Commensal microbial regulation of natural killer T cells at the frontiers of the mucosal immune system

Sebastian Zeissig, Richard S. Blumberg

Abstract

The commensal microbiota co-exists in a mutualistic relationship with its human host. Commensal microbes play critical roles in the regulation of host metabolism and immunity, while microbial colonization, conversely, is under control of host immunity and metabolic pathways. These interactions are of central importance to the maintenance of homeostasis at mucosal surfaces and their perturbation can provide the basis for atopic and chronic inflammatory diseases such as asthma and inflammatory bowel disease (IBD). Recent evidence has revealed that natural killer T (NKT) cells, a subgroup of T cells which recognizes self and microbial lipid antigens presented by CD1d, are key mediators of host-microbial interactions. Mucosal and systemic NKT cell development is under control of the commensal microbiota, while CD1d regulates microbial colonization and influences the composition of the intestinal microbiota. Here, we outline the mechanisms of bidirectional cross-talk between the microbiota and CD1d-restricted NKT cells and discuss how a perturbation of these processes can contribute to the pathogenesis of immune-mediated disorders at mucosal surfaces.

1. Introduction

Recent sequencing-based approaches have revealed an unforeseen complexity of the commensal microbiota, which has co-evolved with its human host, and provides the basis for numerous mutualistic traits [1,2]. At birth, newborns leave an environment considered sterile and are rapidly colonized by microbial organisms derived from the maternal gestational tract as well as the skin of caregivers [3–5]. While initial colonization is rapid, development of an individual, adult-like microbial composition reflects a gradual process, which requires several years for completion [3–6]. It is this dynamic period of early postnatal microbial colonization that is most susceptible to environmental and host genetic perturbations, yet at the same time most critical for microbial-dependent development of the host’s immune system [7]. Thus, the commensal microbiota plays central roles in neonatal immune maturation including the development of cryptopatches and isolated lymphoid follicles, the maturation of Peyer’s patches, the formation of a secretory IgA response, the regulation of intestinal mucus and antimicrobial peptide secretion, and the control of mucosal and systemic T cell differentiation [8]. Accordingly, mice raised in a germ-free (GF) environment or treated at neonatal age with broad-spectrum antibiotics exhibit a variety of alterations of the innate and adaptive immune system, which may predispose to atopic and chronic inflammatory disorders such as asthma and IBD [9,8,7]. While the commensal microbiota is critical for the control of host immunity and the prevention of immune-mediated disorders, host-microbial interactions are bidirectional and the host’s immune system is involved in shaping the composition of the commensal microbiota [1]. In line with this concept, a number of primary immune defects have been described, which are associated with alterations in the composition of the intestinal microbiota and which contribute to microbial-dependent inflammatory as well as metabolic diseases [10–14].

Interestingly, CD1d-restricted NKT cells have emerged as key mediators of host-microbial interactions at mucosal surfaces. Mucosal and systemic NKT cell development and maturation is regulated by the commensal microbiota and the perturbation of this process contributes to the pathogenesis of allergic and inflammatory disorders. Conversely, CD1d and NKT cells actively participate in the regulation of commensal microbial colonization. Here, we provide a brief overview of the classification and function of...
NKT cells, discuss how the commensal microbiota shapes NKT cell development at mucosal surfaces and in the systemic periphery, outline the mechanisms of CD1d-dependent control of the intestinal microbiota, and discuss the consequences of the perturbation of these processes.

