REVIEW

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The microbiota in adaptive immune homeostasis and disease

Kenya Honda[1,2,3] & Dan R. Littman[4,5]

In the mucosa, the immune system's T cells and B cells have position-specific phenotypes and functions that are influenced by the microbiota. These cells play pivotal parts in the maintenance of immune homeostasis by suppressing responses to harmless antigens and by enforcing the integrity of the barrier functions of the gut mucosa. Imbalances in the gut microbiota, known as dysbiosis, can trigger several immune disorders through the activity of T cells that are both near to and distant from the site of their induction. Elucidation of the mechanisms that distinguish between homeostatic and pathogenic microbiota–host interactions could identify therapeutic targets for preventing or modulating inflammatory diseases and for boosting the efficacy of cancer immunotherapy.

Microbiotas that establish mutualistic relationships with their mammalian hosts are able to influence a multitude of physiological functions, often through modulation of the host's immune system. Certain bacteria that inhabit defined niches transmit distinct signals that affect functions of both the innate and adaptive immune systems, which often results in systemic outcomes that are distal to the site of colonization. For example, segmented filamentous bacteria (SFB) induce T helper 17 (T_H17) cells in the small intestine and can trigger autoimmune arthritis in susceptible mice[1,2]. Some species of *Bifidobacterium* can enhance the T-cell-dependent anti-tumour effect of blocking the programmed death 1 (PD-1) pathway[3], and regulatory T (T_{reg}) cells that are induced by bacteria can have systemic anti-inflammatory functions[4,5]. There are only a handful of examples of single species or defined communities of bacteria that can be used to provide insight into the mechanisms by which distinct subsets of lymphocytes are activated and polarized. Efforts to culture and characterize the commensal bacteria of humans and to assess their influence on the host's immune system, which typically involve the colonization of germ-free mice, promise to provide new tools for investigating which cell types and signalling pathways are crucial for the induction of distinct immune responses. The characterization of IgA-coated gut bacteria from mice and humans, which provides a snapshot of the bacteria that are sensed by the cells of the adaptive immune system, has also been a valuable advance. This approach has been used to identify bacteria with potentially colitogenic functions in people with malnutrition[6] and in individuals with inflammatory bowel disease[7] as well as to compare species of bacteria that elicit T-cell-dependent and T-cell-independent IgA-mediated responses in the host[8].

In this Review, we describe progress towards understanding how colonization of the mammalian host by microbes influences the functional diversity and the repertoires of B cells and T cells, with an emphasis on the differentiation of IgA-producing B cells and T cells that carry the CD4 antigen, particularly T_H17 cells and T_{reg} cells that constitute a large proportion of the effector T (T_{eff}) cells of the lamina propria of the intestines. The reciprocal roles of lymphocytes in regulating the microbiota, a topic that has so far received little attention, will also be discussed briefly. It should be noted that insights into the interactions of the microbiota with the immune cells of the host tend to come from studies of mice in controlled environments, which have limited exposure to pathogenic microbes or to the microbiota of wild populations. Housing of laboratory mice together with free-living wild mice results in a constitutive increase in highly differentiated innate and adaptive immune cells, including effector memory T cells that carry the CD8 antigen, in the laboratory mice[9]. The immune profile of these mice matches that of adult humans much more closely than does that of mice kept in specific pathogen-free conditions. The failure of some mouse studies to predict the responses of humans to therapy could therefore be partly because of differences in the microbiotas of the species.

Interactions of the microbiota with B cells and T cells

Studies have suggested roles for diverse species of microbes in regulating the distinct branches of the adaptive immune system. Antigen-specific adaptive immune responses influence the mutualistic relationship between the microbiota and the host, and are mostly directed at the microbes of the gut.

IgA

Mucosal IgA is secreted across the epithelium by binding to the polymeric immunoglobulin receptor, after which it binds to microbes, various components of the diet and to antigens in the lumen of the intestine. IgA coats and agglutinates its targets to prevent their direct interaction with the host[10,11]. This averts potentially harmful stimulation of the immune system in mucous membranes by the contents of the lumen and it also serves to regulate the composition of the microbiota. As well as providing a physical barrier, IgA can control the expression of genes by microbes in the intestine. For example, in the absence of IgA, the commensal bacterium *Bacteroides thetaiotaomicron*, which typically does not trigger inflammation in the human gut, expresses high levels of gene products that are involved in the metabolism of nitric oxide and elicits pro-inflammatory signals in the host[12]. Similarly, mice that are deficient for Toll-like receptor 5 (TLR5) show reduced levels of IgA that is directed against the protein flagellin, which results in aberrant expression of flagella-related genes by a wide range of commensal microbes[13]. IgA that has undergone affinity maturation through somatic hypermutation binds to and selects for particular components of the microbiota, which leads to an increase in the diversity of the microbial community and enhances mutualism between the microbiota and the host[14]. Consistent with this observation, people who are deficient in IgA have more bacteria from taxa with potentially inflammatory properties[15]. Moreover, mice that carry a mutation called

[1]Department of Microbiology and Immunology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan. [2]RIKEN Center for Integrative Medical Sciences, Tsurumi, Yokohama, Kanagawa 230-0045, Japan. [3]AMED-CREST, Chiyoda, Tokyo 100-0004, Japan. [4]The Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, New York 10016, USA. [5]The Howard Hughes Medical Institute, New York University School of Medicine, New York, New York 10016, USA.

