Trends in Immunology

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Review

Tolerance to the Intestinal Microbiota Mediated by ROR(γt)$^+$ Cells

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Harmless microbes colonizing the gut require the establishment of a well-equilibrated symbiosis between this microbiota and its host. However, the immune system is primed to recognize both conserved microbial patterns and foreign antigens, and therefore developed strong tolerance mechanisms to prevent potential fatal immune reactivity to symbiotic microbes. Transcription factor RAR-related orphan-like γt [ROR(γt); encoded by *Rorc*] has been identified as a key determinant of lymphoid tissue organogenesis via expression by lymphoid tissue inducer cells (LTi) and later by proinflammatory type 17 T helper (Th17) cells. Surprisingly, recent research has revealed a contribution of ROR(γt)-expressing cells in a variety of tolerance mechanisms in both the innate and adaptive immune systems.

Diverse Functions of ROR(γt)$^+$ Cells

The highest density of microbial colonization occurs in the gut, and the microbiota allows the host to more efficiently use a wider range of nutrients as energy sources, to prevent infections, and more generally to maintain a better 'fitness' of this collective superorganism [1]. Both the innate and adaptive immune system can recognize diverse foreign molecular patterns or antigens and integrate these signals for the induction of an effective and well-adapted immune response. A special subset in the gut, termed innate lymphoid cells type 3 (ILC3s), together with a subset of innate-like γδ T cells, express the lineage-defining transcription factor ROR(γt) [2,3]. Characteristic for ROR(γt)$^+$ innate cells is the expression of large amounts of the cytokines IL-22 and IL-17, both of which are crucial for the enforcement of the epithelial barrier integrity [4,5]. In addition to constitutive cytokine secretion, ILC3s have been recently shown to express major histocompatibility complex molecules type II (MHC-II) and present microbiota-derived antigens to naïve CD4$^+$ T helper cells [6]. The adaptive arm of the immune system, including T helper (Th) cells, generates its antigen-recognition receptors through random gene rearrangements which are negatively selected for self-recognition. In the periphery, additional passive and active tolerance mechanisms are required to prevent antigen-specific activation by harmless environment-, microbe-, or food-derived antigens. Surprisingly, antigen presentation via ILC3s results in deletion of microbiota-reactive T cells and therefore shapes the T cell receptor (TCR) repertoire of intestinal T helper cells to prevent constant activation of the adaptive immune system [7]. Nevertheless, colonization of the gut with microbes, and especially with epithelium-attaching bacteria, activates the adaptive immune system and leads to the induction of ROR(γt)$^+$ Th17 cells [8–11]. Th17 cells have gained much attention owing to their prominent role in multiple autoimmune diseases and in fighting extracellular infections [12] but, together with ROR(γt)$^+$ ILC3s, they also contribute to the regulation of the intestinal barrier [13]. In addition to Th17 cells, microbe- [14,15] but not food-derived antigens [16] induce a subset of Foxp3$^+$ regulatory T cells (Tregs) co-expressing ROR(γt)

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A high frequency of both innate and adaptive cells that express the transcription factor ROR(γt) characterizes the intestinal immune system, suggesting that 'type 3' immune cells contribute to intestinal homeostasis and tolerance of the intestinal microbiota.

ROR(γt)$^+$ innate lymphoid cells (ILC3s) contribute to the enforcement of the epithelial barrier in multiple ways and help to physically separate the microbial from the host world.

Furthermore, ILC3s have been shown to express MHC-II molecules and influence adaptive T helper cell responses in the gut.

Adaptive ROR(γt)$^+$ Th17 cells are preferentially induced upon colonization with epithelium-attaching bacteria, and may be subdivided into 'pathogenic' and 'tolerogenic' Th17 cells.

ROR(γt)$^+$ Foxp3$^+$ regulatory T cells in the gut are induced upon normal colonization with microbes and contribute to intestinal immune regulation.

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[17,18]. Such ROR(γt)$^+$ Tregs have recently been shown to efficiently protect from intestinal immunopathology in different colitis models [14,15,19]. This raises the question whether the physiological role of a ROR(γt)-directed transcriptional program consists of preventing inappropriate immune activation by the symbiotic microbiota.