2. Natural killer T cells

NKT cells are a diverse group of T cells, which are defined by their ability to recognize self- and non-self-derived lipids in the context of CD1d [15,16]. Various phenotypically and functionally distinct subsets of NKT cells have been described and can be distinguished based, for example, on the lipids recognized by these cells, their T cell receptor (TCR) repertoire, the expression of particular cell surface markers, or the cytokines produced [16]. Most commonly, however, NKT cells are distinguished on the basis of their TCR repertoire. Invariant (i) or type I NKT cells express an invariant TCR-α chain, composed of Vα14/Jα18 in mice and Vα24/Jα18 in humans, paired with a restricted set of TCR-β chains [15,16]. Type I NKT cells can be specifically detected using CD1d tetramers loaded with the marine sponge glycosphingolipid α-galactosylceramide (αGalCer), which has contributed to the extensive characterization of these cells. In addition, mice deficient in iNKT cells through genetic deletion of the Jα18 chain have allowed to delineate the roles of iNKT cells in vivo, although recent work has revealed that these mice harbor additional defects in the TCR repertoire, which may have affected results obtained with these mice [17]. While iNKT cells are adaptive immune cells, which recognize antigens in a TCR-dependent manner, they share various features of innate immune cells. As such, their TCR recognizes diverse CD1d-restricted lipids in a manner reminiscent of pattern recognition receptors (PRRs) [18,19]. Moreover, iNKT cells constitutively transcribe cytokine mRNAs and secrete abundant amounts of cytokines in an instant, innate-like manner upon activation [20]. Invariant NKT cells are thus located at the interface of innate and adaptive immunity, where they act as potent regulators of mucosal and systemic immunity and control antimicrobial immune responses, autoimmunity, and tumor development [16]. At mucosal surfaces, iNKT cells exert beneficial effects through their involvement in antimicrobial immunity, but can also actively contribute to the pathogenesis of chronic immune-mediated disorders. Accordingly, iNKT cells contribute to intestinal inflammation in human IBD and are required for inflammation and inflammation-associated tumorigenesis in mouse models of IBD and colitis-associated cancer [21–26]. Moreover, iNKT cells contribute to pulmonary inflammation and airway hyperreactivity (AHR) in human asthma and mouse models of AHR [27–29]. iNKT cells thus play central roles in mucosal immunity, which are associated with both beneficial and detrimental effects, and which are regulated by the commensal microbiota as further outlined below.

In addition to iNKT cells, a subset of phenotypically and functionally diverse CD1d-restricted T cells expressing non-Vα14/Vα24 TCRs has been described and termed non-invariant or type II NKT cells. A marker specific for type II NKT cells has not been described. For this reason, our knowledge of these cells is based on non-invariant NKT cell clones, the analysis of type II NKT cells with known lipid reactivity using CD1d tetramers, and the indirect characterization of type II NKT cells based on comparative studies of Jα18-deficient mice (lacking type I NKT cells) and CD1d-deficient mice (lacking both type I and II NKT cells). In accordance with the diversity of the non-invariant NKT cell subset, these cells play critical, but often tissue- and subset-specific roles in immune regulation. As such, the sulfatide-reactive subset of type II NKT cells has been demonstrated to suppress autoimmunity in mouse models of multiple sclerosis and non-infectious hepatitis [30,31]. In contrast, type II NKT cells contribute to hepatic inflammation in viral hepatitis [32], while intestinal lysosulfatide-reactive type II NKT cells are involved in inflammation and tissue damage in human ulcerative colitis [22,25,26]. Non-invariant NKT cells thus play diverse roles in immunity and can contribute to chronic immune-mediated disorders such as IBD.

3. The commensal microbiota controls mucosal iNKT cell development

Early studies in the pre-tetramer era suggested that NKT cells exhibit unimpaired development in the absence of the intestinal microbiota [33]. As such, the thymus, bone marrow, spleen, and liver of GF mice harbored similar numbers of NKT cells, as defined by co-expression of the αβ-TCR and the NK marker NK1.1, compared to specific pathogen-free (SPF) mice. NKT cell expression of activation, memory, and NK cell markers also did not differ between GF and SPF mice and the proliferation of spleen cells in response to the CD1d-restricted, iNKT cell-activating lipid αGalCer was indistinguishable between SPF and GF mice [33]. Together, these data suggested that the commensal microbiota is not required for the development of NKT cells. More recent studies, which applied αGalCer/CD1d-loaded tetramers to unequivocally identify iNKT cells, and which studied mucosal compartments in addition to other primary and secondary lymphoid organs, confirmed the notion that iNKT cells, both in humans and mice, are present in the absence of the commensal microbiota [34–38]. However, these studies also revealed a complex interplay of the microbiota and iNKT cells, which is required for control of mucosal iNKT cell homing and proliferation, for the maturation of iNKT cells in the systemic periphery, and for prevention of iNKT cell-associated inflammation at mucosal surfaces [34–38].