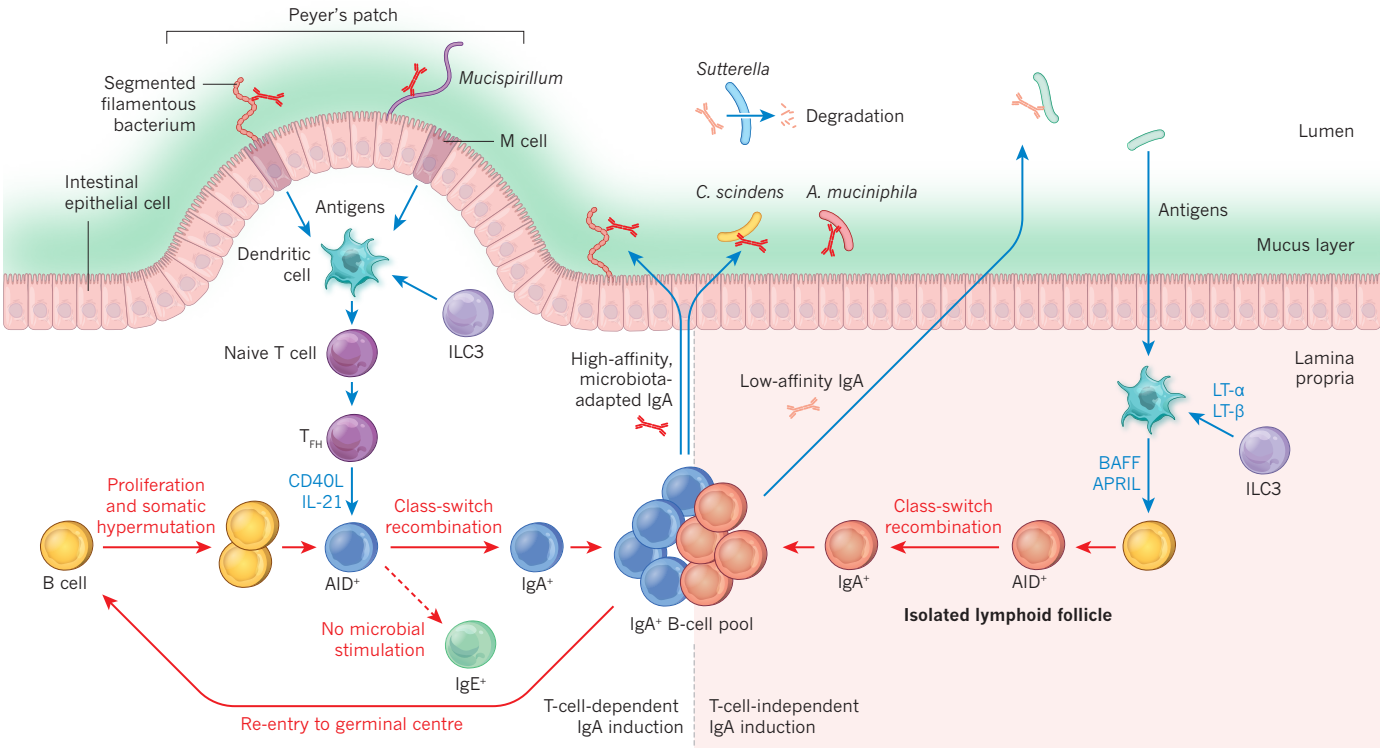

Figure 1 | Induction of IgA in mucosal tissues. T-cell-dependent IgA class-switch recombination (left) takes place mostly in Peyer's patches, in which dendritic cells that are located near to the surface of the epithelium capture antigens from microbes that are transferred by M cells. Dendritic cells induce the differentiation of CD4-expressing T cells into the T follicular helper (T_{FH}) cell subset. CD40 ligand (CD40L) and IL-21 from T_{FH} cells induce the expression of activation-induced cytidine deaminase (AID) in B cells and promote IgA class-switch recombination[11,128]. T-cell-independent IgA class-switch recombination (right) occurs predominantly in the lamina propria and isolated lymphoid follicles (ILFs), where B-cell activating factor (BAFF; also known as TNFSF13B) and its homologue APRIL, which are derived from dendritic cells, promote the induction of AID expression in B cells. Transforming growth factor β (from dendritic cells and stromal cells) and retinoic acid (from dietary vitamin A) play important parts (not shown) in both T-cell-dependent and T-cell-independent pathways. ILC3s that express RORγt also contribute to those pathways, through the expression of lymphotoxin (LT)-α and LT-β, which activate dendritic cells[129]. The gut microbiota affects IgA class-switch recombination in both pathways. The T-cell-independent pathway produces IgA with low affinity but directed towards the microbiota. The T-cell-dependent pathway tends to be activated by bacteria that colonize the surface of the epithelium, such as segmented filamentous bacteria (SFB), *Mucispirillum*, *Clostridium scindens* and *Akkermansia muciniphila*. The IgA-expressing B-cell clones that this pathway induces persist for a long time and can re-enter a germinal centre, where they undergo further somatic hypermutation to produce high-affinity IgA that is adapted to the changing composition of the microbiota.

AID^{G23S} that allows the enzyme activation-induced cytidine deaminase to mediate normal IgA class switching but without somatic hypermutation, harbour a dysbiotic microbiota in their small intestine[16]. Selection of affinity-matured, microbe-specific IgA is therefore crucial for the establishment of a balanced microbiota that, in turn, can restrain inflammatory processes.

Gut plasma cells that produce IgA can be generated by both T-cell-dependent and T-cell-independent mechanisms that involve the cooperation of epithelial cells, dendritic cells and innate lymphoid cells (ILCs) (Fig. 1 and Box 1). In both pathways, the gut microbiota affects the accumulation of cells that express IgA as well as the level and diversity of IgA in the lumen. Indeed, IgA-expressing cells in lymphoid tissue known as Peyer's patches and in the lamina propria are greatly reduced in germ-free animals, and the colonization of germ-free mice with a microbiota quickly triggers the production of IgA. Interestingly, some members of the microbiota, such as species of *Sutterella*, are inversely correlated with the level of IgA in faeces[17]. These members degrade both IgA and a peptide that is required for the stability of IgA in the lumen, known as secretory component. Because microbiota-induced IgAs are directed towards microbial antigens[8], a substantial proportion of the microbiota are coated with IgA and can be detected and characterized through flow cytometry and 16S ribosomal RNA gene sequencing. Known as IgA-SEQ, this combined approach has demonstrated that anatomical location determines whether a particular species of bacterium will elicit an IgA-mediated response in the host[8]. Bacteria that can invade the inner mucous layer of the intestine and colonize regions in proximity to epithelial cells induce high-affinity T-cell-dependent IgA responses[7,8,18]. In particular, SFB and *Mucispirillum* associate intimately with the intestinal epithelium, where they elicit a T-cell-dependent IgA-mediated response and are heavily coated with IgA[8] (Fig. 1). Because SFB have a propensity to induce the production of T_H17 cells, they might also induce follicular helper (T_{FH}) cells with a phenotype that is distinct from those of T_{FH} cells that are induced by other commensal bacteria, thereby resulting in a strong, T_H17-cell-dependent high-affinity IgA response[19]. Mice that are deficient in T cells owing to a lack of T-cell antigen receptor (TCR) chains β and δ, as well as those that lack T_{FH} cells and the T-cell-dependent IgA pathway owing to T-cell-specific inactivation of the gene *Bcl6* in $CD4^+$ T cells, retain an IgA-mediated response that is specific to antigens from commensal bacteria — indicating that the T-cell-independent pathway is also directed at the microbiota[8]. However, this response is characterized largely by the low-affinity binding of IgA to antigens that are shared by multiple species of bacteria[7,11,14].

Induced clones of IgA-producing B cells persist for long periods, even after transient exposure to microbes[20,21] (Fig. 1). Accordingly, an increase in the complexity of the gut microbiota leads to an increase in the diversity of the IgA pool[21]. The repertoire of IgA in the gut is dynamically adjusted in response to changes in the composition of the microbiota[21]. This process of adaptation relies mostly on the re-entry of B-cell clones into a germinal centre and on further somatic hypermutation of B-cell clones that are already established in the pool of plasma cells in the intestine[21]. The types of gut microbes that are targeted by IgA change in accordance with the diet of the host. For example, in mice colonized with the gut

microbiotas of undernourished children and fed a nutrient-poor diet, members of the Enterobacteriaceae are heavily coated with IgA[6]. By contrast, in mice that are colonized by the same microbiotas but fed a nutritionally sufficient diet, IgA binds to taxa other than Enterobacteriaceae, even though the load of Enterobacteriaceae is similar. The transfer of Enterobacteriaceae-enriched consortia of IgA-coated microbes leads to a severe enteropathy that is characterized by disruption of the epithelial barrier of the intestine and by weight loss, which suggests that bacteria that are heavily coated with IgA are colitogenic[6]. Consistent with this idea, IgA-coated bacteria that are isolated from people with inflammatory bowel disease promote dramatically exacerbated development of colitis induced by dextran sulfate sodium[7]. However, enteropathy that is induced by colitogenic bacteria can be prevented by the administration of IgA-targeted species of bacteria from healthy microbiotas, such as *Akkermansia muciniphila* and *Clostridium scindens*[6]. Bacteria that are targeted by IgA are therefore not always colitogenic; they can even be of benefit to the host through contributions to enhancing the barrier function of the mucosa.

