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Regionalized Development and Maintenance of the Intestinal Adaptive Immune Landscape

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The intestinal immune system has the daunting task of protecting us from pathogenic insults while limiting inflammatory responses against the resident commensal microbiota and providing tolerance to food antigens. This role is particularly impressive when one considers the vast mucosal surface and changing landscape that the intestinal immune system must monitor. In this review, we highlight regional differences in the development and composition of the adaptive immune landscape of the intestine and the impact of local intrinsic and environmental factors that shape this process. To conclude, we review the evidence for a critical window of opportunity for early-life exposures that affect immune development and alter disease susceptibility later in life.

Introduction

The intestine is a long tube-like structure stretching from the oral cavity to the anus, and its primary functions are to digest food, absorb nutrients and water, and eliminate waste. The intestine also represents a major site of entry for many bacterial and viral pathogens and is home to a vast and diverse microbial community increasingly recognized to have a major impact on host physiology and pathophysiology. This constantly changing and dynamic environment represents a key challenge to the immune system. It is thus perhaps unsurprising that the intestine contains the greatest number and diversity of immune cells in the body.

The intestine should not be viewed as a single homogeneous organ but rather as consisting of several anatomically and functionally specialized segments with distinct environmental pressures (for a review, see Mowat and Agace, 2014). To maintain local tissue homeostasis, different intestinal segments have adopted unique defense strategies, including alterations in epithelial subset composition and function, mucus layer integrity, and regionalized immune system specialization. Continual crosstalk between environmental signals and the immune system is essential for maintaining local tissue homeostasis, and alterations in such signals (e.g., from nutritional deficiencies or alterations in microbial composition [dysbiosis]) can rewire immune cell composition and functionality, resulting in chronic inflammation and increased risk for infection.

Here, we highlight the contribution of luminal signals in shaping and maintaining regional adaptive immune cell composition and function in the intestine. We further discuss the early environmental factors affecting intestinal immune system development and their long-term impact on health. Collectively, such studies highlight the importance of the environment in maintaining local immune homeostasis and the potential of modulating such signals in early-life and inflammatory settings for improving human health.

Regional Differences in Intestinal Anatomy, Function, and Luminal Content

The small intestine, whose primary function is in nutrient digestion and absorption, starts at the pylorus directly downstream of the stomach and terminates at the ileocaecal valve. In humans, it reaches 6–7 m in length and consists of the duodenum, jejunum, and ileum in descending order. The surface is characterized by long finger-like projections called villi, which become progressively shorter and broader down the small intestine. Villi are surrounded by intestinal crypt invaginations, termed the crypts of Lieberkühn, that contain the multi-potent epithelial stem cells that give rise to the various epithelial lineages covering the intestinal surface. The base of these crypts, particularly in the ileum, is home to specialized epithelial cells, termed Paneth cells, that produce a range of antimicrobial peptides that serve to protect the sterility of the crypt, as well as epithelial growth factors that regulate stem cell differentiation and function. Absorptive epithelial cells, termed enterocytes, coat the villi; their apical surface is covered with microvilli that form a "brush border" for optimal digestion and absorption of foodstuffs. In addition to the luminal content from the stomach, the duodenum receives 0.6–1.0 L of bile per day through the common bile duct. The major constituents of bile include cholesterol, lecithin, bilirubin, and bile salts, the latter of which provide a key detergent action on fat particles in the food and aid in the adsorption of fatty acids, monoglycerides, cholesterol, and other lipids. Absorption of mono-, di-, and trisaccharides, amino acids, dietary fat, and fat (vitamins A, D, E, and K)- or water (vitamins B and C)-soluble vitamins occurs primarily in the duodenum and jejunum, whereas the ileum serves as a major site of bile salt and vitamin B12 absorption. Because many of these luminal constituents have immune-modulatory activity, their varying concentrations along the length of the intestine are likely to have an important impact on regulating regionalized immune cell compartmentalization and functionality.

The ileocecal valve opens into the large intestine, which (in humans) is composed of the caecum (and associated blind-ended

appendix), the ascending, transverse, descending, and sigmoid colon, and the rectum, and it terminates at the anal canal. The human colon is considerably shorter (approximately 1.5 m) and wider than the small intestine. Although also covered by a single layer of columnar epithelial cells, its surface consists entirely of crypts interspersed between flat regions of surface epithelium. Mucus-producing goblet cells make up a relatively small percentage ($\leq$10%) of epithelial cells in the small intestine but represent $\geq$25% of epithelial cells in the large intestine. As a result, the mucous layer, termed the glycocalyx, is diffuse and permeable to bacteria in the small intestine but forms a thick bi-layered structure in the colon, whose inner layer is virtually impenetrable to bacteria. Although the majority of dietary constituents are absorbed in the small intestine, approximately 1.5 L of intestinal fluid passes through the ileocecal valve each day. This fluid consists primarily of water and electrolytes, most of which are absorbed in the upper half of the colon, as well as semi- or undigested foodstuffs, including plant polysaccharides and fiber, which serve as an essential nutritional source for resident microbiota.

The intestinal microbiome plays an essential role in host health and, particularly in the context of this review, in modulating local adaptive immune cell development, composition, and function. Microbial density increases from the upper to the lower gastrointestinal tract until it reaches its peak in the colon, which contains the highest microbial diversity and load. The mechanisms by which the microbiota modulates the intestinal immune system is under intense investigation but include (1) direct microbial interactions with host cells and (2) bacterial generation of immune modulatory molecules either through modification of dietary metabolites or through de novo production. The relative impact of these pathways in modulating immune homeostasis will vary along the length of the intestine depending on local variations in surface physiology (e.g., mucus integrity), microbial load, and microbial diversity.