Physical Separation Between Microbiota and Host Is Regulated by Innate ROR(γt)$^+$ Cells

ROR(γt) was identified in the immune system as an essential transcription factor of LTi cells [2,20] which are now considered as a subset of ILC3s [3]. ROR(γt)$^+$ ILC3s as well as a subset of ROR(γt)$^+$ γδ T cells in the gut share the expression of IL-22 and IL-17A in both mice and humans [21]. Intestinal ILC3s arise from a common precursor in the fetal liver [22] or the bone marrow [23]. Lineage differentiation of ILC3s requires the expression of ROR(γt) in both mice [3,24] and humans [25]. Interestingly, the efficient differentiation of ILC3s is also dependent on a vitamin A metabolite, retinoic acid [26], which in turn regulates the expression pattern of gut-homing chemokines of ILC3s [27]. Even before birth, functional ILC3s populate the intestinal lamina propria, preparing the host for microbial colonization [4]. Mature ILC3s in the gut show a high degree of tissue residency and do not recirculate systemically either at steady-state or during inflammation underlying their local impact on the intestinal barrier [28]. Activation of the IL-23 receptor and signaling via STAT3 are thought to be the main elicitors of IL-22 secretion by ILC3s [5,29]. By contrast, intestinal γδ T cells are able to secrete IL-17A independently of IL-23. The IL-17A provided by these cells is necessary to maintain functional tight junctions between intestinal epithelial cells, an essential parameter for the regulation of barrier permeability during inflammation [30,31]. Nonetheless, inappropriate IL-23 secretion can also ~~produce~~ innate and adaptive colitis [32,33], and transgenic overexpression of IL-23 promotes neonatal pathology and death as a result of expansion and hyperactivation of ILC3s even before birth [34]. Similarly, ROR(γt)$^+$ ILC3s can drive intestinal inflammation in a model of anti-CD40-mediated innate colitis through the combined secretion of IFN-γ, IL-17, GM-CSF, and IL-22 [32,35]. Thus, and similarly to T cells (reviewed in [36]), ROR(γt)-dependent ILCs can acquire a state of shared type 1 and type 3 cytokine secretion that may be considered to be highly pathogenic.

The continuous secretion of IL-22 by ILC3s in the intestine activates epithelial cells to produce antimicrobial peptides (AMP) such as RegIIIγ, RegIIIβ, S100A9, or S100A8 [37,38]. In addition, ILC3s express lymphotoxin β, which triggers IL-23 secretion by dendritic cells (DCs) and thus induces a positive feedback loop [39]. AMPs together with the mucus layer are essential parameters for the spatial separation between the intestinal epithelial barrier and the microbiota [4,38]. In addition, ILC3-derived IL-22 and lymphotoxins enforce host–microbiota symbiosis by fucosylation of intestinal epithelial cells to prevent overgrowth by opportunistic bacteria or pathogens [40,41]. In line with this notion, ILC3s and the cytokine IL-22 have been shown to maintain intestinal stem cells after tissue damage to rapidly restore barrier integrity upon epithelial damage [42].

Not surprisingly, the enforcement of the epithelial barrier plays a central role in the immune response to orally transmitted pathogenic bacteria. For instance, IL-22-producing ROR(γt)$^+$ ILC3s have been shown to promote partial resistance to infection with *Citrobacter rodentium* [43,44], *Salmonella typhymurium* [40], or *Clostridium difficile* [45]. The immune response induced by activated intestinal ILC3s is therefore crucial in fighting oral infections and may prevent the systemic dissemination of life-threatening bacteria early after infection.

In addition to enforcing the epithelial barrier, intestinal ROR(γt)$^+$ ILC3s have been shown to secrete high amounts of granulocyte/macrophage colony-stimulating factor (GM-CSF) that is

responsible for the recruitment of (inflammatory) mononuclear cells to the intestine [35,43,46,47]. GM-CSF production by ILC3s in turn is dependent on IL-1β and IL-23 secretion by CX_3CR1^+ cells triggered upon sensing symbiotic microbes [46,48]. Therefore, ROR(γt)$^+$ ILC3s also shape the myeloid compartment in response to symbiotic microbes.