T_H17 cells

The high-affinity secretory IgA response is proposed to depend largely on T_H17 cells that express RAR-related orphan receptor (ROR)γt[19]. These cells are most abundant in the lamina propria of the intestine, where they account for 30–40% of differentiated memory $CD4^+$ T cells[22–24]. The signature cytokines of T_H17 cells, interleukin (IL)-17A, IL-17F and IL-22, stimulate the production of antimicrobial proteins by intestinal epithelial cells as well as the formation of tight junctions between these cells[25]. They also mediate the transportation of IgA and the recruitment of granulocytes. Consequently, T_H17 cells have an indispensable role in preventing infection by several species of extracellular pathogenic bacteria and fungi. Indeed, genetic defects in the IL-17–IL-17 receptor axis and in RORγt in humans have been linked to susceptibility to chronic mucocutaneous candidiasis[26,27], and a deficiency of both *Il17a* and *Il17f* in mice results in opportunistic infection of mucocutaneous zones by *Staphylococcus aureus*[28]. However, T_H17 cells can also have pathogenic features, particularly following their stimulation with IL-23 and IL-1β[29,30]. Pathogenic T_H17 cells express the pro-inflammatory cytokines interferon (IFN)-γ and granulocyte–macrophage colony-stimulating factor (GM-CSF; also known as CSF2) and exacerbate autoimmune and inflammatory diseases[31–33]. IL-23 is required for the conversion of IL-17-expressing T cells into encephalitogenic and colitogenic T cells that express both IL-17 and IFN-γ or only IFN-γ (known as ex-T_H17 cells, $T_H17.1$ cells or T_H1^* cells)[31,33] and for the onset of disease in mice that are subjected to colitis[34] and to experimental autoimmune encephalomyelitis[29,30]. Although both homeostatic T_H17 cells and pathogenic T_H17 cells are dependent on RORγt in combination with other factors[22,35] for their differentiation, what distinguishes the T_H17 cells that promote homeostatic defence of the gut barrier from those that are involved in pathogenic inflammation is a major unanswered question.

It is unclear whether constituents of the microbiota or other environmental factors direct the differentiation of naive $CD4^+$ T cells into homeostatic or pathogenic T_H17 cells. In experimental models, a multitude of environmental factors have been shown to affect the activation status of intestinal T_H17 cells. For example, a diet that is high in salt enhances the number of T cells in the intestinal lamina propria that express IL-17A and CD4 and increases the risk of T_H17-cell-dependent autoimmunity[36,37]. These phenotypes are ascribed to the salt-mediated induction of serine/threonine-protein kinase Sgk1 (SGK1), which phosphorylates and deactivates forkhead box protein O1, thereby relieving the inhibition of RORγt-mediated transcription of IL-17A and the IL-23 receptor and promoting the generation of pathogenic T_H17 cells[37].

Lipids in the diet have also been implicated in promoting the differentiation of both T_H17 cells and T_{reg} cells[38–40]. Long-chain fatty acids such as lauric acid promote the differentiation of T_H17 cells and induce more severe experimental autoimmune encephalomyelitis, whereas the short-chain fatty acid propionic acid protects animals from disease, in part through the induction of T_{reg} cells[41]. Endogenous fatty acids, which are dependent on the enzyme acetyl-CoA carboxylase 1 for their synthesis, contribute to the differentiation of T_H17 cells and to the development of autoimmune diseases[42]. It has also been suggested that an intermediate in cholesterol biosynthesis acts as an endogenous ligand for RORγt and that enzymes such as CYP51A1 and SC4MOL (also known as MSMO1), which form part of the cholesterol biosynthesis pathway, contribute to T_H17 cell differentiation[43]. These enzymes are upregulated in pathogenic T_H17 cells on their culture with saturated fatty acids, such as palmitic acid, or with IL-23 (ref. 44) (Fig. 2). In the absence of IL-23, non-pathogenic T_H17 cells express the protein CD5L, an inhibitor of fatty-acid synthase, and these cells have elevated levels of polyunsaturated fatty acids at the expense of saturated fatty acids[44]. The mechanism for regulating genes that are the targets of RORγt in the presence of the different types of fatty acids remains unclear, although it is possible that CD5L restricts cholesterol synthesis, which diminishes the endogenous source of RORγt ligands and thus the potential for pathogenicity. Fatty acids that are produced by the microbiota might similarly modulate the activity of RORγt and therefore govern the balance between homeostatic and potentially pathogenic programs of gene expression in T_H17 cells.

The microbiota are the most prominent influence from the environment on the differentiation of T_H17 cells. In germ-free mice, T_H17 cells are scarce in the lamina propria of the intestines as well as in the skin[23,24,45] (Box 2). The number of T_H17 cells in the intestines varies widely between

BOX 1

ILC3s in adaptive immune homeostasis

Signals from the microbiota create complex interactions between epithelial cells, dendritic cells, macrophages and ILC3s. ILC3s contribute to the differentiation of T cells and B cells. For example, ILC3s express lymphotoxin (LT)-α and LT-β and activate dendritic cells, thereby contributing to both T-cell dependent and T-cell-independent pathways of IgA class switching[129]. ILC3s also facilitate the induction of T_H17 cells through the production of IL-22 and other factors. Activation of ILC3s and induction of T_H17 cells have been observed in mice that are colonized by segmented filamentous bacteria (SFB) and other bacteria, including *Citrobacter rodentium*[50,130,131]. Activation of ILC3s by these bacteria requires the TLR-dependent activation of CX_3CR1-expressing cells (derived from monocytes) and their production of IL-23, IL-1β and tumour necrosis factor ligand superfamily member 15 (TNFSF15), which act through receptors on ILC3s[131]. IL-22 from ILC3s then activates epithelial cells to produce serum amyloid A and other factors that are required for the induction of T_H17 cells.

Latent infection of wild-type mice with murine norovirus, which induces pathogenesis in the intestines of mice that lack the gene *Atg16l1* (ref. 132), leads to IL-22 production by ILC3s and the induction of T_H17 cells, while suppressing the expansion of group 2 innate lymphoid cells (ILC2s) — offsetting the deleterious effect of treatment with antibiotics[133]. Viral components of the intestinal microbiota could therefore act with commensal bacteria to reinforce the epithelial barrier through activation of ILC3s and induction of T_H17 cells.

ILC3s that are activated by the microbiota also promote expansion of T_{reg} cells[106]. Gut microbiota induce the production of IL-1β from macrophages in the lamina propria, and this cytokine acts on neighbouring ILC3s to activate their production of CSF2 (ref. 106). CSF2 then acts on CD103-expressing dendritic cells in the colon to enhance the activity of aldehyde dehydrogenase (ALDH) and produce TGF-β and IL-10, which induces T_{reg} cells[106].

BOX 2

The skin microbiota and adaptive immunity

The microbiota influences the differentiation of adaptive immune cells both in the skin and the gut. *Staphylococcus epidermidis*, a commensal bacterium of the skin, potently induces T_H17 cells as well as T cells that express both IL-17A and the antigen CD8 (ref. 134). Both cross-presenting dendritic cells that are dependent on basic leucine zipper transcription factor ATF-like 3 (Batf3) and cells derived from monocytes are required to induce a response from cells that express IL-17A and CD8 to *S. epidermidis* in the skin[134]. On infection with the cutaneous pathogenic protozoa *Leishmania major*, local commensal bacteria are necessary to elicit protective immunity (which manifests as inflammation and necrosis), and monoassociation with *S. epidermidis* is sufficient to promote this response[45]. Importantly, T_H17 cells in the skin are affected by the skin microbiota independently of the gut microbiota[45], which suggests that T_H17 cells of the mucosa are regulated in a compartmentalized manner by local commensal bacteria. The production of IL-17A by T cells in the skin requires the expression of IL-1R but not IL-23R, which is in contrast to the requirements of T_H17 cells in the intestines and is consistent with compartment-specific mechanisms for T-cell regulation[45]. Although immunological cross-communication has been shown to occur between mucosal tissues such as the intestine and the lung[135] and the nasopharynx and the uterus[108], there seems to be a compartment-specific regulation of immunity in the skin. This might be because the skin is faced with challenges from the environment that differ from those faced by mucosal sites and therefore requires distinct pathways to control its local immune responses.