Regionalized Variation in Intestinal Inductive Compartments

The intestine is composed of four major layers: the mucosa, the submucosa, the muscularis, and the serosa. The mucosa, which is proximal to the intestinal lumen, consists of a single layer of columnar epithelial cells and an underlying lamina propria (LP) and contains the vast majority of immune cells. Immune cells are located within organized lymphoid structures, termed gut-associated lymphoid tissues (GALTs), embedded within the intestinal LP and sub-mucosa or are diffusely distributed throughout the epithelium and LP. GALTs, together with intestinal draining lymph nodes (LNs), serve as the major sites of adaptive immune cell priming in the intestine. GALTs include the macroscopically visible Peyer's patches (PPs) of the small intestine, caecal patches, colonic patches, and smaller structures collectively referred to as solitary isolated lymphoid tissues (SILTs), which include cryptopatches (CPs) and more mature isolated lymphoid follicles (ILFs) (Hamada et al., 2002; Pabst et al., 2005). In humans, PPs are primarily located in the terminal ileum; their numbers peak in the midteens (approximately 250) and then wane with age (Cornes, 1965). Additional large GALT structures are found throughout the appendix. Although CPs have not been identified in the human intestine, there are an estimated 30,000 ILFs, whose highest densities are in the ileum and distal colon (Moghaddami et al., 1998; O'Leary and Sweeney, 1986). Large GALT structures in mice include PPs, which are more numerous in the distal small intestine, a single large GALT in the caecum (the caecal patch), and two to five colonic patches (Baptista et al., 2013; Masahata et al., 2014). Mice also have 1,000–1,500 SILTs distributed throughout the small intestine and mature SILTs concentrated in the ileum, consistent with the finding that SILT maturation in the small intestine requires signals from the microbiota (Herbrand et al., 2008). Although SILT numbers in the colon have yet to be enumerated, they appear more abundant in the distal colon (Baptista et al., 2013).

GALTs lack afferent lymphatics and sample luminal particulate antigens through specialized microfold (M) cells located within an overlying follicular-associated epithelium. Antigens sampled through M cells are subsequently taken up by antigen-presenting cells in the underlying sub-epithelial dome of GALTs for processing and presentation to lymphocytes. In contrast, adaptive immune cell priming in intestinal draining LNs involves the transport and presentation of luminal or self-derived antigen by LP-derived migratory dendritic cells (DCs) (Macpherson and Uhr, 2004). Several mechanisms by which DCs acquire luminal antigen in the LP have been proposed, including (1) direct antigenic uptake via goblet-cell-associated antigen passages (McDole et al., 2012) or trans-epithelial extended DC-derived dendrites (Farache et al., 2013; Rescigno et al., 2001) or (2) indirect uptake via tissue-resident macrophages (Mazzini et al., 2014), apoptotic epithelial cells (Huang et al., 2000), or villous M cells (Jang et al., 2004). The relative contribution of these pathways in adaptive immune cell priming, and whether differential antigen uptake results in distinct adaptive immune responses, remains unclear.

Development of the Inductive Sites in the Intestine

The development of PPs is initiated during fetal life and is guided by intrinsic and programmed events, although maturation is completed postnatally and is influenced by external factors. In contrast, CPs and ILFs develop and mature after birth. In addition, the development of GALT structures in the small intestine and colon is guided by different signals.

In mice, the development of PPs and colonic patches is initiated on embryonic day 12.5 (Adachi et al., 1998). For PPs, the primary event is initiated by $CD45^+CD4^-CD3^-CD127^-cKit^+CD11c^+$ lymphoid tissue initiator (LTin) cells expressing LTβ and RET (Fukuyama and Kiyono, 2007; Veiga-Fernandes et al., 2007) (Figure 1A). Stimulation of LTin cells by the RET ligand artemin (ARTN) expressed on VCAM-1^+ stromal cells initiates upregulation of LTα1β1 and clustering of LTin cells, resulting in activation of lymphoid tissue organizer (LTo) cells and recruitment of lymphoid tissue inducer (LTi) cells (Patel et al., 2012). In PPs, this initial event is dependent on RET and cellular adhesion but not on chemokines. It is not yet clear whether RET signaling is also involved in the development of colonic patches. LTi cells originate in the fetal liver, although recently the fetal gut has been shown to contain pre-LTi ($IL\text{-}7R\alpha^+CD4^-ROR\gamma t^-$) and LTi_0 ($IL\text{-}7R\alpha^+CD4^-ROR\gamma t^+$) cells that mature locally in response to retinoic acid (RA) signaling (van de Pavert et al., 2014). Increasing maternal retinoid intake or blocking RA signaling in