In general, innate ROR(γt)$^+$ cells enforce the epithelial barrier and regulate the physical separation from symbiotic microbes at steady-state. Nevertheless, no massive inflammation is observed in mice with constitutive deficiency of ILC3s or their key cytokines. Thus, the function of ROR(γt)$^+$ ILC3s may be more important after an abrupt alteration of the intestinal homeostasis – as may naturally occur during infection or epithelial injury. However, hyperactivation of ROR(γt)$^+$ ILC3s has been shown to contribute to intestinal inflammation depending on the disease model applied. These models differ dramatically in their mode of action, and it is likely that each model mimics only particular aspects of intestinal inflammation in humans – and may thus serve as surrogates to study immune-triggered pathologic symptoms rather than causative mechanisms, for example intestinal barrier dysfunction (DSS-colitis), hyperactivation of innate immunity (anti-CD40 injection, IL-23 transgenic overexpression), or infection with pathogens (*C. rodentium*, *C. difficile, S. typhimurium*).

ILC3s Shape Adaptive Immune Responses to the Microbiota

The adaptive immune response to the microbiota is directly or indirectly regulated by ROR(γt)$^+$ ILC3s. A prototype example is the genetic knockout of the arylhydrocarbon receptor (AhR) in ROR(γt)$^+$ ILC3s which results in the expansion of a particular member of the microbiota, termed segmented filamentous bacteria (SFB) [49]. These bacteria in turn promote the induction and expansion of ROR(γt)$^+$ Th17 cells in the intestine [10,50]. Furthermore, AhR-deficient ILC3s are not able to induce intestinal lymphoid follicles [51], and hence prevent the generation of affinity-matured IgA [51,52]. Physical separation and regulation of gut microbiota composition is mediated by a gradient of microbiota-reactive IgA molecules that are transcytosed into the intestinal lumen [53]. Noteworthy, ROR(γt)$^+$ ILC3-derived lymphotoxins have also been shown to promote the generation of T cell-independent and -dependent IgA [54,55]. Therefore, ROR(γt)$^+$ ILC3s indirectly regulate expansion of Th17 via limitation of SFB colonization and control IgA responses to oral pathogens and commensals.

Recently, it was shown that ROR(γt)$^+$ ILC3s express MHC-II molecules in both mice and humans, and this enables them to present foreign antigens [6,7]. Genetic knockout of MHC-II molecules in ROR(γt)$^+$ ILC3s was sufficient to deregulate intestinal adaptive $CD4^+$ T helper cells and induce spontaneous intestinal inflammation. The improvement after treatment with broad-spectrum antibiotics suggests that most $CD4^+$ T helper cells respond to microbiota-derived antigens, although a purely adjuvant effect of the microbiota, for example on the antigen-presenting cells (APCs), cannot be completely excluded. Among other T effector cells, Th17 cells specific for microbiota-derived antigens were expanded in mice lacking MHC-II on ILC3s [6]. Nevertheless, ILC3-derived GM-CSF was shown to regulate oral tolerance to dietary antigens by augmenting the myeloid cell production of cytokines and retinoic acid that are required for the efficient induction of Tregs [46]. Importantly, antigen presentation by ILC3s themselves did not result in the induction of Tregs but rather in the deletion of a commensal-reactive T cell clones through induction of apoptosis [7]. However, genetic ablation of MHC-II on conventional DCs also induces intestinal inflammation characterized by impaired T follicular helper cell accumulation and consequently reduced IgA induction [56]. In contrast to mice lacking MHC-II molecules on ROR(γt)$^+$ ILC3s, the DC-specific ablation of MHC-II results in reduced frequencies of induced Tregs in the gut and associated lymphoid organs. Mucida and colleagues recently confirmed that classical DCs are necessary to induce antigen-specific tolerance to dietary antigens through the induction of peripheral Tregs [57].