animal facilities, even in genetically identical mice that have been reared in specific pathogen-free conditions, and often reflects whether mice have been colonized with SFB[1] (Fig. 2). Such bacteria are potent modulators of the immune-cell functions of the host: as well as inducing T_H17 cells, they also stimulate IgA synthesis[8,46,47] and fucosylation of the epithelium through the activation of group 3 innate lymphoid cells (ILC3s)[48]. SFB that are indigenous to mice and rats are genetically distinct host-specific members of the gut microbiota[49]. On their monocolonization of germ-free mice or rats, populations of SFB can expand in the gut lumen of either species; however, the bacteria bind to epithelial cells of the small intestine and induce T_H17 cells in a strictly host-specific manner[50]. The physical interaction of SFB with the gut epithelium is therefore probably essential for T_H17-cell differentiation. The causality of the relationship between the adhesion of bacteria to the epithelium and the induction of T_H17 cells is further supported by analysis of T_H17-cell induction by the intestinal pathogenic bacteria *Citrobacter rodentium* and *Escherichia coli* O157:H7 (ref. 50). On monocolonization of mice, these species triggered T_H17-cell responses, whereas adhesion-defective mutants fail to do so. Moreover, 20 strains of bacteria that were isolated from the faeces of a person with ulcerative colitis exhibit characteristics that enable their adhesion to epithelial cells and induction of T_H17 cells in the colons of mice[50].

Colonization with adherent SFB elicits a unique program of gene expression that includes the upregulation of two isoforms of the protein serum amyloid A in the epithelial cells of the small intestine. This induction is largely restricted to the terminal ileum, the site at which SFB attach to the epithelium[51]. The genes that encode serum amyloid A are also induced when SFB and epithelial cell lines are cultured together *in vitro*[52], which suggests that their direct interaction initiates a signalling pathway that results in gene expression. In parallel, SFB activate ILC3s to produce IL-22 through the intermediary expression of IL-23 by myeloid cells[51] (Fig. 2). The expression of serum amyloid A in the epithelial cells of the small intestine is dependent on the secretion of IL-22 from ILC3s, by way of phosphorylation of signal transducer and activator of transcription 3 (Stat3) in epithelial cells[50,51]. *In vivo* induction of serum amyloid A might therefore require both adhesion of SFB to epithelial cells and activation of the IL-22 receptor.

Polarization of T_H17 cells that are specific to SFB occurs in the mesenteric lymph nodes, in which RORγt is upregulated before T cells migrate to the lamina propria[51]. T_H17-cell polarization is dependent on monocyte-derived CX_3CR1^+ cells rather than classic dendritic cells[53], although a role for dendritic cells that express CD103 and CD11b and are dependent on Notch2 and IRF4 for their development has also been proposed[54–56]. Polarized T cells that express RORγt and CD4 are distributed broadly throughout the intestine and are even found in the spleen, although most IL-17A expression is confined to the ileum, where serum amyloid A seems to act as an adjuvant and contributes to the induction of IL-17A[51] (Fig. 2).

The mechanism through which serum amyloid A stimulates T_H17 cells has yet to be resolved. In a feed-forward process, myeloid cells including those that carry CX_3CR1 can respond to serum amyloid A by producing cytokines that activate ILC3s, which promotes T_H17-cell differentiation[50] (Box 1). Serum amyloid A might also stimulate T cells directly to enhance RORγt function and upregulate IL-17A expression[51]. Serum amyloid A is a carrier of both high-density lipoprotein and retinol[57], and it can deliver these immunomodulatory molecules to antigen-presenting cells and T cells. The potential regulation of T_H17-cell differentiation by lipids suggests that serum amyloid A might function unconventionally to modulate inflammatory functions in these cells. Together, these findings indicate that the differentiation of T_H17 cells directed by SFB is mediated through a complex circuitry of interactions between epithelial cells, dendritic cells and ILC3s to generate cells that are poised to acquire effector functions in the appropriate microenvironment (Fig. 2). Because SFB have not yet been definitively identified in the human intestine[58], whether this circuitry applies more generally to microbiota-mediated T_H17-cell-induction in humans requires further investigation[58].

Intestinal T_H17 cells and autoimmunity

Most of the T_H17 cells that are elicited by SFB have TCRs that specifically bind to antigens that are expressed by adhesive forms of these bacteria[50]. Two major antigens have been identified as being responsible for this induction[59]. These antigens might be preferentially taken up by the cells of the host when SFB adhere to epithelial cells. Colonization with these bacteria, and the consequent induction of T_H17 cells with TCRs that are specific for SFB antigens, helps to protect the host from intestinal pathogenic species such as *C. rodentium*[1]. However, SFB-induced T_H17 cells might promote pathogenesis in hosts that have a genetic predisposition to autoimmune diseases. In the K/BxN mouse model of autoimmune arthritis, colonization with commensal microbes is required for the development of disease[2]. Monocolonization with SFB enhances the production of autoantibodies and accelerates the progression of disease through the generation of T_H17 cells[2], although a microbiota-induced T_{FH}-cell-dependent process can also precipitate disease[60]. Mice that harbour SFB are more susceptible to experimental autoimmune encephalomyelitis than are germ-free mice[61]. By contrast, the presence of SFB is strongly correlated with a diabetes-free state in non-obese diabetic mice[62]. The influence of such bacteria on the development of autoimmune diseases is therefore dependent on context. The conditions that determine whether intestinal T_H17 cells play a beneficial or harmful part in the host are not yet fully understood. Interestingly, germ-free mice that are colonized with SFB show a striking genotype-specific difference in the induction of T_H17 cells. For instance, BALB/c mice have fewer T_H17 cells but a greater amount and diversity of IgA in their faeces than do C57BL/6 mice[50,63]. Therefore, a combination of genetics and the composition of the gut microbiota affects the status of the immune system and an individual's susceptibility to disease.

In the K/BxN mouse model of autoimmune arthritis, self-reactive T_H17 cells that express a transgenic TCR that is specific for a self antigen can