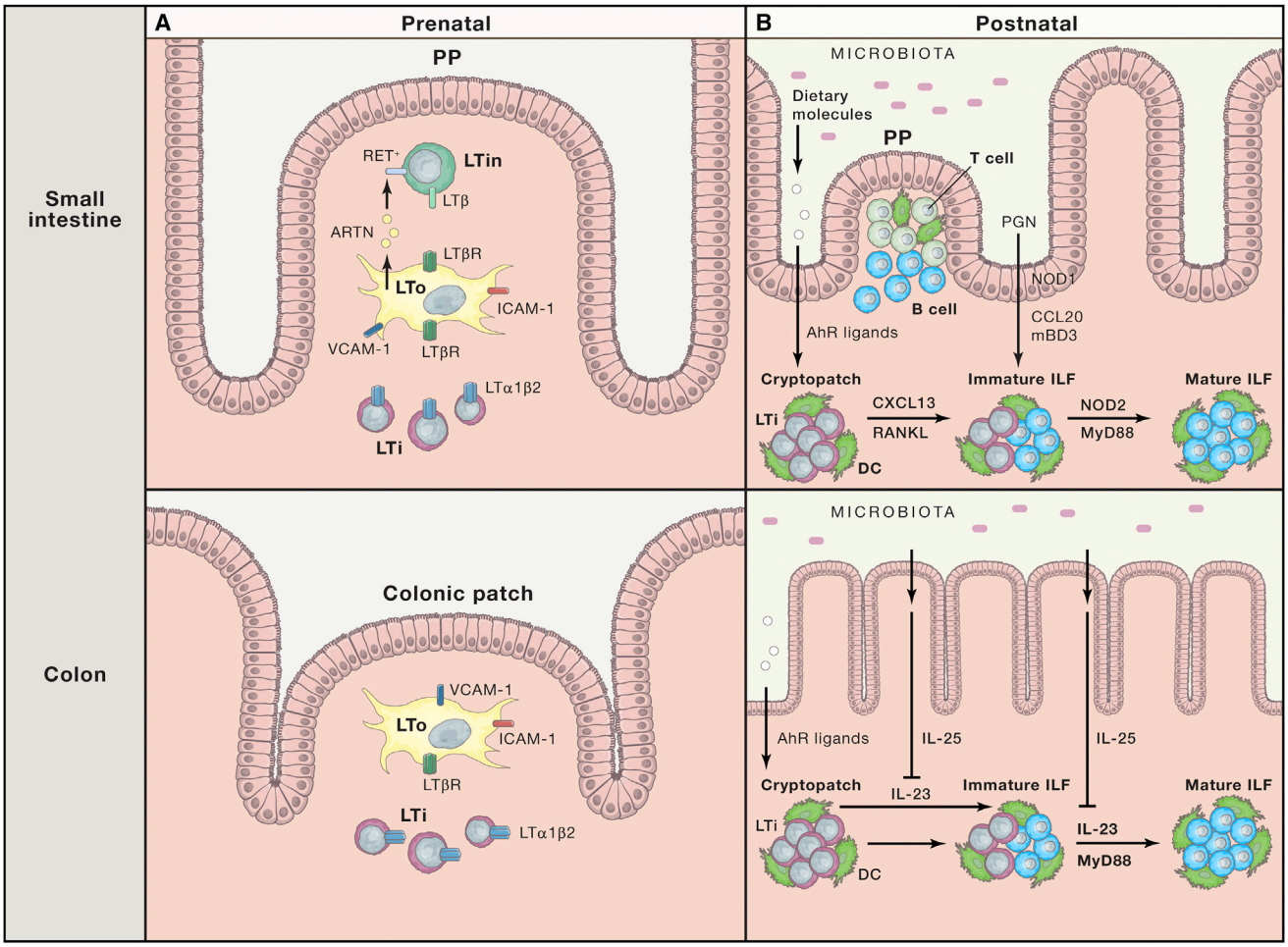

Figure 1. Pre- and Postnatal Development of GALTs

(A) PP development in the small intestine is initiated prenatally after interaction betweeen LTβ$^+$RET$^+$ LTin cells and ARTN-producing VCAM-1$^+$ICAM-1$^+$ LTo stromal cells. LTβ interaction with LTβR upregulates VCAM-1 and ICAM-1 expression on LTo cells and stimulates production of cytokines CCL19, CCL21, CXCL13, and IL-7, the latter of which recruits IL-7Rα$^+$ LTi cells and upregulates LTα1β1 expression. It is not known whether LTβ$^+$RET$^+$ LTin cells are required for the development of colonic patches.

(B) CPs and ILFs develop postnatally in the colon and small intestine. CPs form first as clusters of LTi cells with CD11c$^+$ cells and survive and expand in response to AhR ligands. In the small intestine, the transition of CPs to immature ILFs requires RANKL and CXCL13 and microbial stimulation of NOD1 on epithelial cells, leading to the production of CCL20 and mBD3, ligands of CCR6. Further maturation involves NOD2 and the adaptor molecule MyD88. In the colon, the transition of CPs to ILFs does not require CXCL13, RANKL, CCR6, or the microbiota. Here, IL-23 production from CD11c$^+$ cells in the CPs drives maturation, which is inhibited by the IL-25 that is produced by epithelial cells in response to the microbiota.

pregnancy alters PP development, indicating that in addition to the intrinsic and programmed events that guide PP development, maternal dietary signals can also play a role (van de Pavert et al., 2014).

In PPs and colonic patches, LTβR signaling on LTo cells leads to activation of NFκβ (Dejardin et al., 2002) and stimulates expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), mucosal vascular addressin cell adhesion molecule 1 (MadCam-1), CXCL13, CXCL12, CCL19, CCL21, and IL-7 (Bénézech et al., 2010; Cupedo et al., 2004; Dejardin et al., 2002; Vondenhoff et al., 2009). These interactions create a positive-feedback loop leading to further recruitment of IL-7Rα$^+$ LTi and other hematopoietic cells, including B and T cells. IL-7Rα signaling on LTi cells is necessary for PP development (Adachi et al., 1998; Luther et al., 2003; Yoshida et al., 1999). Whereas B and T cells are rapidly recruited to small intestinal PPs during development, recruitment to the colonic patches is delayed (Baptista et al., 2013), suggesting differential signaling between the small intestine and the colon.

In mice, CPs and ILFs develop postnatally in a process that is influenced by dietary and microbial factors, whereas in humans, ILFs are present before birth (Hoorweg and Cupedo, 2008). In mice, CPs form around day 14 after birth and first appear as clusters of LTi cells surrounded by CD11c$^+$ cells (Figure 1B). The microbiota appears not to be required for CP formation given that such structures also form in germ-free mice (Kanamori et al., 1996). In contrast, CPs are severely reduced in the absence of the aryl hydrocarbon receptor (AhR), most likely because of a lack of expansion or survival of RORγt$^+$ innate lymphoid cells (ILCs) (Kiss et al., 2011; Lee et al., 2011). Natural AhR ligands can be endogenous, provided through diet, or produced by the microbiota through tryptophan metabolism (Cella and Colonna, 2015).

In the small intestine, the transition of CPs to ILFs requires stromal cell expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates CXCL13 production by stromal cells and CD11c$^+$ DCs (Knoop et al., 2011). Deficiency of either CXCL13 or CXCR5 leads to an absence of ILFs (but not CPs) in the small intestine (McDonald et al., 2010; Velaga et al., 2009). In contrast, colonic ILFs do not require RANKL or CXCL13 for development (Baptista et al., 2013; Knoop et al., 2011). The transition of CPs to immature ILFs in the small intestine occurs in response to peptidoglycan (PGN) derived from gram-negative bacteria. PGN ligation of nucleotide-binding oligomerization domain-containing protein 1 (NOD1) on epithelial cells leads to secretion of CCL20 and β-defensin 3 (mBD3), which are both ligands for CCR6, and a deficiency of CCR6, CCL20, or mBD3 leads to loss of ILFs in the small intestine (Bouskra et al., 2008). Further maturation is then stimulated by activation of Toll-like receptors (TLRs), myeloid differentiation factor 88 (MyD88), and NOD2 (Bouskra et al., 2008). In contrast to the small intestine, the colon of germ-free mice harbors immature and mature ILFs, indicating that, despite the increased bacterial load of the colon, microbial-derived signals are not essential for their development (Baptista et al., 2013; Donaldson et al., 2015). Nevertheless, the microbiota is still able to regulate colonic ILFs. For example, the number of ILFs in the colon is increased in NOD1-deficient mice (Bouskra et al., 2008), suggesting that NOD1-independent pathways can stimulate colonic ILF development. Chemokine and cytokine involvement in ILF development also differs between the small intestine and colon; colonic ILFs are present in CCR6-deficient mice (Baptista et al., 2013), indicating that the CCR6-CCL20 pathway is less important in the colon. In addition, despite their presence in germ-free mice, mature colonic ILFs still require the adaptor molecule MyD88 (Baptista et al., 2013), suggesting that signaling through members of the IL-1R family rather than TLRs could be important at this site. Furthermore, IL-23 has been shown to be a colon-specific regulator of ILFs, which is supported by the observation that IL-23p19-deficient mice show a specific reduction in colonic ILFs (Donaldson et al., 2015). Microbial stimulation of epithelial cells leads to IL-25 production, which then inhibits IL-23 expression by CD11c$^+$ cells enriched with colonic ILFs (Donaldson et al., 2015). Although microbiota stimulate IL-25 production in both the small and large intestines, tissue specificity of the response could be due to the scarcity of the IL23-expressing CD11c$^+$ cells in small intestinal ILFs.