The question emerges of why ROR(γt)$^+$ ILC3s and professional APCs such as DCs or macrophages show a division of labor in the regulation of T helper cells in response to symbiotic microbes. First, ROR(γt)$^+$ ILC3s are generally found in cryptopatches and lymphoid follicles, or are dispersed in the intestinal lamina propria, and do not have direct contact with the microbiota at steady-state [2,47]. By contrast, specific dendritic cell subsets are able to extend their dendrites into the intestinal lumen and constantly sample antigens from microbes in close proximity to the epithelium [58,59]. In addition, DCs sample antigens beneath the M cells in Peyer's patches and may acquire phagocytosed antigens from CX_3CR1^+ macrophages via gap junction transfer [60]. CD103$^+$ DCs are thought to frequently migrate to draining lymph nodes where the local milieu including resident stromal cells preferentially drives the induction of Foxp3$^+$ Tregs at steady-state [61,62]. Therefore, different accessibilities to antigens and presentation at different anatomical sites may influence the impact of the respective APC type on the adaptive Th cell response. Second, the functional outcome of antigen presentation is fundamentally different between DCs and ILC3s because the latter provoke deletion of commensal reactive T cells rather than activation and differentiation to Treg or T effector phenotypes [6,7]. Similarly to thymic epithelial cells during negative selection of T cells – and in contrast to classical APCs – ROR(γt)$^+$ ILC3s lack the dynamic regulation of costimulatory molecules such as CD40, CD80, or CD86 upon stimulation with TLR ligands, and may thus induce 'negative selection' of microbiota-reactive T cells [6].

Q3 In summary, presentation of microbial antigens by DCs may be more regulated and tightly controlled to induce efficient tolerogenic or effector T cell responses, whereas antigen presentation by ROR(γt)$^+$ ILC3s can be seen as a 'safeguard' to ~~prevent~~ T cells reactive against luminal antigens ~~from reaching~~ the host side.

Pathogenic and Protective ROR(γt)$^+$ Th17 Cells at Barrier Sites

The differentiation of IL-17-secreting T cells from naïve T cells is induced by the cytokines IL-6 and TGF-β1, and ROR(γt) expression is generally seen as a master regulator of Th17 cell differentiation [12,50]. In addition to their very prominent role in autoimmune diseases, Th17 cells are found in non-inflammatory tissues at steady-state where, together with ROR(γt)$^+$ ILC3s, they contribute to maintain epithelial barrier integrity [13]. Interestingly, even in some autoimmune diseases, the accumulation of Th17 cells can be associated with alteration of epithelial barriers, for example psoriasis-like diseases or Crohn's disease (CD). Th17 cells secrete a variety of cytokines, including IL-17A and IL17F, IL-22, IL-26, and GM-CSF, which has led to the idea of targeting Th17 cells themselves [e.g., via the transcription factor ROR(γt)] rather than single cytokines for the treatment of autoimmune disorders (reviewed in [63]). Interestingly, transient inhibition of ROR(γt) ~~produced~~ a reduction of IL-17A production from Th17 cells and intestinal inflammation after *C. rodentium* infection, while preserving functionality of ILC3s [64]. However, complete ROR(γt)-deficient animals show an enhanced fraction of bacteria-specific IgG immunoglobulins at steady-state and suffer from aggravated inflammation in DSS-mediated chronic colitis despite the absence of potentially proinflammatory Th17 cells [17]. Furthermore, ex-Th17 cells giving rise to follicular Th cells in Peyer's patches have been implicated in the generation of high-affinity IgA molecules upon oral immunization [65]. Along these lines, Foxp3$^+$ Tregs have also been shown to promote diversification of the intestinal microbiota and regulation of IgA selection, both mandatory for intestinal homeostasis [66]. Whether ROR(γt)$^+$Foxp3$^+$ Tregs (see below) contribute to regulation of IgA selection remains to be established but is not unlikely given their close relationship to the microbiota [14,15,67].

The highest frequencies of Th17 can be found in the ileum of mice harboring epithelium-attaching SFBs [10,11]. The presence of SFB or a complex microbiota has been associated with more severe Th17-associated autoimmune reactions at different anatomical sites [68,69] but it seems unlikely that this process is antigen specific because most SFB-induced Th17 cells

are SFB-specific and therefore not the result of a polyclonal bystander activation [70,71]. Another possibility for the microbiota/Th17 cell axis to contribute to autoimmune disorders is molecular mimicry of the antigen: antigens from the microbiota have been shown to activate retina-specific Th17 cells preceding the migration to the immune privileged eye and induction of autoimmune uveitis [72].