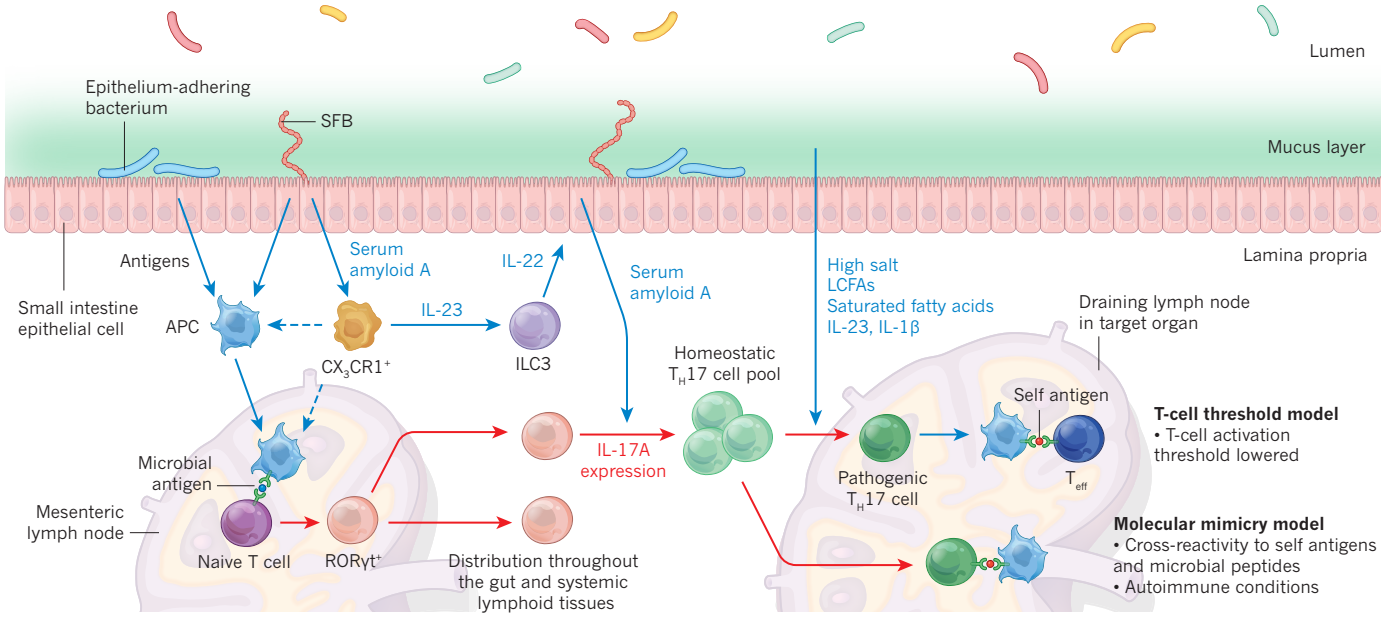

Figure 2 | Microbiota-mediated induction of T_H17 cells and autoimmunity. Epithelium-adhering bacteria initiate the differentiation of naive $CD4^+$ T cells into RORγt-expressing T cells (T_H17 polarized cells) (red) in the mesenteric lymph node through as-yet-undefined antigen-presenting cells (APCs). T_H17 polarized cells then accumulate and further differentiate into IL-17-expressing homeostatic T_H17 cells (green) in the lamina propria of the small intestine. These homeostatic T_H17 cells then stimulate epithelial cells to enhance the integrity of the intestinal mucosal barrier. The adhesion of segmented filamentous bacteria (SFB) elicits a unique program of gene expression in the epithelial cells, including the upregulation of serum amyloid A. Serum amyloid A from epithelial cells of the small intestine seems to function as a cytokine and it modulates CX_3CR1-expressing cells (that are derived from monocytes) to produce IL-23, which stimulates the production of IL-22 by ILC3s. As well as its effects on CX_3CR1-expressing cells, serum amyloid A can stimulate RORγt-expressing T cells directly to upregulate the expression of IL-17A. Dendritic cells that express the antigens CD11b and CD103 have also been implicated in the expansion and maintenance of T_H17 cells (not shown). T_H17 cells become pathogenic when they are stimulated with IL-23, IL-1β, higher concentrations of salt, long-chain fatty acids (LCFAs) and saturated fatty acids. Pathogenic T_H17 cells can migrate to the draining lymph nodes of target organs, where they contribute to autoimmune disease through cross-reactivity between peptides from microbes and self antigens (the molecular mimicry model). Alternatively, microbiota-specific T_H17 cells migrate to the lymph nodes and lower the threshold of activation of auto-reactive T cells such as T_{eff} cells (the T-cell threshold model).

migrate out of the intestine and into the spleen[64]. Self-reactive but gut-microbiota-activated T_H17 cells might contribute to other autoimmune disorders, including uveitis[65] and encephalomyelitis[66]. Such T-cell-mediated autoimmune conditions could be caused by cross-reactivity between microbial peptides and self antigens[67], a process known as molecular mimicry (Fig. 2). This model is consistent with the fact that the genes of the major histocompatibility complex (MHC) are the most important genetic susceptibility loci for many autoimmune disorders. Alternatively, microbiota-specific T_H17 cells might mediate some kind of bystander effect. This is because autoimmune disorders often affect more than one organ, and the genes that encode the signalling molecules that act downstream of TCRs are important determinants of genetic susceptibility to various autoimmune disorders in humans, including rheumatoid arthritis[68]. The T-cell threshold model proposes that gut-microbiota-activated T_H17 cells might migrate into the draining lymph nodes of the target organs and either lower the threshold of activation of autoreactive T cells or have their own activation threshold lowered. Indeed, T_H17 cells that are specific to SFB and that are primed in gut-draining lymph nodes can be found in other lymph nodes and in the spleen[59]. When produced aberrantly in some organs, molecules such as serum amyloid A might serve an adjuvant function and contribute to the heightened activity of such T cells (Fig. 2).

The potential for detrimental inflammation suggests that the responses of T cells and B cells to the gut microbiota must be tightly regulated. This is achieved through a number of mechanisms, including T-cell depletion and anergy. In this context, expression of MHC class II molecules by ILC3s has been found to restrain the expansion of T_H17 cells[69]. This could occur through the presentation of antigens that are derived from commensal bacteria to induce apoptosis of the antigen-specific T cells[69], although an antigen-presenting function for ILC3s is yet to be demonstrated. Beyond this context, however, one of the most crucial mechanisms for restraining inflammation in the gut is the induction of $CD4^+$ T_{reg} cells that express forkhead box protein P3 (Foxp3).

Induction of T_{reg} cells by the microbiota

T_{reg} cells that express both CD4 and Foxp3 can be found in every organ of the body, and they comprise a high proportion of the T cells of the lamina propria of the intestine[5,70–73]. Intestinal T_{reg} cells play an important part in maintaining immune tolerance to dietary antigens and the gut microbiota[74,74] as well as in suppressing tissue damage inflicted by immune responses against pathogenic bacteria such as *C. rodentium*[76] that are mediated by T_{eff} cells. The intestine contains both thymus-derived T_{reg} (tT_{reg}) cells and peripherally differentiated T_{reg} (pT_{reg}) cells; pT_{reg} cells are substantially enriched in the colon, mainly express RORγt and generally lack the zinc-finger protein Helios and the receptor neuropilin 1 (Nrp1) (refs 77–79) (Fig. 3). Because pT_{reg} cells disappear under germ-free conditions, they are probably induced by the microbiota[77–79]. Consistent with this, T_{reg} cells that express RORγt show the restricted TCR repertoire of cells that have proliferated in response to peripheral stimuli, but their TCR sequences overlap with those of $CD4^+$ T cells that lack Foxp3 (ref. 79). Experiments to track the fate of immature T cells that express a transgenic TCR cloned from colonic T_{reg} cells demonstrate that the expansion and differentiation of the transgenic T cells into T_{reg} cells occurs in the colon in the presence of cognate commensal bacteria and not in the thymus[80]. A considerable fraction of $ROR\gamma t^+$ T_{reg} cells express IL-10 (ref. 77), which is also produced by many other types of cell, including type 1 regulatory (Tr1) cells and myeloid cells, which have important roles in maintaining homeostasis in the intestines[81,82]. T_{reg}-cell-derived IL-10 is essential for suppression of the aberrant activation of myeloid cells, γδ T cells and T_H17 cells[83–85]. T_{reg} cells that express RORγt also express high levels of cytotoxic T-lymphocyte protein 4 (CTLA-4) (ref. 77) and are more effective than RORγt-negative T_{reg} cells in restraining immune pathogenesis