The analysis of iNKT cells at mucosal surfaces, and in particular in the intestine, suggested that their abundance is indirectly related to bacterial density with higher iNKT cell numbers observed in the small intestine compared to the large intestine and in the lamina propria compared to the intraepithelial compartment [36]. Consistent with the concept of negative regulation of intestinal iNKT cell numbers by the commensal microbiota, increased relative and absolute numbers of iNKT cells were found in the intestine but not the spleen, liver, and thymus of GF compared to SPF mice [35,36]. Microbial regulation of intestinal iNKT cell numbers was shown to occur early in postnatal development with effects persisting for life, as evidenced by the restoration of intestinal iNKT cell numbers upon microbial colonization of neonatal but not adult GF mice [35]. Interestingly, these principles were not specific to the intestine but similarly observed in the lung, where iNKT cell numbers were found to be increased in GF mice, which could be restored by microbial colonization at neonatal but not at adult age [35]. In addition, microbiota-dependent regulation of mucosal iNKT cells differed from that observed for conventional TCRαβ T cells, which were significantly decreased in numbers in intestinal compartments of GF mice and rats and which expanded in response to microbial colonization [39–42].

Early and persistent effects of the commensal microbiota on mucosal iNKT cells are dependent on CD1d and are achieved both through the regulation of homing of iNKT cells to the mucosa as well as the control of proliferation of resident iNKT cells within the mucosal tissue. Olasz et al. reported that a CpG island 5' of the Cxcl16 gene is hypermethylated in the colon and lungs of GF mice, which is associated with increased epithelial expression of CXCL16, and CXCL16-dependent mucosal homing of iNKT cells (Fig. 1) [35]. Thus, microbiota-dependent epigenetic control of the Cxcl16 locus regulates iNKT cell homing to the colon and lung. An additional pathway of intestinal iNKT cell regulation was
Thereby negatively regulating neonatal expansion of resident mucosal iNKT cells, the immune system of the host may not be able to confer protection against microbial pathogens and when the immature adaptive immune response is still developing. This iNKT cell-dependent defense may be critical during neonatal life, when the unstable commensal microbiota regulates mucosal iNKT cell numbers through effects on iNKT cell homing and the proliferation of resident iNKT cells.

While most studies that investigated microbial regulation of mucosal iNKT cell development focused on model organisms, elegant studies by Loh and colleagues recently extended these findings to humans [38]. The authors investigated fetal human intestinal tissues to study iNKT cell development during the prenatal period and thus before microbial colonization of the host. Intriguingly, similar to observations in GF mice [35,36], the human fetal small intestine contained large numbers of iNKT cells, which were phenotypically and functionally mature as characterized by the expression of activation, memory, and NK cell markers and the secretion of TNF-α and IFN-γ. Furthermore, in line with the observation of local mucosal iNKT cell proliferation in neonatal mice [37], fetal human small intestinal iNKT cells readily proliferated in response to CD1d-restricted antigens and/or cytokines. Thus, similar to findings in mice, human intestinal iNKT cells can develop and mature in the absence of the commensal microbiota and strongly proliferate in response to lipid antigens. These observations are in contrast to conventional intestinal T cells, which are present in intraepithelial and lamina propria compartments before birth, but exhibit significant expansion during postnatal development, likely in response to microbial colonization [43].

One potential interpretation of the negative regulation of mucosal iNKT cells by the microbiota is that microbiota-independent prenatal and neonatal mucosal iNKT cell development provides a first-line, innate-like defense at mucosal surfaces during the early postnatal period. This iNKT cell-dependent defense may be critical during neonatal life, when the unstable commensal microbiota may not provide sufficient ‘colonization resistance’ against invasion by microbial pathogens and when the immature adaptive immune system of the host may not be able to confer protection against these pathogens. With the emergence of a stable commensal microbiota and the maturation of host adaptive immunity, iNKT cell-mediated antimicrobial immunity at mucosal surfaces may become less critical and may in fact contribute to mucosal inflammation, as further discussed below. As such, negative regulation of mucosal iNKT cell development by the commensal microbiota may represent an important developmental switch between a period of neonatal iNKT cell-dependent antimicrobial immunity and the prevention of iNKT cell-mediated pathology later in life.