The intestinal microbiota undergoes constant alterations depending on environmental changes, e.g., food intake, use of antibiotics, infections, behavior and personal hygiene. Recently, even circadian rhythms were found to alter the relative composition of the microbiota [73]. Similarly, Th17 cell differentiation varies with the circadian rhythm in an NFIL3-dependent manner which blocks transcription of ROR(γt) in Th17 cells [45,74]. Thus, both Th17 cell differentiation and symbiotic microbes are subjected to the same biological mechanism even though no direct link has been identified [74].

The prominent role of Th17 cells in autoimmune disorders and their close association to microbiota and epithelial barriers raises the question whether both 'pathogenic' and 'tolerogenic' Th17 cells exist. Indeed, Th17 'subpopulations' have been identified in the analysis of human *Staphylococcus aureus* – and *Candida albicans* – specific Th17 cell clones that show a unique co-expression pattern with IL-10 or IFN-γ, respectively [75]. Single-cell analysis further revealed a remarkable diversity among Th17 cells that can be separated into 'pathogenic' and 'non-pathogenic' cells [76,77]. For example, the expression of CD5L correlated with a 'non-pathogenic' state of Th17 cells and links this state with an altered metabolic state and availability of ROR(γt) ligands [76]. The pathogenic potential of Th17 cells is further regulated by IL-23-induced genes such as Blimp-1 and TGF-β3 [78,79]. Regulatory networks controlling Th17 cell specification and identity may in the future provide evidence for different transcriptional regulation of protective and pathogenic Th17 cells [80,81].

Relationship and Stability of Th17 and Treg Cells

As discussed before, Th17 cells are a diverse population and pathogenicity may be dictated solely by TCR specificity against 'self' versus 'foreign' antigens. This notion has important consequences for the observed plasticity between Th17 and Tregs: First, both subsets have been thought to share the requirement of TGF-β for differentiation [82] but pathogenic Th17 cells may differentiate even without TGF-β [83]. Further, pathogenicity of murine Th17 cells during experimental autoimmune encephalomyelitis (EAE) can be explained by ROR(γt)-driven expression of GM-CSF, but not IL-17A or IL-17F whereas GM-CSF expression in humans is independent of ROR(γt) [84–86]. Co-expression of Foxp3 and ROR(γt) in a subpopulation of Th cells has led to the idea of an intermediate state of differentiation between Th17 and Treg cells explaining a subsequent equilibrium between both subsets [87]. This equilibrium or the equilibrium between Th17 cells and ROR(γt)$^+$ Tregs may be regulated by the availability of IL-2 and IL-6 in the microenvironment [88–90]. Second, Th17 cells specific for (foreign) pathogenic antigens may convert more rapidly into a non-inflammatory state after resolution of the infection than self-reactive Th17 cells: Fate reporter systems demonstrated that cells with a history of IL17A expression show a marked difference among fate-mapped Th17 cells after chronic EAE or acute *Candida albicans* infection because ex-Th17 cells transdifferentiated into pathogenic T effector cells during EAE in an IL-23-dependent manner, whereas *Candida*-experienced ex-Th17 cells did not [91]. Th17 cells are further able to convert into IL-10-secreting Foxp3$^-$ Tregs (Tr1) during the resolution of polyclonal anti-CD3-mediated or autoimmune inflammation [92]. Interestingly, IL-10 signaling via Stat3 in Tregs themselves and in Th17 cells is also required for efficient suppression of Th17-mediated inflammation [93–95]. Moreover, ROR(γt)$^+$ Th17 cells can be recruited to the small intestine after anti-CD3 injection, where they are deleted or acquire a regulatory phenotype [92,96]. Conversely, CD25^{lo}Foxp3$^+$ Tregs are able to convert into

pathogenic Th17 cells in an IL-6-dependent manner in a model of autoimmune arthritis [97]. Nonetheless, CD25^{lo} Tregs probably do not represent *bona fide* Tregs, irrespective of Foxp3 expression, as revealed by analysis of epigenetic modifications in the non-coding regions of the *Foxp3* gene [98].

Overall, these results demonstrate the relatively high plasticity of Th17 cells and emphasize the idea that the ~~name-giving~~ IL-17 and ROR(γt) expression by T cells is probably not a key determinant of Th17 pathogenicity in most scenarios. Instead, a heterogeneous population of ROR(γt)$^+$ T cells may acquire tissue-protective or pathogenic functions according to yet to be determined mechanisms.

