)GalCer is a synthetic GSL compound that is able to strongly activate iNKT cells when bound to CD1d. Furthermore, researchers have been able to probe the functions of these cells using iNKT cell-deficient mice, either \(Ja18^{-/-}\) mice, which lack only iNKT cells, or \(Cd1d^{-/-}\) mice, which lack iNKT and the vNKT cells that have more diverse TCRs. Even as they differentiate in the thymus, iNKT cells express a pattern of cell surface markers (CD69\(^{+}\), CD44\(^{+}\), CD11a\(^{+}\), CD62L\(^{+}\), CD122\(^{+}\)) typically associated with activated or memory T cells. After activation, iNKT cells rapidly gain cytotoxic activity and produce both T helper type 1 (TH1) cytokines, such as IFN-\(\gamma\) and TNF, and T helper type 2 (TH2) cytokines, such as IL-4, IL-10, and IL-13. Recent evidence suggests that subsets of iNKT cells can also produce IL-17 (34).

Because of their rapid initiation of effector functions, iNKT cells have been reported to be crucially involved in the early phases of a dazzling variety of different immune reactions, ranging from self-tolerance and development of autoimmunity to include responses to pathogens and tumors (30, 62). In the different studies, iNKT cell have been shown to be either beneficial or harmful, depending on whether they polarize the immune response either toward a TH1 or TH2 direction, and the beneficial or detrimental effects of such a cytokine polarization in the context of different models. For this reason, others have referred previously to their “Janus-like” character.

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![Fig. 1. Pictorial representation of some of the main features of canonical natural killer T (\(\alpha\)NKT) cells. DN, double negative; \(\alpha\)GalCer, \(\alpha\)-galactosylceramide; iNKT, invariant natural killer T cells; \(\alpha\)ManCer, \(\alpha\)-mannosylceramide; mNKT, mucosal natural killer T cells.](http://ajpgi.physiology.org/)
NKT CELL ACTIVATION BY SELF ANTIGENS

The effector or memory phenotype of NKT cells and their constitutive expression of mRNA for IL-4 and IFN-γ suggest that they undergo a strong antigenic stimulation during their differentiation. This is consistent with the hypothesis that true TCR agonists mediate their positive selection (30). In line with this idea is the observation that mature NKT cells can, under some circumstances, recognize endogenous glycolipids bound to CD1d. However, elucidation of the glycolipids involved in this endogenous recognition process has proven to be quite challenging. Several suggestions have been put forward (for reviews, see Refs. 30, 62); however, for these cases, only a minor fraction of the NKT cells were responsive. The best candidate to date for a more general endogenous ligand or autoantigen for NKT cells is isoglobotrihexosylceramide (iGb3), a GSL found in the lysosomes of some cell types. The majority of NKT cells can recognize iGb3 when bound to CD1d (69). The inability of mutant mice to synthesize iGb3 in lysosomes, through catabolism of a tetrasaccharide precursor, has been linked to Vα14/NKT cell deficiency (69). However, recent data have raised doubts about whether iGb3 is the only endogenous antigen driving NKT cell development (31). Particularly striking is the finding that mice deficient for iGb3 synthase have normal NKT cell number and function (47). It seems possible that several different self-antigens can drive CD1d-restricted NKT cell development.

NKT CELL ACTIVATION BY MICROBIAL ANTIGENS

Abundant evidence has established an important role for NKT cells in the host defense against several pathogens, especially in the early phases of infection (30, 62, 68). Before the discovery of iGb3, however, the only compound presented especially in the early phases of infection (30, 62, 68). Before this endogenous recognition process has proven to be quite challenging. Several suggestions have been put forward (for reviews, see Refs. 30, 62); however, for these cases, only a minor fraction of the NKT cells were responsive. The best candidate to date for a more general endogenous ligand or autoantigen for NKT cells is isoglobotrihexosylceramide (iGb3), a GSL found in the lysosomes of some cell types. The majority of NKT cells can recognize iGb3 when bound to CD1d (69). The inability of mutant mice to synthesize iGb3 in lysosomes, through catabolism of a tetrasaccharide precursor, has been linked to Vα14/NKT cell deficiency (69). However, recent data have raised doubts about whether iGb3 is the only endogenous antigen driving NKT cell development (31). Particularly striking is the finding that mice deficient for iGb3 synthase have normal NKT cell number and function (47). It seems possible that several different self-antigens can drive CD1d-restricted NKT cell development.