### 4. Commensal microbial mediators in the regulation of mucosal iNKT cell development

Several questions arise from the observation of commensal microbial regulation of mucosal iNKT cells. Are bacterial lipid antigens, microbe-associated molecular patterns (MAMPs) or microbial metabolites involved in this process? Are the observed effects specific for bacteria or for particular bacterial phyla, families or species? Are there distinct but functionally redundant pathways of commensal microbial regulation of mucosal iNKT cells? Some of these questions have been addressed in recent studies, the findings of which will be discussed in this section.

MAMPs can potently activate iNKT cells through indirect pathways involving toll-like receptor (TLR) signaling by dendritic cells (DCs) and cytokine-dependent stimulation of iNKT cells by DC-derived cytokines such as IL-12- and IL-18 [44-46]. While these indirect pathways can lead to iNKT cell activation in the absence of lipid antigen presentation [45], iNKT cells are most potently activated by a combination of cytokine signals and CD1d-restricted lipid antigen presentation [44,46]. As such, these pathways have been suggested to act as the predominant mode of iNKT cell activation in the context of microbial infection [44,46]. However, although the commensal microbiota provides rich sources of MAMPs, indirect TLR- and cytokine-dependent effects on iNKT cells do not seem to be the primary mechanism responsible for commensal-dependent regulation of mucosal iNKT cells. Thus, mice deficient in IL-12 or the TLR adaptor MyD88 do not exhibit alterations in mucosal iNKT cell numbers or the iNKT cell phenotype [35,36]. Moreover, recent studies have presented evidence for direct, lipid antigen-dependent regulation of mucosal iNKT cells. An et al. demonstrated that monoclonalization of neonatal GF mice with B. fragilis but not with a mutant strain deficient in serine palmitoyltransferase, an enzyme required for sphingolipid biosynthesis, restores mucosal iNKT cell levels [37]. Lipidomics and subsequent functional studies revealed that B. fragilis contains an abundant α-galactosylceramide (BF717), which binds to CD1d but fails to activate iNKT cells [37]. In this manner, BF717 limits CD1d-dependent colonie mucosal iNKT cell proliferation during early postnatal development and contributes to mucosal homeostasis (Fig. 1). The observation of increased CD1d-dependent expansion of mucosal iNKT cells in the absence of commensal-derived inhibitory CD1d lipids such as BF717 also indicates that host-derived iNKT cell-activating CD1d lipids contribute to mucosal iNKT cell proliferation during neonatal development. Although little is known about potential self antigens involved in mucosal iNKT cell regulation, it was recently reported that non-invariant intestinal NKT cells exhibit broad reactivity to the host-derived sphingolipid lysocteramide, which suggests that similar self lipid antigens may exist for iNKT cells [26].

Further complexity arises from the observation that microbial-derived CD1d lipids differ in their ability to elicit iNKT cell activation and proliferation. Thus, Wieland Brown et al. reported an α-GalCer derived from B. fragilis, which activated iNKT cells and promoted their expansion, in contrast to BF717 derived from the same bacterial species (Fig. 1) [47]. Moreover, a CD1d-binding choleste-
ryl glucoside derived from *Helicobacter pylori* (PI57) was shown to be associated with the expansion of a regulatory subset of iNKT cells in the lung, which contributed to prevention of AHR in mice [48]. Given the complexity of the commensal microbiota, future studies are likely to reveal a large number of additional microbial-derived CD1d lipids involved in the modulation of mucosal iNKT cell development. In addition, further work is required to delineate the structure–function relationships underlying the observed divergent effects of microbial lipids on iNKT cells.

Finally, it is worthy to note that, in contrast to iNKT cells, little is known about commensal microbial regulation of type II NKT cells. However, recently described strategies to detect a least a subset of type II NKT cells, based for example on CD1d tetramers [31,26] or the detection of spontaneous IL-4 transcription by type II NKT cells in mice lacking iNKT cells [32,49], will enable such analyses in the near future and will provide insight into the microbial regulation of non-invariant NKT cells at mucosal surfaces.