Identification and Characterization of ROR(γt)$^+$ Tregs

A subpopulation of T helper cells co-expressing the regulatory T cell transcription factor Foxp3 and ROR(γt) has been identified, and was shown to have potent suppressive function *in vitro* [18,89]. The expression of Foxp3 is thought to inhibit ROR(γt)-driven IL-17A expression at steady-state [18,99], but some Foxp3$^+$ROR(γt)$^+$ cells expressing IL17A have been identified in both mice and humans, albeit at lower levels than in Foxp3$^-$ROR(γt)$^+$ T cells [100–102]. Nevertheless, ROR(γt)$^+$ Tregs express high levels of regulatory molecules including IL-10, CTLA-4, and ICOS [15,100]. Transfer experiments and analysis of Treg-specific demethylation region (TSDR) revealed that ROR(γt)$^+$ Tregs probably represent a relatively stable lineage and not merely a Th17/Treg intermediate differentiation state [19,100]. The induction of ROR(γt)$^+$ Treg crucially depends on symbiotic microbes and increases from oral to aboral along the intestinal tract [14,15,67,89]. Individual members of the microbiota differ in their ability to induce ROR(γt)$^+$ Tregs [14], but it is unclear whether microbiota-derived metabolites such as short-chain fatty acids or other determinants regulate their induction [15]. Tregs isolated from the colon – most of which co-express ROR(γt) – but not from secondary lymphoid organs have been shown to recognize microbiota-derived antigens [103]. Even so, Tregs generated and selected in the thymus may contribute to intestinal tolerance [104]. The anatomical site of Treg induction (thymus vs periphery) has been used to discriminate between Tregs selected to recognize self and non-self antigens. In line with this idea, mice lacking peripherally induced Tregs as a result of deletion of a conserved non-coding element next to the *Foxp3* promoter (CNS1) show spontaneous inflammation at mucosal surfaces [105]. Two markers have been proposed to discriminate between thymic and peripherally induced Tregs, namely Helios [106] and neuropilin-1 [107,108], but their usefulness has been recently questioned [109]. Nevertheless, ROR(γt)$^+$ Tregs express only low levels of both markers [14,15]. Interestingly, Tregs specific for neo self-antigens are still generated in the periphery but do express Helios [110]. The expression level of neuropilin-1 and ROR(γt) in Tregs has been further used to delineate peripheral-induced Tregs specific for food- or microbe-derived antigens [16]. Thus, Helios and neuropilin-1, together with ROR(γt), may be used to distinguish between self-reactive versus foreign-reactive Tregs in the gut rather than as markers of the site of differentiation.

ROR(γt)$^+$ Tregs as Novel Players in Intestinal Homeostasis

To elucidate the function of ROR(γt)$^+$ Tregs, several groups generated mouse strains with a selective knockout of ROR(γt) in Tregs [14,15,100]. Such mice show more pronounced inflammation in different models of chemically induced colitis as well as increased type 1, type 2, or type 3 cytokine secretion by T cells [14,15]. In addition, ROR(γt)$^+$ Tregs were more efficient than their ROR(γt)$^-$ Treg counterparts in suppressing transfer colitis [19]. ROR(γt)$^+$ Tregs may be more effective in suppressing microbe-reactive T effector cells because, similarly to T effector cells, microbial recognition by Tregs may be required for suppression of transfer colitis [111]. Thus, Treg quality seems to be more relevant than Treg quantity for the suppression of colitis.

In addition to these tolerogenic effects of ROR(γt)$^+$ Tregs, the absence of ROR(γt)$^+$ Tregs can also result in stronger immune responses that are beneficial in the case of parasitic infections or detrimental in the case of foreign antigen-induced nephritis [15,100]. Nevertheless, Tregs with reduced Gata3 expression as a result of lack of defective IL-33 signaling also fail to efficiently suppress transfer colitis [112]. In light of the mutually exclusive expression of Gata3 and ROR(γt) in Tregs [113], this raises the question whether these two Treg subpopulations use different modes of action for the suppression of colitis.