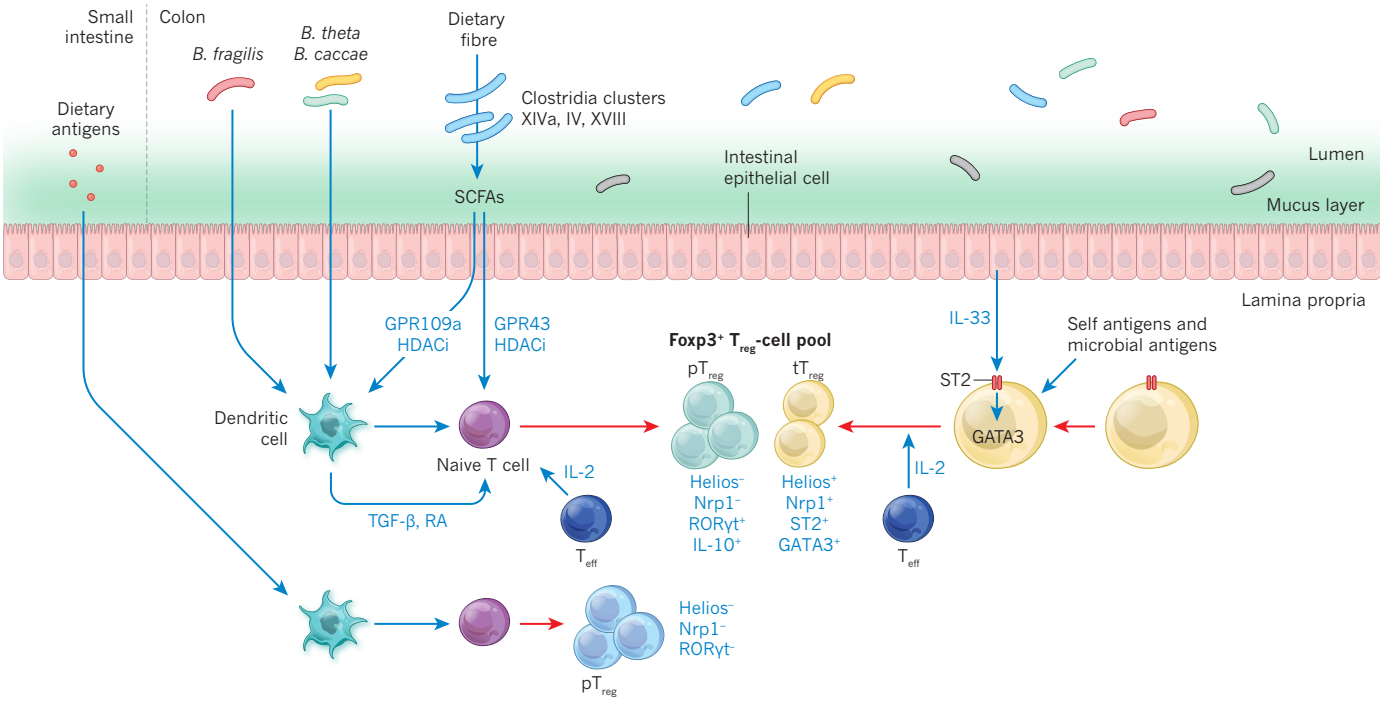

Figure 3 | Influence of the microbiota and diet on subsets of regulatory T cells in the intestine. Foxp3-expressing T_{reg} cells in the intestine can be subdivided into at least three subsets on the basis of their expression of RORγt, GATA3, Helios and Nrp1. T_{reg} cells that express RORγt but not Nrp1 are induced at peripheral sites by antigens derived from the microbiota. Known as pT_{reg} cells, they are the main producers of IL-10, which suppresses the aberrant activation of myeloid cells, γδ T cells and T_H17 cells. Dendritic cells produce mediators of pT_{reg}-cell differentiation, including TGF-β and retinoic acid (RA). Short-chain fatty acids (SCFAs), which are produced from dietary fibre by certain members of the microbiota, particularly species of Clostridia, also contribute to the induction of pT_{reg} cells. On binding to the G-protein-coupled receptor (GPR)109a on dendritic cells, short-chain fatty acids induce the expression of aldehyde dehydrogenase (ALDH), which metabolizes vitamin A into RA. SCFAs entering dendritic cells act as inhibitors of histone deacetylase (HDACi) to suppress the expression of pro-inflammatory cytokines. They also directly act on naive T cells through GPR43 or the upregulation of Foxp3 expression through HDAC inhibition. IL-2 derived from T_{eff} cells probably helps to stabilize the differentiation of T_{reg} cells. Several species of Bacteroides contribute to the induction of pT_{reg} cells that express RORγt but not Nrp1 through dendritic cells. A second pool of pT_{reg} cells expresses neither RORγt nor Nrp1; these T_{reg} cells are induced by, and maintain immune tolerance to, dietary antigens. It should be noted that induction of pT_{reg} cells through dietary antigens occurs largely in the small intestine, whereas the induction of pT_{reg} cells by the microbiota occurs largely in the colon. T_{reg} cells that express both GATA3 and Nrp1 are thought to be generated in the thymus and are known as tT_{reg} cells. GATA3$^+$ T_{reg} cells express ST2 (a component of the IL-33 receptor that is also known as IL1RL1). IL-33, which is probably released from the epithelial cells of the intestine at steady state, is markedly upregulated under conditions of inflammation. IL-33 acts with IL-2 (from T_{eff} cells) to induce the expression of GATA3 in T_{reg} cells.

in models of colitis[78,79]. Conditional inactivation of RORγt using the Cre–Lox recombination system in Foxp3$^+$ intestinal T cells in mice results in T_H2-cell-mediated inflammation[77] or in the expansion of T_H17 cells[78]. It should be noted that some intestinal T_H17 cells lose IL-17A expression in the presence of SFB and a fraction of these ex-T_H17 cells express Foxp3 (ref. 86). Foxp3$^+$ Cre–Lox mice in which RORγt has been inactivated might therefore reflect their RORγt deficiency in ex-T_H17 cells as well as microbiota-induced pT_{reg} cells.

The intestine also contains a subpopulation of T_{reg} cells that expresses the transcription factor GATA3 (ref. 87) (Fig. 3). These cells are distinct from RORγt$^+$ T_{reg} cells, and most express Nrp1 and Helios and are unaffected by the absence of the gut microbiota, which suggests that they mainly derive from tT_{reg} cells[78]. T_{reg} cells that express GATA3 co-express the IL-33 receptor ST2 (also known as IL1RL1) (ref. 88). IL-33, which is produced by the epithelial cells of the intestine at high levels under conditions of inflammation, works with IL-2 and the process of TCR engagement to induce the expression of GATA3 in T_{reg} cells. GATA3 upregulates the expression of Foxp3 and ST2 in a feed-forward process that promotes the proliferation and maintenance of T_{reg} cells[88]. T_{reg} cells that express Foxp3 but that lack RORγt and Nrp1 constitute one-third of the T_{reg}-cell population and are uniquely abundant in the lamina propria of the small intestine[89] (Fig. 3). This subpopulation is unaffected by the absence of the gut microbiota but disappears in germ-free mice that are fed an antigen-free diet[89]. Such cells therefore seem to be pT_{reg} cells that are induced by dietary antigens, and they constitute a subpopulation that can be distinguished from microbiota-induced, RORγt$^+$ pT_{reg} cells and from GATA3-expressing tT_{reg} cells. Mice that lack this subpopulation exhibit an increased susceptibility to food allergies[89]. Certain pT_{reg}-cell and tT_{reg}-cell subpopulations might have complementary and context-dependent functions, such as immune regulation at steady state in response to components of the microbiota (by RORγt$^+$ T_{reg} cells that lack Nrp1) and of the diet (by RORγt-negative T_{reg} cells that also lack Nrp1) and under conditions of inflammation that is triggered by self antigens (by GATA3$^+$ T_{reg} cells that express Nrp1).