Regionalized Variation in Intestinal Adaptive Immune Cell Effector Compartments

The intestinal epithelium and underlying LP are the major sites of adaptive immune cell accumulation within the intestine. Although the overwhelming majority of adaptive immune cells in the epithelium are CD8$^+$ intraepithelial lymphocytes (IELs) (Cheroutre and Madakamutil, 2004), the LP contains large numbers of functionally diverse CD4$^+$ T cell subsets and plasma cells (PCs) and a relatively minor fraction of CD8$^+$ T cells. Collectively, these cells play an essential role in maintaining intestinal homeostasis; however, their composition differs markedly between distinct intestinal segments. Below, we highlight some of the major differences in adaptive immune cell composition between intestinal segments to emphasize the importance of local signals in shaping regional immune specialization. Most studies have been performed in mice, and although it is tempting to extrapolate such findings to the human setting, far more work is required for assessing variations in immune cell subset composition within different segments of the human intestine under distinct physiological and pathophysiological conditions.

Variations in IEL Composition along the Length of the Intestine

IELs can be broadly divided into two major subsets: type A and type B, the proportions of which vary with age, species, and location along the length of the intestine (Figure 2). Type A IELs derive from conventional naive CD8αβ$^+$TCRαβ$^+$ or CD4$^+$TCRαβ$^+$ T cells that have undergone activation in GALTs or LNs and subsequently homed to the intestine to generate an epithelial resident effector memory T cell population. Type b IELs, on the other hand, do not express CD8β or CD4; instead, they express the CD8αα homodimer and either an αβ or γδTCR. CD8αα$^+$ TCRαβ$^+$ IELs are autoreactive and appear to derive from agonist-selected double-positive thymocytes that seed the epithelium as CD4$^-$CD8$^-$ cells, where they upregulate CD8αα (Gangadharan et al., 2006; Leishman et al., 2002; Pobezinsky et al., 2012). The origin of CD8αα$^+$TCRγδ$^+$ IELs is less clear, although recent evidence suggests that Vγ7$^+$ IELs, representing the major population of TCRγδ IELs in mice, undergo selective maturation and expansion within the intestine (Di Marco Barros et al., 2016). In both humans and mice, IEL numbers are greatest in the proximal small intestine and decrease down the length of the intestine. In mice, type B IELs represent a larger fraction of the IEL compartment in the small intestine than in the colon, whereas in humans, type B IELs represent only a minor fraction of IELs in each intestinal segment (Beagley et al., 1995; Boll et al., 1995; Camerini et al., 1993; Ibraghimov and Lynch, 1994; Lundqvist et al., 1995; Suzuki et al., 2002).

Germ-free mice have a marked reduction in IELs, particularly type A IELs (Bandeira et al., 1990; Kawaguchi et al., 1993; Suzuki et al., 2002), most likely as a result of reduced lymphoid tissue maturation and antigen-dependent naive T cell priming. The mechanism(s) by which the microbiota promote type B IEL homeostasis is less clear but could include bacterial-mediated NOD2-dependent IL-15 production by intestinal antigen-presenting cells (Jiang et al., 2013). Although the role of MyD88 in IEL homeostasis remains controversial (Iiyama et al., 2003; Ismail et al., 2011; Qiu et al., 2016; Yu et al., 2006), bacterial-induced MyD88 signaling in small intestinal epithelial cells appears important in maintaining type B TCRγδ IEL functionality (Ismail et al., 2011).

Germ-free mice still have large numbers of type B IELs and maintain a bias for these cells in the small intestine (Suzuki et al., 2002), indicating a role for additional tissue-specific cues in regulating regionalized IEL subset composition (Figure 2). One such factor is the chemokine CCL25. CCL25 is constitutively expressed by epithelial cells in the small intestine but not the colon (Kunkel et al., 2000; Svensson et al., 2002), and its expression, which is microbiota independent, decreases from the proximal to the distal small intestine (Ericsson et al., 2004; Stenstad et al., 2007). Mice deficient in CCL25 or its receptor CCR9 have reduced numbers of small intestinal IELs and TCRγδ IEL in particular (Wurbel et al., 2007; Wurbel et al., 2001). The CCL25-CCR9 axis is also required for optimal