An absence of ROR(γt)$^+$ Tregs can lead to more-efficient primary or secondary immune responses to parasites and ~~they~~ must therefore be tightly controlled. Taking into account the random generation of the TCR repertoire, it is highly likely that most naïve T cells reacting to commensal- or food-derived antigens undergo anergy induction or deletion simply due to the high number of potential antigens and the restricted 'niche' for Tregs. How the discrimination between deletion and Treg induction is made (e.g., by the microenvironment, the nature of the antigen, or APC status) remains to be discovered, but has certainly important consequences for

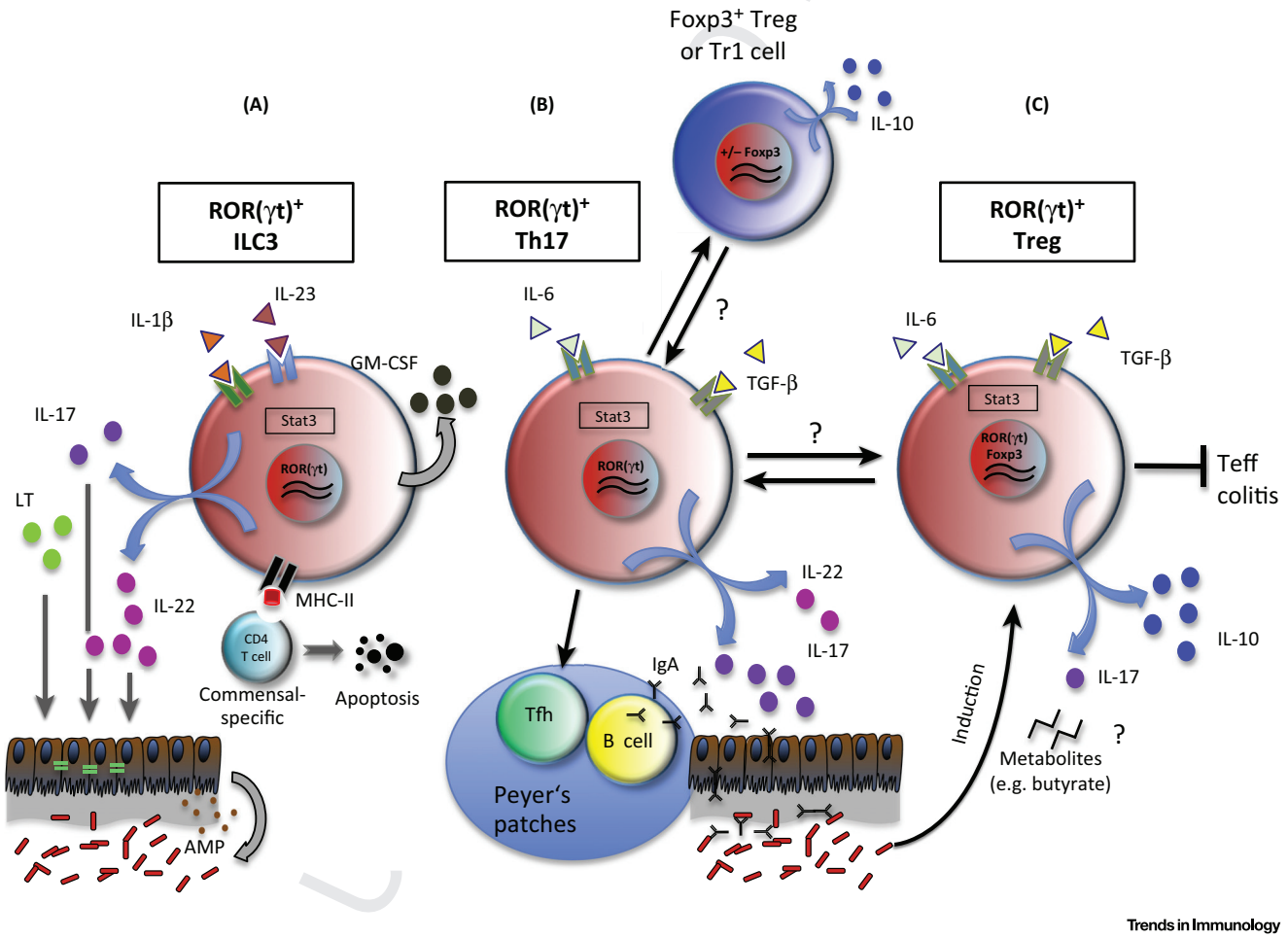

Figure 1. The Role of ROR(γt)$^+$ Cells in Intestinal Tolerance. (A) Innate ROR(γt)$^+$ cells secrete large amounts of IL-22 and IL-17 which, together with lymphotoxins (LT), induce the production of antimicrobial peptides (AMP) for the efficient spatial separation between the intestinal microbiota and the host. Presentation of microbiota-derived peptides by MHC-II molecules on RORγt$^+$ innate lymphoid cells (ILC3s) leads to the induction of apoptosis of microbiota-reactive T helper cells. The secretion of GM-CSF alters the function of intestinal myeloid cells. (B) Intestinal ROR(γt)$^+$ T helper cells secrete mainly IL-17 to regulate barrier integrity. ROR(γt)$^+$ T helper cells can further transdifferentiate into follicular helper T cells (Tfh) in Peyer's patches where they induce IgA class-switching in B cells. Furthermore, transdifferentiation to IL-10-secreting regulatory Tr1 or Foxp3$^+$ regulatory T cells (Tregs) is possible. (C) ROR(γt)$^+$Foxp3$^+$ T cells are induced by the intestinal microbiota and are essential for suppressing effector T cell responses and colitis.

the prevention and therapy of diseases associated with loss of intestinal tolerance. In this context, it will be important to study tissue-resident memory of regulatory and effector T cells in the gut in light of a potentially dynamic microbiota.

Concluding Remarks

Increasing evidence suggests that the transcription factor ROR(γt) – or type 3 immunity – plays a central role for the regulation of mucosal immune responses in response to the intestinal microbiota (Figure 1). The central role of this transcription factor is highlighted by the firm ROR(γt)-dependence of the development and function of the respective cell types. Surprisingly, ROR(γt)$^+$ cells are implicated in a variety of tolerance mechanisms to harmless antigens which – in addition to their role in fighting