NKT CELLS IN THE INTESTINE

Many of the studies reporting the presence of NKT cells in the intestine have relied on the coexpression of the TCR/CD3ε complex and NK cell receptors, such as NK1.1 in mice and CD161 in humans, which does not allow for the distinction of the four NKT cell populations shown in Table 1. An unequivocal identification is to date only possible for the cNKT cells, either by measuring mRNA for the canonical α-chain TCRs (mNKT and iNKT cells) or by flow cytometry using CD1d tetramers loaded with αGalCer (iNKT cells).

Up to 4% of mouse small intestine (SI) intraepithelial lymphocytes (IELs) (4, 18, 25, 39), 8–10% of large intestine (LI) IELs (4, 25), and 7% of LI lamina propria lymphocytes (LPLs) (20) have been reported to coexpress the TCR-CD3ε complex and NK cell receptors. Most of these cells were CD8α+, and two-thirds thereof were CD8αβ+ (2, 25). It has been noted that a significant portion of these CD3εNK1.1+ cells were γδ T cells (25) and therefore not part of the four NKT cell populations defined here. Furthermore, most of these cells were CD1d independent (4, 25). With the use of CD1d tetramers loaded with αGalCer, a few (1%) iNKT cells have been detected in SI IELs (33), but they were more prevalent (2%) in LPLs (49). Furthermore, cells derived from colitic LI LPLs responded to αGalCer (20). Interestingly, 80% of the iNKT cells were NK1.1+ (33). However, in other studies, no tetramer-positive cells, invariant mRNA, or responsiveness to αGalCer could be detected in IELs (49). From these reports, one might conclude that the majority of NKT cells in the mouse intestine are CD1d independent (εNKT cells) and that iNKT cells are mainly found in LPL.

In humans, the values for NKT cells have been expressed as percentages of CD3ε+ cells, rather than total lymphocytes. The following proportions of T cells were also positive for CD161: 50–70% for SI IELs (23, 41), 40–45% for LI IELs (23, 41), and 9% for LI LPLs (14). Of these cells, 60–80% were CD8α+ (23, 41). The dependence on CD1d could not be addressed; however, only 1.6–1.7% of the CD3ε/CD161+ IEL cells expressed Vα24 (23, 41), and immunohistochemistry data suggested that the majority of the Vα24+ cells in the SI are actually located in the lamina propria (16). Furthermore, the majority of these Vα24+ cells did not express the Vβ11 TCR chain associated with the invariant TCR (41). Therefore, the great majority of CD161+ T cells in human intestine are not NKT cells, similar to results obtained by analysis of human peripheral blood mononuclear cells (PBMCs). Together with αGalCer-loaded CD1d tetramer data from LPL (14), one can estimate that the proportion of NKT cells in the human intestine is <0.4% of all T cells and that they are, like in the mouse, mainly localized in the lamina propria. Nonetheless,
these iNKT cells were functionally active and produced cytokines after αGalCer stimulation (41).

**CD1d EXPRESSION IN THE MUCOSAL IMMUNE SYSTEM**

As noted above, an important role for iNKT cells is possible even in the absence of CD1d expression within the local environment because of cytokine-mediated or indirect activation. However, CD1d expression obviously is a prerequisite for the local, antigen-specific activation of iNKT and γδT cells. Because most hematopoietic cells express CD1d, CD1d also is likely to be found on antigen-presenting cells in the intestine. An example of biologically relevant CD1d expression on antigen-presenting cells in the intestine is provided by studies of mesenteric lymph node B cells. It had been found that colitis in TCRα−/− mice is exacerbated when these mice are also deficient for B lymphocytes (37). There also was an increase in colitis when the TCRα−/− mice were CD1d−/−, and a subset of B cells in the mesenteric lymph node increased their expression of CD1d under inflammatory conditions (37). Transfer of this B cell subset to TCRα−/− mice that were also B cell deficient prevented the increased colitis observed in these mice (37). Transfer of mesenteric lymph node B cells also prevented colitis induction in the Gaα2−/− model (66); however, in both of these cases, it is uncertain whether T cell reactivity to the CD1d abundantly expressed by the B cells was required for their beneficial effect.