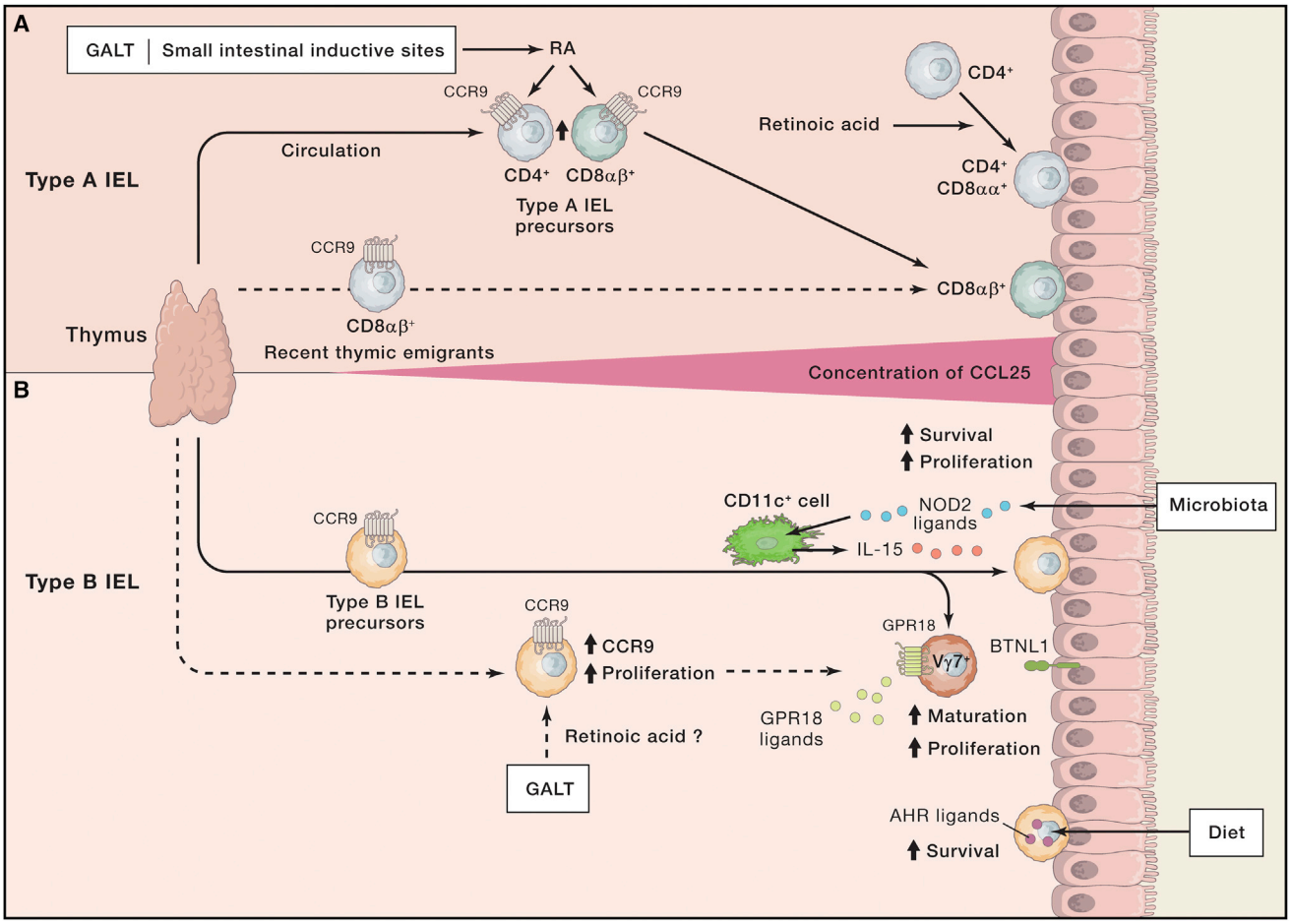

Figure 2. Factors Implicated in Regulating Intraepithelial Lymphocyte Composition along the Length of the Intestine

CCL25 is constitutively expressed by epithelial cells in the small intestine, but not the colon, particularly in the proximal small intestine. CCL25 recruits CCR9-expressing IEL precursors from the blood stream into the small intestinal epithelium.

(A) Most type A IELs derive from naive conventional $CD4^+$ or $CD8\alpha\beta^+$ T cells that acquire CCR9 expression after their activation in small intestinal inductive sites and exposure to RA. $CD8\alpha\beta^+$ recent thymic emigrants also express CCR9 and can gain direct access to the small intestinal epithelium. RA is also required for the conversion of $CD4^+$ type A IELs into MHCII-restricted cytotoxic $CD4^+CD8\alpha\alpha^+$ IELs.

(B) CCR9-expressing type B IEL precursors are recruited directly from the thymus, although some might undergo prior circulation and activation in small intestinal GALTs and acquire higher expression of CCR9. After their entry into the small intestinal epithelium, type B IEL numbers are maintained by multiple local factors, including (1) microbial-derived NOD ligands that drive resident antigen-presenting cells to produce IL-15, a proliferation and survival factor for type B IEL; (2) selective expression of BTNL1 by small intestinal epithelial cells to promote $V\gamma7^+$ IEL maturation and proliferation; (3) GPR18 ligands that promote type B IELs, particularly $V\gamma7^+$ IEL maturation; and (4) AhR ligands that directly promote type B IEL survival.

$CD8\alpha\beta^+$ T cell migration to the small intestinal epithelium (Johansson-Lindbom et al., 2003; Svensson et al., 2002; Wurbel et al., 2007), especially the proximal small intestine (Stenstad et al., 2007). CCR9 is induced on $CD8\alpha\beta^+$ T cells during their activation in intestinal inductive sites (Johansson-Lindbom et al., 2003; Svensson et al., 2002), a process that is dependent on the vitamin A metabolite RA (Iwata et al., 2004; Jaensson-Gyllenbäck et al., 2011; Svensson et al., 2008). Vitamin A obtained through diet is found at higher concentrations in the small intestine than in the colon (Jaensson-Gyllenbäck et al., 2011) and is locally converted to RA by small intestinal epithelial cells (McDonald et al., 2012) and local stromal cells (Vicente-Suarez et al., 2015). DCs in the small intestine constitutively receive stronger RA signals than do colon DCs (Jaensson-Gyllenbäck et al., 2011), imprinting them with an ability to generate RA and, after their migration to intestinal draining LNs, induce CCR9 on responding T cells. Some type B IEL precursors, particularly $V\gamma7^+$ T cells, can also proliferate and acquire enhanced CCR9 expression in small intestinal GALTs prior to their entry into the small intestinal epithelium (Guy-Grand et al., 2013). RA is also required for the differentiation of $CD4^+$ type A IELs into major histocompatibility complex II (MHCII)-restricted cytotoxic $CD4^+CD8\alpha\alpha^+$ IELs (Reis et al., 2014). Indeed, given the pleiotropic effects of RA on immune cells, its broader impact on small intestinal IEL functionality warrants further study.

The butyrophilin-like (btnl) molecules are an additional family of mediators involved in regulating region-specific lymphocyte composition in epithelial tissues (Abeler-Dörner et al., 2012). Murine BTNL1, BTNL4, and BTNL6 are selectively expressed in the small intestine by post-mitotic enterocytes; BTNL1 is predominately present in the proximal and middle segments of the small intestine and reaches maximal expression 14 days

after birth (Bas et al., 2011; Di Marco Barros et al., 2016). Enterocyte-expressed BTNL1 is required for the maturation and proliferation of Vγ7$^+$ IELs after their entry into the epithelium, a process that is independent of the thymus, the microbiota, and dietary proteins (Di Marco Barros et al., 2016). Induced expression of a *Btnl1* transgene in young (7- or 21-day-old) but not adult (11-week-old) *Btnl1*-deficient mice rescues Vγ7$^+$ IEL proliferation and numbers, suggesting that BTNL1-mediated Vγ7$^+$ IEL proliferation is temporally restricted to early life. G-protein-coupled receptor 18 (*Gpr18*)-deficient mice also have reduced numbers of small intestinal IELs, particularly Vγ7$^+$ IELs (Wang et al., 2014). Similar to BTNL1, GPR18 appears to function after γδ$^+$ T cell entry into the epithelium, and γδ$^+$ IELs in *Gpr18*-deficient mice appear immature (Wang et al., 2014). Although the identity of the GPR18 ligand(s) remains unclear, these results indicate that GPR18 could function to promote Vγ7$^+$ IEL interactions with BTNL1-expressing enterocytes. Interestingly, although the genetic locus encompassing *Btnl1* and *Btnl6* is lacking in humans, BTNL3 and BTNL8 are constitutively expressed by epithelial cells in the human colon, and in vitro studies indicate their potential role in supporting human Vγ4$^+$ IEL maturation (Di Marco Barros et al., 2016).