The parts played by individual members or defined communities of the gut microbiota in the accumulation and functional maturation of T_{reg} cells of the intestine are starting to be illuminated. For example, strains that fall within clusters IV, XIVa and XVIII of Clostridia have a strong capacity for inducing the accumulation of T_{reg} cells in the colon[4,5] (Fig. 3). Oral administration to germ-free mice of a mixture of 46 strains of Clostridia that were derived from the faeces of conventional mice[90] leads to the strong induction of T_{reg} cells in the colon[5]. Similarly, a mixture of 17 strains of Clostridia that were isolated from a healthy person strongly induces T_{reg} cells in the colons of mice and rats[4]. This mixture preferentially enhances the accumulation of RORγt-expressing T_{reg} cells that lack Helios, rather than of GATA3-expressing T_{reg} cells[4,77]. Strains of Clostridia can also facilitate the expression of IL-10 and CTLA-4 by T_{reg} cells[4,5], and mice with an abundance of strains of Clostridia in their intestines exhibit resistance to experimental colitis[4,5]. In mouse models of graft-versus-host disease, the introduction of 17 strains of T_{reg}-inducing Clostridia reduces severity of the disease[91]. These Clostridia also stimulate ILC3s to produce IL-22, which helps to reinforce the epithelial barrier

and reduces the permeability of the intestine to dietary proteins[73]. Mice colonized by a microbiota that includes Clostridia therefore display a suppressed response to food allergens[73]. Clostridia-induced T_{reg} cells support the production of IgA in the intestine, which contributes to increased diversity of the microbiota and, in particular, of Clostridia[14].

One species of Clostridia, *Faecalibacterium prausnitzii*, is underrepresented in people with inflammatory bowel disease[92] and it promotes the accumulation of IL-10-expressing T cells that are positive for both CD4 and CD8αα in the colon[93]. A population of lymphocytes from the intestinal epithelium that is positive for both such antigens could have a similar immune regulatory role in the small intestine of the mouse. These microbiota-dependent T cells differentiate in the periphery on loss of the expression of the CD4-lineage transcription factor ThPOK and upregulation of the CD8-lineage transcription factor Runx3[94,95]. How these cells function in preventing the differentiation of inflammatory cells in the small intestine is yet to be determined.

A small consortium of microbes known as altered Schaedler flora, which contains strains of Clostridia, is also capable of increasing the number of T_{reg} cells in the lamina propria of the mouse colon[71]. The precise mechanism through which Clostridia stimulate the induction of T_{reg} cells in the colon remains to be elucidated. One possible mechanism is the cooperative production of short-chain fatty acids through fermentation of dietary fibre[4,39,96] (Fig. 3). For example, the collective genomes of the 17 strains of T_{reg}-cell-inducing Clostridia contain numerous genes that are predicted to be involved in the biosynthesis of short-chain fatty acids[96]. Short-chain fatty acids suppress the expression of pro-inflammatory cytokines in dendritic cells through the inhibition of histone deacetylases (HDACs)[40] and through the activation of the G-protein-coupled receptor (GPR)109a (also known as HCAR2) (ref. 97). They can also stimulate the proliferation of T_{reg} cells directly by activating GPR43 (FFAR2) (ref. 38) and the differentiation of naive $CD4^+$ T cells into pT_{reg} cells through HDAC inhibition, which results in histone H3 acetylation at the conserved non-coding sequence (CNS)1 element of the gene *Foxp3* (ref. 39). *In vitro* stimulation of T_{reg} cells with short-chain fatty acids upregulates the expression of GPR15 (ref. 38), which promotes the recruitment of T_{reg} cells to the colon[76], although this has not been demonstrated *in vivo*.

Programs for the induction of T_{reg} cells can also be activated by non-Clostridia members of the microbiota. *Lactobacillus reuteri* and *L. murinus* have been shown to increase the proportion of T_{reg} cells in mice[98–100]. Infection with *Helicobacter hepaticus* induces IL-10-producing T_{reg} cells that inhibit the development of colitis in an *H. hepaticus* antigen-specific manner[101]. *Bacteroides fragilis* boosts the production of IL-10 by T_{reg} cells of the colon, and this activity is mediated by polysaccharide A[70] from the bacterium's capsule. Outer-membrane vesicles containing polysaccharide A that are released by *B. fragilis* might also be taken up by dendritic cells of the intestine to stimulate their production of IL-10 through TLR-2 signalling[102]. The IL-10 from these dendritic cells might then induce T_{reg} cells to also produce IL-10. Several other species of *Bacteroides*, including *B. caccae* and *B. thetaiotaomicron*, also induce the accumulation of $Foxp3^+$ cells, particularly RORγt-expressing pT_{reg} cells in the colon[78,103]. Collectively, there is considerable overlap between the responses of T_{reg} cells to Clostridia, *Lactobacillus* and *Bacteroides*, which indicates that different pathways for the regulation of T_{reg} cells converge in the intestine. The induction and maintenance of T_{reg} cells might be a common and crucial mechanism for maintaining the homeostatic and beneficial relationship between the microbiota and the host.

It has been suggested that tolerogenic dendritic cells that carry the CD103 antigen contribute to the induction of T_{reg} cells[104,105]. CSF2 that is produced in response to the microbiota by ILC3s might also act on dendritic cells of the colon to promote the expansion of T_{reg} cells[106] (Box 1) (Fig. 3). The ablation of MHC class II expression in conventional dendritic cells, which include $CD103^+$ dendritic cells, results in reduced induction of pT_{reg} cells and in spontaneous inflammation[107]. The TCR repertoires of pT_{reg} cells and tT_{reg} cells differ substantially. In one study, at least half of the TCRs that were cloned from T_{reg} cells of the colon and expressed in a reporter hybridoma cell line responded to autoclaved contents of the intestines of mice, and two TCR clones were stimulated by strains of *Parabacteroides distasonis* or by an uncharacterized species of Clostridia[80]. Consistent with this, at least some of the T_{reg} cells that were induced by the mixture of 17 strains of human-derived Clostridia reacted to Clostridia antigens[4]. Whether there is a role for antigen specificity in T_{reg}-cell-mediated tolerance at mucosal surfaces, however, is an important question that still needs to be answered.

Cells from the adaptive immune system that are primed at microbiota-sensing mucosa can take up residence in and protect other mucosal surfaces. For example, intranasal vaccination is particularly effective at eliciting protective memory-T-cell responses against *Chlamydia trachomatis* in the female reproductive tract[108]. However, when ultraviolet-inactivated *C. trachomatis* is delivered intramucosally, antigens accumulate preferentially in CD103-expressing dendritic cells that lack CD11b and induce antigen-specific T_{reg} cells, and no protective immunity is elicited[108]. By contrast, immunization with ultraviolet-inactivated *C. trachomatis* conjugated to adjuvant nanoparticles that target CD103-lacking dendritic cells that express CD11b provides effective, antigen-specific memory responses by mucosa-resident T_H1 cells[108]. The immune responses that mucosal bacteria elicit therefore differ according to the route and context of antigen delivery. Elucidation of the mechanisms by which commensal microbes deliver antigens for presentation in an immunogenic versus a tolerogenic context might enable the development of effective mucosal vaccines.

Implications for disease and therapeutics

Members of the gut microbiota have distinct effects on homeostasis of the host's adaptive immune system. Differences in the composition of the community therefore contribute to variability in immune responses and susceptibility to infection, autoimmune disorders, allergy and other immunological conditions. Understanding the development of the mucosal immune system and its dysregulation in relation to normal and dysbiotic microbiotas is important for the development of drugs, probiotic supplements, vaccines and cancer immunotherapies.