Conflicting reports have been made regarding the expression of CD1d on human and mouse intestinal epithelial cells (IECs). One reason for the controversy is the unusual form of CD1d that IECs were reported to express. Interestingly, the majority of the CD1d expressed by IECs is nonglycosylated and not associated with β2-microglobulin, and it is localized mainly inside the cells, with surface expression restricted to the apical surface (2, 28, 29, 56). Its function remains to be elucidated, and there is no evidence for the recognition of this form of CD1d by NKT cells, although it has been suggested that some T cells, presumably γδT cells, recognize β2-microglobulin-independent CD1d molecules (3, 10, 43). Mouse monoclonal antibodies that recognize native CD1d made by several groups do not detect this β2-microglobulin-independent form of CD1d; consequently, they do not detect CD1d expression by IECs, contributing to the above-mentioned controversy. The native β2-microglobulin-associated CD1d molecule is weakly expressed by human IECs with a preference for the basal surface (56). IECs have been reported to bind (50) and present αGalCer (64), suggesting that this low amount of native CD1d expression is significant, but IECs could not present derivatives requiring carbohydrate antigen processing in lysosomes (64).

Contradictory data regarding the expression of CD1d in patients with colitis have been reported. Whereas one study reported higher expression of CD1d in affected tissue (42), two other reports found lower expression (15, 46). The production of the anti-inflammatory cytokine IL-10 has been linked to CD1d expression by IEC lines and primary cells, and this required an intact cytoplasmic domain of CD1d, implying a signaling function for this molecule (11, 37). Furthermore, mice lacking microsomal triglyceride transfer protein, an endoplasmic reticulum resident lipid transfer protein, display a defective loading and presenting capability of CD1d and were largely protected against oxazolone-induced colitis (8). This protection was linked to IECs, as microsomal triglyceride transfer protein within the intestine is preferential expressed by IECs; however, the authors did not directly address the role for CD1d expressed by IEC.

**NKT CELLS IN INFLAMMATORY BOWEL DISEASE**

Most studies addressing the role of iNKT and γδT cells in the intestine were concerned with inflammatory bowel disease (IBD) model systems. IBD denotes a variety of what are likely to be T cell-dependent diseases of different parts of the intestine, as a consequence of an inflammatory response to antigens derived from the gut lumen. This inflammation may follow perturbation of the epithelial layer, either through genetic alterations or the administration of exogenous agents. NKT cells have been implicated in several animal models of IBD, and, although the data are not entirely consistent, the findings correlate with a protective role for NKT cells in Th1-mediated IBDs and a deleterious one in Th2 IBDs.

Crohn disease is characterized by a chronic and discontinuous inflammation of both the SI and LI, with high levels of Th1 cytokines. Clinical studies have reported a significant reduction of iNKT cells, measured as Vα24/Vβ11+ or αGalCer-loaded CD1d tetramer-positive cells, in the peripheral blood of affected patients (17, 65) and reduced Vα24 mRNA levels and Vα24-expressing cell numbers in the intestine (17). A decrease of iNKT cells in the PBMC and of Vα24+ T cells in the intestine has also been reported for patients with celiac disease (16). However, as noted above, the majority of these Vα24+ cells were not iNKT cells, as judged by the absence of Vβ11 expression (41).

For three mouse colitis models characterized by a Th1 immune response, the involvement of NKT cells has been reported, including 1) adoptive cell transfer of naïve CD4 T cells into immune-deficient hosts, 2) oral challenge with dextran sodium sulfate (DSS), and 3) rectal instillation of the hapten trinitrobenzene sulfonic acid (TNBS). In the transfer model of colitis, cotransfer of T cells expressing the NK receptor DX5 with the pathogenic CD4 T cells ameliorated disease, and this effect could be blocked by injection of an anti-CD1d antibody (21). However, because only a minority of DX5+ NKT cells are CD1d dependent (45), it is not certain which NKT cell population was responsible for the protection, and, given the induction of IL-10 by anti-CD1d, it is possible that the antibody triggered CD1d functions independent of their interactions with subsets of NKT cells. DDS-induced colitis could be ameliorated by treatment with the iNKT cell agonists αGalCer (38, 50) or the closely related compound OCH (63). OCH has been reported to shift the immune response stimulated by iNKT cell activation to the Th1 cytokine pattern (36); consistent with this, the protection in the DSS colitis model was directly linked to the increased production of IFN-γ (63). A similar protection by OCH treatment was observed for TNBS-induced colitis; importantly, treatment with OCH was even effective at ameliorating an ongoing disease in the DSS model (63). Although in one study a single injection of αGalCer led to a shift to a protective Th2 response (50), two later studies reported that repetitive challenges were necessary (38, 63). This is in line with the reported Th2 shift by repetitive αGalCer treatment (9). These data clearly demonstrate the protective