extracellular infections – illustrates their role in barrier immunity and tolerance. Therefore, ROR(γt)$^+$ cells and associated transcriptional networks may have evolved to tolerate the symbiotic microbiota by enforcing physical separation, deletion of microbiota-reactive T cells, and induction of Tregs. Nevertheless, a variety of questions remain open: how does the immune system fine-tune the reactivity to individual members of the microbiota? Why is there a potential division of labor for presentation of microbial antigens between DCs and ILC3s in the gut? In that regard, better models for the genetic ablation of ILC3s may be mandatory to answer these questions. In addition, it is still unclear how the decision is made between induction of apoptosis and differentiation towards a ROR(γt)$^+$ Th17 or a ROR(γt)$^+$ Treg cell upon TCR-mediated recognition of microbiota-derived antigens by naïve T cells? For ROR(γt)$^+$ Th17 and ROR(γt)$^+$ Tregs, it is not entirely clear which factors determine the respective differentiation pathway because both subsets are dependent on a ROR(γt)-driven transcriptional program (see Outstanding Questions). For instance, do microbial or fungal metabolites contribute to this decision or is this decision fixed early during activation and the recognition of conserved molecular structures by APCs? Finally, it will be important to understand if and how intestinal tolerance by ROR(γt)$^+$ Tregs can be exploited therapeutically to treat chronic intestinal inflammations such as irritable bowel disease (IBD), and whether these cells are also crucial mediators of oral immune defense.

Outstanding Questions

Which mechanism regulates the decision between the induction of apoptosis versus the induction of Treg differentiation upon TCR-mediated recognition of microbiota-derived antigens by naïve T cells?

What factors ~~determine~~ the differentiation ~~of~~ ROR(γt)$^+$ Th17 cells versus the differentiation ~~of~~ ROR(γt)$^+$ Tregs? Do microbial or fungal metabolites contribute to regulating this decision? Q6

What is the functional difference between Gata3- and ROR(γt)-expressing Tregs in the suppression of intestinal inflammation?

Do microbiota-induced Tregs contribute to the suppression of immune responses at different mucosal sites (e.g., the lung)? Can the results from murine studies be extrapolated to humans?

May ROR(γt)$^+$ Tregs be used in the future as a measure of the status of intestinal tolerance? Can this knowledge be exploited therapeutically?

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