A crucial window of time

The microbiota is established in early life. Indeed, an absence of microbiota during this period of development leads to increases in the number of invariant natural killer T (iNKT) cells and in susceptibility to colitis and asthma[109] in animal models. Early exposure to the gut microbiota suppresses the abundance of iNKT cells in the gut and lung, partly through the epigenetic suppression of the gene that encodes the chemokine CXCL16 (ref. 109). Colonization with commensal bacteria during the neonatal period also results in the recruitment of T_{reg} cells to mucosal sites and the establishment of long-lasting tolerance to the microbes[110]. Treatment with antibiotics results in an increase in susceptibility to asthma in perinatal, but not adult, mice through a decrease in the accumulation of T_{reg} cells in the colon and an enhanced IgE response[111]. In the absence of colonization by a microbiota at an early age, B cells preferentially undergo isotype switching to IgE, rather than IgA[112,113]. The elevated concentration of IgE in the blood serum of germ-free mice is accompanied by an increase in circulating basophils and exaggerated basophil-mediated T_H2-cell responses and allergic inflammation[112]. The induction of IgE is not suppressed by colonization with a microbiota later in life or by early colonization with a microbiota of limited complexity[113].

A cohort of children who had a high risk of developing atopy and asthma were found to have microbiotal dysbiosis that is characterized by a reduction in four specific genera of bacteria: *Faecalibacterium*, *Lachnospira*, *Veillonella* and *Rothia* — collectively known as FLVR[114]. Colonization of mice with FLVR mitigated airway inflammation in a model of allergic asthma, which raises the prospect that atopy or asthma could be averted by early therapy to correct dysbiosis[114]. There is a crucial window of time in early life, therefore, during which exposure to diverse microbiota is extremely important for the suppression of iNKT cells and IgE-expressing cells, the induction and expansion of T_{reg} cells

and the establishment of systemic tolerance to a large spectrum of environmental antigens.

Dysbiosis and aberrant adaptive immunity

Microbiotal dysbiosis can be caused by genetic predisposition, infections and changes in diet and nutritional status, as well as the use of antibiotics, agents that suppress gastric acid and anticancer drugs. Although there is convincing evidence to suggest that dysbiosis causes or promotes disease, the underlying mechanisms are not fully understood. Several reports describe the association of particular species of bacteria with autoimmune and inflammatory conditions[115,116]. In a mouse model, the administration of a diet rich in milk fat induces a bloom of taurocholic-acid-consuming *Bilophila wadsworthia*, which enhances the response of T_H1 cells and accelerates the onset of colitis[115]. Adherent-invasive *E. coli* (AIEC) is frequently observed in people with inflammatory bowel disease and can induce an active response by T_H17 cells in mice[116]. Mutations in the gene *NOD2*, found in subsets of people with inflammatory bowel disease, are associated with shifts in the composition of the gut microbiota[117]. *Nod2* deficiency in mice results in the expansion of the commensal bacterium *Bacteroides vulgatus*, which is accompanied by an excessive IFN-γ response from intraepithelial lymphocytes[118]. Colonization of the intestinal mucosa by bacteria from the mouth, such as Veillonellaceae and Fusobacteriaceae, is one of the earliest events in children with new-onset Crohn's disease[119]. Similarly, there is an increased prevalence of *Prevotella copri* in the faecal microbiota of people with new-onset rheumatoid arthritis[120]. However, the ability of these bacteria to trigger disease is yet to be established.

As well as the activation of T_{eff} cells in response to potentially pathogenic bacteria, compromised barrier function of the epithelium and dysregulated responses to the commensal microbiota are important features of chronic inflammatory diseases that are associated with dysbiosis. For instance, infection with HIV leads to chronic dysbiosis with a reduction in Clostridia and Bacteroidia and an enrichment of taxa that produce enzymes for tryptophan catabolism, and is accompanied by heightened permeability of the mucosa, elevated levels of T-cell activation and diminished numbers of IL-17-secreting mucosal T cells[121]. These events might contribute collectively to the chronic inflammation that is observed in individuals who are infected with HIV. In a mouse model, infection with the protozoa *Toxoplasma gondii* and subsequent disruption of the epithelial barrier induces memory T_H1 cells specific for commensal Clostridia that normally induce T_{reg} cells and IgA-secreting B cells[122,123]. Similarly, responses to flagellin antigens (known as CBir) that are expressed by commensal species from the Clostridia cluster XIVa have been detected in people with Crohn's disease[124]. Importantly, the transfer of CD4-expressing T cells that are specific for CBir into immunodeficient mice that have been colonized with commensal Clostridia causes severe colitis[124]. Disruption of the epithelial barrier owing to the complex interplay between a dysbiotic microbiota and pathogenic bacteria might therefore lead to dysregulated immune responses to commensal microbes, chronic inflammation and the stabilization of a pro-inflammatory community of microbes.

Cancer immunotherapy

The importance of the composition of the microbiota in how tumour-carrying hosts respond to chemotherapy or checkpoint blockade immunotherapy has been highlighted in several studies. Reductions in the growth of sarcomas in mice following treatment with the chemotherapeutic drug cyclophosphamide can be compromised after exposure to antibiotics, and this has been attributed to the loss of anti-tumour T_H17-cell-inducing commensal bacteria, the growth of which is favoured by the chemotherapy[125]. However, it is unknown whether the beneficial anti-tumour properties of microbiota-dependent T_H17 cells are broadly applicable. Similarly, antibiotics compromise the anti-tumour response that follows CTLA-4 blockade in mice[126]. In this case, anti-CTLA-4 immunotherapy favours the dominance of species of *Bacteroides*, such as *B. fragilis* and *B. thetaiotaomicron*, in both mice and people. These bacteria are of benefit because they enhance the efficacy of the CTLA-4 blockade, possibly through an anti-tumour response mediated by T_H1 cells[126]. In another mouse model, colonization of the gut with Bifidobacteria has been found to contribute to the control of implanted syngeneic tumours by CD8-expressing T cells following anti-PD-L1 cancer immunotherapy[3]. The mechanism for the improved anti-tumour response might involve activation of the functions of antigen-presenting cells, followed by improved infiltration of tumours by cytotoxic T cells, although it remains to be determined whether microbiota-regulated $CD4^+$ T cells also have a role in restraining the growth of tumours.

Outlook

Studies of how the mutualistic relationship between cells of the adaptive immune system and members of the microbiota affect health and disease are in their infancy. Most efforts have strived to establish reductionist approaches that can be exploited to elucidate cellular and molecular mechanisms. From a translational perspective, models of humanized microbiota in germ-free mice and pigs have been established[127]. It is possible that these efforts will permit the design of bacterial consortia and metabolic products that durably activate or suppress specific programs of adaptive immunity, which will result in the development of improved vaccines and therapeutic drugs for disorders that involve the immune system — including infections, autoimmunity, allergies and cancer. It should be noted, however, that the interactions between the microbiota and the host are influenced to a large extent by host genetics, cooperation and competition between pathogenic and commensal microbes and multiple environmental variables, including diet, circadian factors and the climate. The 'one microbe, one response' approach will probably need to be supplanted by more integrative systems analyses that require the development of advanced technologies and computational tools. Improved characterization of metabolites or other microbial effectors, coupled with computational pathway analyses, might enable the design of synthetic organisms or postbiotic products that can shape immune responses. Elucidation of the role of viruses and phages might provide further approaches for targeting components of the microbiota or host cells for therapeutic purposes. The role of the microbiota in shaping adaptive immunity should therefore become an increasingly fertile area for basic and translational investigation. ■

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