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CANONICAL NKT CELLS IN MUCOSAL IMMUNITY AND INFLAMMATION
potential of iNKT cells that have been activated by potent agonists to shift the immune response from the deleterious T\(\gamma\)1 cytokine pattern in these diseases to a protective T\(\gamma\)2 response. There is also a profound lack of IL-4 production by mononuclear cells purified from the affected intestines of patients with Crohn disease (26, 67). However, it is important to note that the depletion of NK and NKT cells had no effect on the course of TNBS-induced colitis (20), and, in the absence of activation by potent synthetic agonists, it remains to be demonstrated in these models whether the normal functioning of NKT cells influences colitis.

Oxazolone-induced colitis is an IBD model in which T\(\gamma\)2 cytokines induce pathology. The mucosal immune response following oxazolone exposure is initially dominated by IL-4, which is soon superseded by an IL-13 response (20). The IL-13 following oxazolone exposure is initially dominated by IL-4, cytokines induce pathology. The mucosal immune response genes

The mucosal immune response. After intragastric infection of patients with ulcerative colitis, it was found that activation prevented disease development (8, 20). However, in infected C57BL/6 mice, but not in other strains, this T\(\gamma\)1 immune response leads to lethal acute ileitis, due to overwhelming IFN-\(\gamma\) production. The potential of NKT cells to produce copious amounts of IFN-\(\gamma\) after stimulation makes them a candidate for the source of the pathogenic IFN-\(\gamma\) in T. gondii-infected C57BL/6 mice. In accordance with this, it has been shown that Ja\(18^{+/−}\)/C57BL/6 mice are less susceptible to ileitis after infection with T. gondii (49). The deleterious effect of NKT cells in wild-type C57BL/6 mice could almost entirely be blocked by pretreatment with αGalCer (49).

Systemic challenge of the mice with αGalCer 1 day before T. gondii infection led to a pronounced T\(\gamma\)2 cytokine shift of the NKT cells, with markedly reduced IFN-\(\gamma\) and increased amounts of IL-4 and IL-10 (49). IL-10, produced mainly by NKT cells and by CD4/CD25\(^+\) regulatory T cells, was shown to be the main agent for protection from runaway inflammation (49). Surprisingly, however, the same report also showed that Cd1d\(^−/−\) mice had entirely the opposite phenotype compared with the Ja\(18^{+/−}\) mice. After T. gondii infection, the Cd1d\(^−/−\) animals had an increased mortality (49), and similar data on Cd1d\(^−/−\)/C57BL/6 mice were reported by another group (55). Both reports are examples of the surprising differences between Ja\(18^{+/−}\) animals, specifically lacking NKT cells, and Cd1d\(^−/−\) mice, which not only lack all CD1d-reactive iNKT and vNKT cells but also other potentially important CD1d-dependent pathways, for example those based on CD1d-mediated signal transduction (see above).

Despite the clear involvement of iNKT and vNKT cells in many different systemic and intestinal infections and their rapid activation by pathogenic and even nonpathogenic microbes such as Sphingomonas spp., iNKT cell-deficient mice have not been reported to be more susceptible to spontaneous infections with what are normally commensal bacteria. Furthermore, NKT cells themselves are not dependent on commensal bacteria, as even in germ-free animals iNKT cells are phenotypically activated/memory T cells that function normally after antigenic stimulation (44).

mNKT CELLS

Less is known about a second population of canonical NKT cells found in humans and mice. As noted, these cells express an invariant Vα14-Jo33 (mouse)- or Vα2-7-Jo33 (human)-rearranged TCR α-chain (for reviews, see Refs. 60, 61). The invariant TCR contains only a few N-nucleotide additions, and the coexpressed V\(\beta\) usage is skewed to V\(\beta\)13 and V\(\beta\)2 in human and V\(\beta\)8.1/8.2 and V\(\beta\)6 in mice (51, 57).