Finally, a major dietary determinant of type B IEL homeostasis is dietary AhR ligands (Li et al., 2011). Mice deficient in AhR have a selective reduction in type B IELs in the small intestine. Wild-type mice fed a synthetic diet have reduced numbers of type B IELs, but these can be restored by dietary supplementation with the AhR ligand precursor indole-3-carbinol (I3C). Mechanistically, direct AhR signaling in IELs appears important for type B IEL survival after their entry into the epithelium. Whether AhR signaling contributes to type B IEL homeostasis in other parts of the intestine remains to be explored.

Regional Compartmentalization of LP CD4$^+$ T Cell Subsets

The selective induction of homing molecules in intestinal inductive sites is a major contributor to the generation of regionalized LP T cell compartments. CCR9 induction on CD4$^+$ and CD8$^+$ T cells after activation in small intestinal inductive sites promotes their homing to the small intestinal LP, particularly to the proximal small intestine (Campbell and Butcher, 2002; Stenstad et al., 2006; Stenstad et al., 2007; Svensson et al., 2002). Conversely, the G-protein-coupled receptor GPR15 is expressed on colonic but not small intestinal T cells, and in mice, it promotes effector and regulatory T cell recruitment to the colonic LP (Kim et al., 2013; Nguyen et al., 2015). In contrast to CCR9, where and how activated T cells acquire expression of GPR15 remains unclear, although it is attractive to speculate that this could occur in colonic GALT or colon draining LNs. Administration of broad-spectrum antibiotics in mice reduces GPR15 expression on colonic T cells (Kim et al., 2013), and SCFAs induce expression of GPR15 on anti-CD3-treated human CD4$^+$ T cells in vitro (Fischer et al., 2016), suggesting a potential role for the microbiota in GPR15 induction. The GPR15 ligand(s) mediating T cell recruitment to the colon are unknown, although GPR15-dependent T cell homing to the colon is unaffected in germ-free mice, indicating that the ligands are not microbial derived (Kim et al., 2013). In contrast to murine GPR15, the human protein is expressed on intestinal effector cells but not Foxp3$^+$ Treg cells, suggesting that GPR15 is a potential target for the treatment of colitis (Fischer et al., 2016; Nguyen et al., 2015).

Although distinct homing pathways help focus effector and regulatory T cell responses to the small intestine or colon, there are also marked differences in the composition of LP T helper (Th) subsets, particularly in the proportions of FoxP3$^+$ Treg and Th17 cells along the length of the mouse intestine. The proportions of FoxP3$^+$ Treg cells in the intestinal LP are higher than in other tissues; they range from 10%–20% of CD4$^+$ T cells in the small intestine and 20%–40% in the colon, depending on housing conditions. Strikingly, Foxp3$^+$ Treg cell numbers are reduced in the colon but not small intestine of germ-free or antibiotic-treated mice (Atarashi et al., 2011; Geuking et al., 2011), whereas germ-free mice fed an antigen-free diet have dramatically reduced numbers of small intestinal FoxP3$^+$ Treg cells (Kim et al., 2016a). Thus, most FoxP3$^+$ Treg cells in the small intestine appear to develop in response to dietary antigens, whereas a large proportion of colonic FoxP3$^+$ Treg cells develop in response to the microbiota. During homeostasis, LP FoxP3$^+$ Treg cells can be divided into two major subsets: (1) FoxP3$^+$ Treg cells that co-express neuropilin 1 (NRP1) and Helios and are believed to primarily consist of thymically derived natural (n)Treg cells and (2) Helios$^-$NRP1$^-$FoxP3$^+$ Treg cells that appear to represent peripherally induced Treg (pTreg) cells. pTreg cell subsets can be further divided into RORγt-expressing FoxP3$^+$pTreg cells and RORγt$^-$FoxP3$^+$ pTreg cells (Ohnmacht et al., 2015; Sefik et al., 2015; Yang et al., 2016). RORγt$^+$FoxP3$^+$ pTreg cells represent the major FoxP3$^+$ Treg cell subset in the colon and, in contrast to natural Treg (nTreg) or RORγt$^-$FoxP3$^+$ pTreg cells, are dramatically reduced in germ-free mice or after antibiotic treatment, suggesting that they develop in response to the microbiota (Kim et al., 2016a; Ohnmacht et al., 2015; Sefik et al., 2015; Yang et al., 2016). Consistent with this, mono-colonization of germ-free mice with bacteria from a range of phyla and genera induces colonic RORγt$^+$FoxP3$^+$ pTreg cell generation, albeit to varying degrees (Sefik et al., 2015); a dominant species possessing such activity in both humans and mice appears to be the Clostridia that promote mucosal TGFβ production and RORγt$^+$FoxP3$^+$ pTreg cell generation independently of MyD88 (Atarashi et al., 2013; Atarashi et al., 2011; Ohnmacht et al., 2015). Interesting, RORγt$^+$FoxP3$^+$ Treg cell numbers are also reduced in IL-6- and IL23α-deficient mice, suggesting that they follow a developmental pathway at least partially overlapping that of Th17 cells (Ohnmacht et al., 2015). The microbiota has also been suggested to maintain the intestinal nTreg cell niche under conditions of pTreg cell deficiency in an MHCII-independent manner (Korn et al., 2014). In contrast, RORγt$^-$FoxP3$^+$ pTreg cells dominate in the small intestine and represent only a minor proportion of FoxP3$^+$Treg cells in the colon, and their numbers are selectively reduced in mice fed an antigen-free diet, suggesting that they develop in response to dietary antigen (Kim et al., 2016a). Global absence of FoxP3$^+$pTreg cells in the intestine results in spontaneous allergic Th2-type inflammation along the length of the intestine (Josefowicz et al., 2012); however, RORγt$^+$ and RORγt$^-$ pTreg cell subsets appear to play distinct roles in maintaining homeostasis given that the specific absence of RORγt$^+$FoxP3$^+$ pTreg cells results in enhanced intestinal Th2 (Ohnmacht et al., 2015) or Th17 and Th1 cell responses (Sefik et al., 2015), whereas the absence of