5. Microbial regulation of NKT cell-mediated mucosal inflammation

The intestinal microbiota plays critical but often complex roles in the pathogenesis of immune-mediated diseases such as IBD and asthma. Infections can elicit flares of these diseases in humans, and disease models in mice are either dependent on the presence of the intestinal microbiota (IBD) or actively elicited by microbial antigens (asthma) [7]. However, antibiotic use in childhood is associated with an increased risk for development of IBD and asthma, suggesting that interference with commensal microbial colonization may promote disease susceptibility [7]. Given that iNKT cells play central roles in inflammation and tissue damage in IBD and asthma [25], the observation of microbial regulation of iNKT cells raised the question of whether alterations in commensal microbial colonization may promote iNKT cell-dependent inflammation at mucosal surfaces. In line with this concept, it was recently reported that GF mice, which harbor increased numbers of mucosal iNKT cells [35,36], exhibit severe CD1d-dependent tissue damage and inflammation in mouse models of asthma and IBD [35,37]. Susceptibility to disease was prevented by commensal microbial colonization of GF mice at neonatal but not at adult age in accordance with early and persistent effects of the microbiota on mucosal iNKT cell expansion by the commensal microbiota [52], these results suggested a potential microbial origin of the observed differences in iNKT cells. Consistently, GF mice as compared to SPF mice also exhibited differences in iNKT cell phenotype and function [22,26]. In accordance with a pathogenic role of type II NKT cells [53], together, these data suggest that control of mucosal iNKT cell homing and proliferation by the commensal microbiota is critical for prevention of iNKT cell-mediated pathology at mucosal surfaces.

Remarkably, the observation of severe, CD1d-dependent inflammation in GF mice also indicates that iNKT cell-mediated inflammation does not require microbial antigens and may indeed be elicited by self-derived lipids in accordance with a *bona fide* autoimmune disease. This would further indicate that inhibition of mucosal iNKT cell expansion by the commensal microbiota is critical to prevent pathogenicity elicited by autoreactive iNKT cells, which have escaped negative selection in the thymus. Consistent with the concept of NKT cell-mediated autoreactivity and tissue damage, recent work by the Strober group demonstrated that a major fraction of lamina propria cells in human ulcerative colitis, a subgroup of IBD, recognizes the *self*-derived glycosphingolipid lysosulfatide in the context of CD1d, which is associated with IL-13 production and cytotoxicity against the intestinal epithelium [22,26]. Lysosulfatide reactivity was CD1d-restricted thus confirming an NKT cell-dependent response. However, lysosulfatide-reactive T cells could not be stained with α-GalCer-loaded CD1d tetramers in line with the presence of non-invariant or type II NKT cells [22,26]. In accordance with a pathogenic role of type II NKT cells in intestinal inflammation, Liao and colleagues previously observed that transgenic expression of a non-invariant NKT cell TCR is associated with spontaneous intestinal inflammation in mice, particularly in the presence of transgenic CD1d expression [50]. Inflammation in this model, however, was dependent on the intestinal microbiota as demonstrated by protective effects of broad-spectrum antibiotics, which is in contrast to observations made for iNKT cells. These findings reveal the complexity of commensal microbial NKT cell regulation at mucosal surfaces, which is dependent on the respective subgroup of NKT cells, the nature of microbial lipid antigens, and the timing of microbial exposure. Furthermore, recent observations suggest that the type of antigen presenting cell (APC) involved in CD1d-restricted antigen presentation to NKT cells also influences the outcome of these interactions. Thus, CD1d-restricted interactions of iNKT cells with intestinal epithelial cells (IECs) promote IL-10 secretion and mucosal homeostasis, while CD1d-dependent interactions with bone marrow-derived APCs contribute to intestinal inflammation [51]. Whether these divergent responses are the sole consequence of differences in the expression of costimulatory molecules by IECs and professional APCs or whether cell type-specific differences in CD1d trafficking and lipid acquisition contribute to this process is currently unclear.