Although mNKT cells have been detected in blood, lymph nodes, liver, spleen, and bone marrow (27, 51, 53, 58), they are most prominent under steady-state conditions in mucosal sites of the gut and the lung (27, 59–61), hence the appellation mNKT cells, also called mucosal-associated invariant T (MAIT) cells (59). By PCR analysis for the invariant rearrangement, mNKT cells are especially enriched in the lamina propria and mesenteric lymph node and virtually absent in the
intestinal epithelium (27, 59, 60). In this context, the observation that Vα19i mNKT cells are absent in germ-free mice (59, 60) is quite interesting because it suggests that microbially derived stimuli, either directly or indirectly, are required for their selection and/or expansion. Vα19i mNKT cells are absent in athymic nu/nu (nude) mice (58), and hematopoietic cells mediate their thymic-positive selection, rather than the cortical epithelial cells that positively select conventional T cells (59). Furthermore, Vα19i mNKT cells are absent in B cell-deficient mice and in patients with a mutation in Bruton’s tyrosine kinase, which leads to a profound B cell deficiency (59). Innate-like B1-B cells and plasma cells are not involved, however, in forming the mNKT cell population (59, 60). Whether this dependency reflects a requirement for the few thymic B cells to select Vα19i mNKT cells in the thymus, or perhaps more likely their expansion, homeostasis, or homing in the periphery, is so far unresolved (59–61). In line with the latter hypothesis, the few intestinal IgA⁺ B cells that develop in μ-heavy chain-deficient (μMT⁻/⁻) mice were sufficient for the development of Vα19i mNKT cells (59).

Vα19i mNKT cells are restricted by MR1 (59), a β2m-dependent nonpolymorphic MHC class I-like molecule encoded by a gene linked to the Cd1d gene (Fig. 1). Considering the entire family of antigen-presenting molecules, MR1 shows the highest degree of conservation between mouse and human (19, 35). Although MR1 mRNA is ubiquitous (19), cellular expression of the protein is still incompletely characterized. In MR1-transfected cell lines, most of the protein is retained in the endoplasmic reticulum; therefore, it has been suggested that attainment of a native conformation leading to MR1 surface expression might be limited by the availability of a specific MR1-binding ligand(s) (35). Consistent with this hypothesis, addition of a surplus of serum components could increase the surface expression of MR1 (22, 35). This and other indirect evidence (22, 35) make it very likely that MR1 acts as an antigen-presenting molecule. Furthermore, because some Vα19i mNKT cell hybridomas were activated spontaneously by MR1-transfected cell lines (22, 59), there may be a substantial degree of autoreactivity by Vα19i mNKT cells, similar to their CD1d-dependent iNKT cell counterparts. Endogenous MR1-binding ligands for potentially self-reactive mNKT cells are not known so far, but it has recently been reported that α-mannosylceramide and particularly some related compounds with modifications of the ceramide lipid can activate Vα19i NKT cells after binding to MR1 (40, 52–54).

Consistent with their potential autoreactivity, mNKT cells display an effector/memory phenotype in the periphery characterized by the expression of CD27⁺, CD44high, CD45RA⁻, and CD57⁻ in humans (59) and CD25low, CD44high, CD45RBhigh, CD62L⁺, and CD69⁺ in mice (27). Most mNKT cells are CD4⁻ CD8⁻ (double negative) (27, 58), ~60–80% in mice (27). Although in mice Vα19i mNKT cells are never CD8α⁺ (27, 58), some human Vα7.2i NKT cells express CD8α (58). Furthermore, mNKT cells express several costimulatory molecules such as CD28, CD154, and inducible costimulator (ICOS) (12, 27, 51, 59), as well as NK cell receptors such as NK1.1 in mice and CD161 in humans (12, 24, 27, 51, 53, 59). Finally, Vα7.2i mNKT cells have been shown to express the integrin α₄β₇, which is required for gut homing (59).