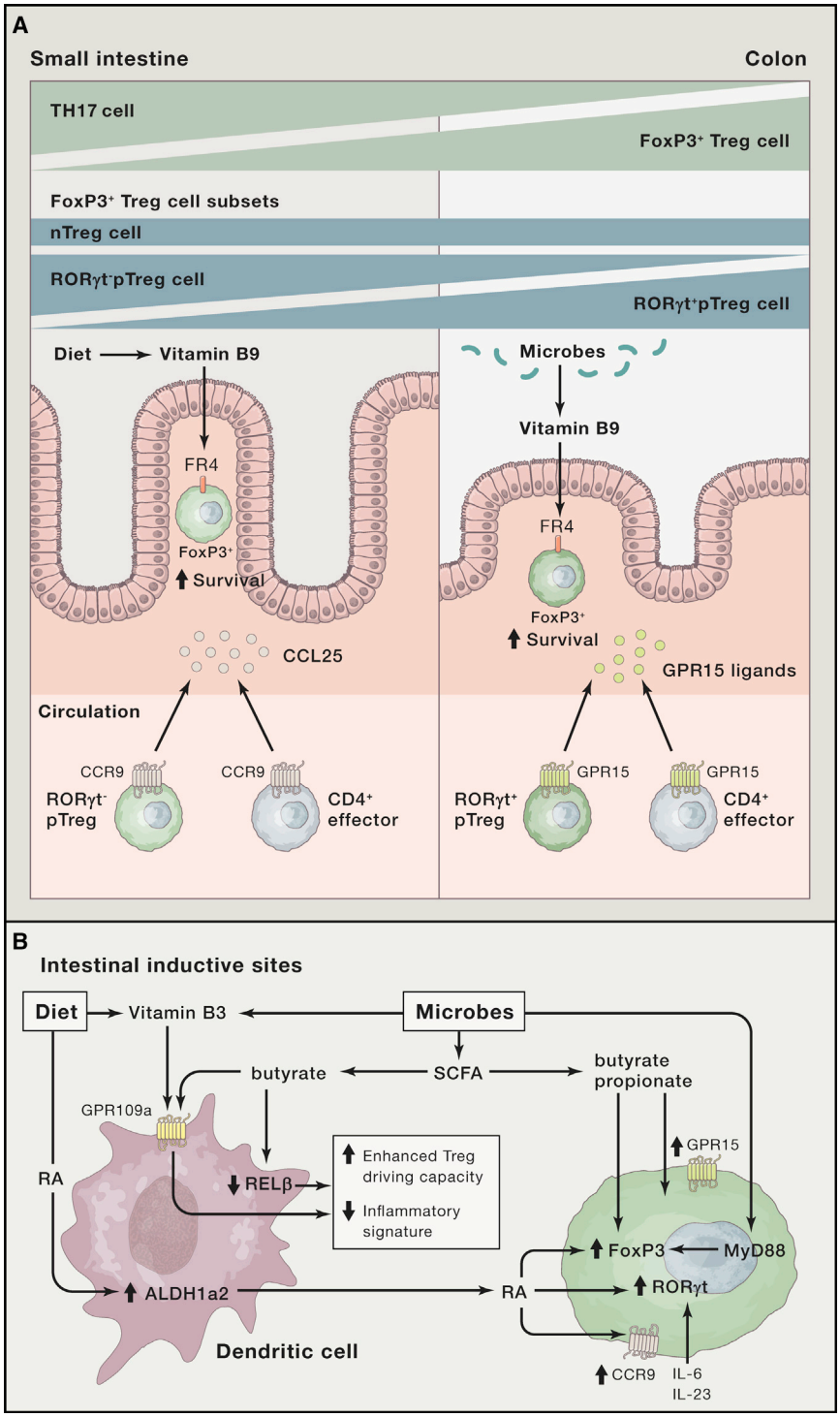

Figure 3. Impact of Environmental Signals on the Generation and Distribution of Peripherally Induced FoxP3$^+$ Treg Cells

(A) FoxP3$^+$ Treg cells, although found throughout the intestinal LP, are highest in number in the colon and inversely correlate with intestinal Th17 cell numbers. FoxP3$^+$ Treg cells can be divided into Helios$^+$NRP1$^+$ thymically derived nTreg cells and Helios$^-$NRP1$^-$ pTreg cells. pTreg cells can be further divided into RORγt$^+$pTreg cells, which are heavily dependent on the microbiota and dominate in the colon, and RORγt$^-$pTreg cells, which are dependent on dietary antigen and dominate in the small intestine. Dietary or microbial-derived vitamin B9 signals through folate receptor 4 (FR4) to promote FoxP3$^+$ Treg cell survival in the intestinal LP. FoxP3$^+$ Treg cell and effector CD4$^+$ T cell migration to the small intestine involves the CCL25-CCR9 axis, whereas FoxP3$^+$ Treg cell (mouse only) and effector CD4$^+$ T cell migration to the colon is mediated by GPR15, whose ligands remain to be determined.

(B) pTreg cells are generated from naive CD4$^+$ T cells in intestinal inductive sites, and their development requires MHCII expression by CD11c$^+$ DCs. The generation of pTreg cells is enhanced by environmental signals acting directly on CD4$^+$ T cells during their differentiation or indirectly on DCs promoting their tolerogenic capacity. For RORγt$^+$ pTreg cells, such signals include microbial-dependent MyD88 signaling and SCFA signaling in T cells to promote FoxP3 transcription and potentially expression of the colon homing receptor GPR15. Generation or maintenance of RORγt$^+$pTreg cells also requires IL-6 and IL-23, which promote RORγt expression, and RA, which promotes FoxP3 expression and could prevent these cells from differentiating to the Th17 cell lineage. The ability of DCs to drive FoxP3$^+$ Treg cell differentiation is promoted by RA, which in a feed forward loop enhances the capacity of DCs to make RA and vitamin B3 (niacin), which functions via GPR109a, to reduce the inflammatory potential and promote the regulatory potential of these cells. Butyrate signals through GPR109a in a similar manner or in a G-protein-coupled-independent manner to downregulate RelB expression and enhance the Treg-cell-driving capacity of DCs. The generation of RORγt$^-$ pTreg cells is independent of the microbiota, IL-6, and IL-23 but is promoted by RA. RA synergizes with TGFβ to drive RORγt$^-$pTreg cell development and simultaneously induces expression of CCR9, priming these cells with a small intestinal homing capacity.