6. Commensal microbial control of iNKT cells at systemic sites of immunity

Intriguing recent work has revealed that commensal microbial regulation of iNKT cell development is not restricted to mucosal surfaces but also extends to systemic sites of immunity. Wingender et al. noted that genetically identical inbred mice obtained from different commercial vendors exhibited differences in TCR Vβ usage and cytokine production by thymic, splenic, and hepatic iNKT cells [36]. These differences in iNKT cell phenotype and function disappeared upon co-housing of newborn mice from different vendors thus confirming the involvement of a transmissible factor [36]. As mice from these commercial vendors had been reported to harbor a distinct intestinal microbiota [52], these results suggested a potential microbial origin of the observed differences in iNKT cells. Consistently, GF mice as compared to SPF mice also exhibited differences in iNKT cells including an altered TCR Vβ usage of thymic and splenic iNKT cells, reduced expression of activation markers by thymic, splenic, and hepatic iNKT cells, and impaired cytokine secretion by splenic iNKT cells in response to CD1d-restricted antigen presentation (Fig. 2) [36]. Interestingly, similar findings were made for fetal human iNKT cells, which develop in the absence of the commensal microbiota. Thus, while fetal small intestinal iNKT cells were phenotypically and functionally mature, their splenic counterparts exhibited reduced expression of activation, memory, and NK cell markers as well as increased expression of naïve T cell markers [38]. In contrast to iNKT cells, TCR Vβ usage of conventional T cells in the thymus as well as cytokine secretion by splenic conventional T cells was unimpaired in GF mice, again suggesting that microbial regulation of iNKT cells differs from that observed for conventional T cells [53]. Together, these fascinating observations demonstrate that the commensal microbiota not only influences iNKT cell development at mucosal surfaces but also elicits distant effects, which govern iNKT cell development and maturation in the systemic periphery as well as in primary lymphoid organs.

The mechanisms underlying distal effects of the commensal microbiota remain to be defined but may include microbial metabolites, microbe-associated molecular patterns, bacterial-derived CD1d lipids as well as changes in host-derived CD1d lipids as a consequence of microbial colonization. While cytokine secretion by professional APCs in response to PRR engagement can indirectly activate iNKT cells as discussed above, unimpaired iNKT cell devel-
Thymic iNKT cell selection (?)  
Thymic & peripheral iNKT maturation

Liver  
Microbial lipids?  
Microbial metabolites?  
Cytokines, chemokines?

Intestine

Fig. 2. The commensal microbiota shapes the systemic development of iNKT cells. Compared to SPF mice, GF mice show an altered TCR Vβ usage of thymic and splenic iNKT cells, reduced expression of activation markers by thymic, splenic, and hepatic iNKT cells, and impaired cytokine secretion by splenic iNKT cells [36]. Similarly, human fetal splenic iNKT cells exhibit reduced expression of activation, memory, and NK cell markers as well as increased expression of naive T cell markers compared to iNKT cells in peripheral blood of adults [38]. These observations suggest that the commensal intestinal microbiota regulates the maturation of iNKT cells in the systemic periphery. In addition, altered thymic TCR Vβ usage suggests that the maternal or neonatal commensal microbiota may influence thymic iNKT cell selection and development. The mechanisms of systemic iNKT cell regulation by the commensal microbiota are unknown. Colonization of GF mice with bacteria containing CD1d lipids, but not with those devoid of CD1d lipid antigens, can restore iNKT cell development, which indirectly suggests the involvement of microbial-derived CD1d lipids in this process [36]. Alternatively, microbial metabolites or host-derived cytokines and chemokines secreted upon microbial recognition may contribute to systemic iNKT cell development.