mNKT cells are ~5–10 times more frequent in humans than in mice, accounting for ~15% of all double-negative T cells in PBMCs (i.e., 0.1–0.2% of all T cells) and for 10% of double-negative T cells in the intestine (59–61). Because of their paucity in mice, the generation of Vα19i transgenic animals has greatly facilitated the investigation of this subpopulation (27, 40), complemented by the generation of MR1-deficient mice (59). Although the high evolutionary conservation of mNKT cells and their prominence in the intestine suggest an important role in the mucosal immune system, no nonredundant function of mNKT cells in the intestinal mucosa has yet been described. For example, the frequency of Vα7.2i NKT cells is not altered in patients with Crohn disease or ulcerative colitis (61).

Similar to iNKT cells, mNKT cells rapidly produce effector cytokines like IL-4, IL-5, IL-10, IL-17, and IFN-γ after activation via MR1 or αCD3ε antibody in vitro and in vivo (12, 27, 52, 53, 59). mNKT cells also have the capacity to migrate to sites of inflammation, where their cytokines could influence the inflammatory process. It has been reported that they accumulate in some central nervous system lesions and in the cerebral fluid of patients with multiple sclerosis, as well as in peripheral nerve samples of patients with chronic inflammatory demyelinating polyneuropathy (24). The same group demonstrated a protective role of Vα19i NKT cells during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (12). Overexpression of Vα19i NKT cells ameliorated the disease, whereas depletion of Vα19i NKT cells exacerbated it (12). Furthermore, donor Vα19i NKT cells could transfer protection (12). The data indicate that the interaction of NK1.1⁺ Vα19i T cells and B cells via ICOS/ICOS-L, and to a lesser extent via CD40/CD154, induced the production of protective IL-10 by both cell populations (12). In this setting, the interaction of TCR and MR1 seemed not to be required (12).

SUMMARY

For iNKT and mNKT cells, there are a number of striking similarities. Both are selected in the thymus by nonpolymorphic MHC class I-like molecules expressed by hematopoietic cells. Interestingly, CD1d and MR1 are encoded in close proximity on chromosome 1 (19), perhaps suggesting a similar evolutionary selection and function. Both cNKT cell populations display a phenotype characteristic of activated or memory cells, they rapidly secrete effector cytokines, and they exhibit a degree of autoreactivity. Both cNKT cell populations have been reported to be specific for glycolipids, although the antigens recognized by mNKT cells are not yet well characterized. Additionally, both populations are prominent in sites other than the lymph node, such as the intestine for mNKT cells or the liver, which collects circulation from the intestine, for iNKT cells. iNKT cells require CD1d in the periphery for their final differentiation, and mNKT cells depend on MR1 expression on B cells and the gut flora for their homeostasis and localization to the intestine. Selection by hematopoietic cells in the thymus might imprint the properties that cNKT cells share. In this context, it is of interest to note that the forced selection of MHC class II-reactive CD4 T cells in transgenic mice that express MHC class II only on thymocytes also led to the development of T cells bearing an effector/
memory phenotype and the ability to rapidly secret cytokines after activation (32).

From their shared properties, it has been suggested that NKT and mNKT cells might play similar roles, although in different environments (60). It could be hypothesized that the continuous presence of different endogenous or exogenous ligands presented by CD1d or MR1 guide differential NKT and mNKT cell homing and retention. However, although NKT and mNKT cells are enriched in different organs, it should be kept in mind that there is by no means a complete segregation of these two populations.

There is evidence for a role for NKT cells and CD1d expression in mucosal immunity and inflammation. CD1d-dependent NKT cells maybe even more prevalent and more important in humans, however, as suggested by a study of cells from patients with ulcerative colitis. By contrast, the function of mNKT cells in the intestine has yet to be established. So far, it has not been possible to produce MR1 tetramers, and the paucity of reagents for detecting and purifying mNKT cells surely has hampered progress in their study. However, given their prevalence in the lamina propria, their ability to produce effector cytokines, and their demonstrated ability to regulate inflammation in other sites including the nervous system using mouse models, it is likely that an important role for mNKT cells in mucosal immunity will be uncovered soon.

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