7. CD1d in the regulation of commensal microbial colonization

As discussed above, the commensal microbiota controls the development and function of NKT cells at mucosal and systemic sites of immunity. Since host immunity shapes the composition of the intestinal microbiota [11], these findings raise the question of whether the CD1d-NKT axis may also exert control of the commensal microbiota in the sense of a feedback mechanism involved in host-microbial mutualism. Indeed, recent evidence suggests that CD1d and NKT cells are not only critical for the control of microbial pathogens but also regulate commensal microbial colonization dynamics and the composition of the intestinal microbiota. Nie-uwenhuis and colleagues observed that CD1d-deficient mice exhibited increased colonization of the lung upon exposure to Pseudomonas aeruginosa, a Gram-negative bacterium associated with clinically relevant infections of the airways and the urinary tract [54]. Based on these observations, the authors investigated whether similar mechanisms of CD1d-mediated control of bacterial colonization also apply to the intestine and whether they extend to members of the commensal microbiota. Reminiscent of findings made in the lung, CD1d-deficient compared to WT SPF mice exhibited increased small but not large intestinal colonization with P. aeruginosa. Studies in GF mice further revealed that CD1d is required for the control of intestinal colonization by both Gram-negative and Gram-positive members of the commensal intestinal microbiota. As such, E. coli, Staphylococcus aureus, and Lactobacillus gasseri exhibited a dramatically accelerated course of intestinal colonization in GF CD1d-deficient mice compared to WT littermates [54]. Interestingly, bacteria seemed to persist at higher numbers in the proximal small intestine but not the ileum, cecum or colon of CD1d-KO mice, suggesting that colonization defects may originate from the small intestine. Indeed, Paneth cells (PCs), which are located at the bottom of small intestinal crypts and which are involved in microbial control through the secretion of antimicrobial peptides (AMPs), exhibited defects in the morphology of AMP-containing granules as well as impaired AMP release in response to bacterial colonization in CD1d-KO mice. In addition, administration of the iNKT cell-activating lipid α-GaCer was associated with degradation of PCs in WT but not CD1d-KO mice. Together, these results demonstrate that interactions between CD1d and NKT cells are required for PC function and PC-mediated control of microbial colonization.

PC-derived AMPs influence the composition of the intestinal microbiota and limit the translocation of bacteria into mesenteric lymph nodes (MLNs) [55,11]. Impaired AMP secretion in CD1d-KO mice thus raised the question of whether these mice also exhibit alterations in bacterial trafficking and the composition of the intestinal microbiota. Indeed, monoclonization of GF CD1d-KO but not WT mice with E. coli was associated with bacterial translocation into MLNs [54]. Moreover, CD1d-KO mice maintained under SPF conditions harbored a distinct fecal microbiota compared to WT littermates, which included a higher percentage of Bacteroidetes and a lower percentage of the Bacilli class of Firmicutes [54]. CD1d thus regulates the composition of the intestinal microbiota and limits transepithelial bacterial translocation in the intestine.

The precise mechanisms through which CD1d-dependent interactions between APCs and NKT cells regulate PC function remain to be identified. NKT cells release abundant amounts of cytokines, some of which influence the composition of the intestinal microbiota. It is therefore possible that forward signaling by CD1d associated with NKT cell activation and cytokine production governs NKT cell-mediated control of the microbiota. In addition, CD1d is expressed by the intestinal epithelium including PCs [56,57] and is subject to STAT3-dependent retrograde epithelial signaling upon engagement of CD1d by NKT cells [51]. It is therefore tempting to speculate whether reverse signaling by epithelial CD1d contributes to PC function. Thus, further work is required to elucidate the mechanisms of CD1d- and NKT cell-mediated control of intestinal microbial colonization.

8. Conclusion and perspective

Recent work has revealed a complex network of interactions between the commensal microbiota and CD1d-restricted NKT cells,
which together govern microbial colonization and control NKT cell-dependent immunity and immunopathology. The relevance of these interactions is highlighted by the observation that perturbation of the cross-talk between NKT cells and the microbiota provides the basis for severe NKT cell-mediated inflammation at mucosal surfaces. Despite these advances, further work is required to delineate the spectrum of commensal-derived lipids involved in CD1d-restricted regulation of iNKT cells and to define the structural elements, which dictate the functional consequences for iNKT cell activation and function. In addition, the application of recently developed tools for the identification of type II NKT cells as well as CD1a-, CD1b-, and CD1c-restricted T cells will be critical to investigate whether commensal microbial regulation is specific for iNKT cells or similarly observed for non-invariant NKT cells and other groups of lipid-reactive T cells.

Are cell type-dependent effects of CD1d-NKT cell interactions [51] the consequence of differences in the CD1d-associated lipid repertoire? Are temporal changes and regional differences in the commensal microbiota associated with distinct effects on NKT cells? Are other exogenous factors such as nutrition-derived metabolites involved in prenatal development of mucosal NKT cells, similar to observations made for innate lymphoid cells [58]? And finally, is CD1d-dependent regulation of PCs the consequence of retrograde CD1d signaling or of forward signaling by NKT cell-derived cytokines? These are critical questions, which remain to be addressed, and which may ultimately provide novel opportunities for the prevention and treatment of immune-mediated diseases through interference with commensal microbial NKT cell regulation.

References