RORγt$^-$FoxP3$^+$ pTreg cells enhances susceptibility to food antigen (Kim et al., 2016a).

In addition to providing a steady source of antigen, diet and the microbiota support intestinal Treg cell homeostasis by a wide variety of mechanisms, many of which remain incompletely understood (for a recent review, see Tanoue et al., 2016) (Figure 3). Direct microbiota-induced MyD88-dependent signaling in FoxP3$^+$ Treg cells is important for optimal pTreg cell induction potentially via demethylation of conserved non-coding sequence 2 (CNS2) of the *Foxp3* promotor, which is required for stable *Foxp3* expression. As a result, mice with MYD88 deficiency specifically in FoxP3$^+$ Treg cells have reduced numbers of intestinal FoxP3$^+$ Treg cells (Wang et al., 2015). Short-chain fatty acids (SCFAs), bacterial metabolites generated from the fermentation of undigested fiber and present in high levels in the colon (Cummings et al., 1987), also promote colonic FoxP3$^+$Treg cell homeostasis. Butyrate acts directly on CD4$^+$ T cells to drive H3 acetylation of the *FoxP3* promotor and its CNS1 enhancer element, required for

intestinal pTreg cell generation (Arpaia et al., 2013; Furusawa et al., 2013), whereas propionate enhances pTreg cell generation (Arpaia et al., 2013) and colonic nTreg cell proliferation and functionality, the latter via GPR43 (Smith et al., 2013). Butyrate also promotes FoxP3$^+$ Treg cell generation indirectly through DCs, either in a G-protein-coupled-receptor-independent manner to suppress RelB and pro-inflammatory gene expression (Arpaia et al., 2013) or via the butyrate and niacin (vitamin B3) receptor GPR109a (Singh et al., 2014), enhancing their ability to drive de novo FoxP3$^+$ Treg cell differentiation. Consistent with the latter, mice deficient in *Niacr1* (which encodes GPR109a) display a marked reduction of colonic but not small intestinal FoxP3$^+$ Treg cells (Singh et al., 2014). Certain commensals have also adapted unique mechanisms to promote intestinal Treg cell homeostasis and host-microbial mutualism. Perhaps the best characterized mechanistically is polysaccharide A (PSA) from the human commensal *Bacteroides fragilis*; it promotes IL-10 production by intestinal FoxP3$^+$ Treg cells via TLR2-dependent direct effects on CD4$^+$ T cells (Round and Mazmanian, 2010) or via indirect effects on plasmacytoid DCs (Dasgupta et al., 2014) or conventional DCs (Shen et al., 2012).

Several dietary metabolites that promote FoxP3$^+$ Treg cell generation have also been identified. In addition to inducing CCR9 on pTreg cells, RA synergizes in vitro with TGFβ to promote pTreg cell differentiation (Kang et al., 2007; Sun et al., 2007), and consistent with this finding, mice kept on a vitamin-A-deficient diet appear defective in the induction of oral tolerance (Cassani et al., 2011). RA also promotes the generation of RORγt$^+$FoxP3$^+$ pTreg cells in vitro (Lochner et al., 2008), and blocking RA signaling in vivo prevents the development of these cells (Ohnmacht et al., 2015). Nevertheless, RA has pleiotropic roles in regulating immune cell functionality (for a review see, Brown and Noelle, 2015), and the direct role of RA signaling in T cells for intestinal nTreg and pTreg cell homeostasis in vivo remains to be determined. The water-soluble vitamin B subtype vitamin B9 (folic acid [FA]) also affects FoxP3$^+$ Treg cell homeostasis. FA is obtained exclusively from diet or through the microbiota, and mice kept on an FA-deficient diet display a selective reduction in intestinal FoxP3$^+$ Treg cells (Kinoshita et al., 2012; Kunisawa et al., 2012). Local concentrations of FA promote FoxP3$^+$ Treg cell survival through folate receptor 4, which is highly expressed by FoxP3$^+$ Treg cells (Kinoshita et al., 2012; Yamaguchi et al., 2007). Although additional dietary derivatives have been implicated in promoting Treg cell induction, most notably tryptophan metabolites (Mezrich et al., 2010; Singh et al., 2014) and the vitamin D3 metabolite 1-25 dihydroxyvitamin D3 (Kang et al., 2012), their impact on regionalized intestinal FoxP3$^+$ Treg cell homeostasis remains to be elucidated.

In mice, the total number and proportions of intestinal Th17 cells along the length of the intestine inversely correlate with those of FoxP3$^+$ Treg cells, such that the highest Th17 cell numbers are found in the proximal small intestine and steadily decline toward the colon (Denning et al., 2011). Th17 cells are virtually absent from the intestine of germ-free mice but are found in normal numbers in *Myd88*$^{-/-}$*Trif*$^{-/-}$ mice, demonstrating a key role for the microbiota, but not TLR signaling, in intestinal Th17 cell homeostasis (Atarashi et al., 2008; Ivanov et al., 2008). Treatment of mice with broad-spectrum antibiotics results in a severe depletion of intestinal Th17 cells, suggesting that the microbiota is also critical for maintenance of the intestinal Th17 cell compartment (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2008). The best characterized microbial inducer of intestinal Th17 cell responses is the gram-positive segmented filamentous bacteria (SFB) (Ivanov et al., 2009). SFB adhere to epithelial cells in the terminal ileum and drive a dramatic increase in Th17 cell numbers within the ileal LP. Mechanistically, SFB induce an antigen-specific CD4$^+$ T cell response that is heavily skewed toward the RORγt$^+$ Th17 cell lineage (Geem et al., 2014; Goto et al., 2014; Yang et al., 2014) and dependent on MHCII expression by CD11c$^+$ cells (Geem et al., 2014; Goto et al., 2014) and monocyte-derived macrophages (Panea et al., 2015). SFB-specific Th17 cell differentiation can occur independently of GALT and MLN (Geem et al., 2014; Lécuyer et al., 2014) but most likely requires the tertiary lymphoid tissues that develop as a result of SFB colonization (Lécuyer et al., 2014). Recent data suggest that newly generated SFB-specific RORγt$^+$ CD4$^+$ T cells disseminate throughout the gut (Sano et al., 2015) and that epithelial-derived serum amyloid A (SAA) and reactive oxygen species (ROS) promote IL-17 production by infiltrating RORγt$^+$CD4$^+$ T cells within the ileum (Atarashi et al., 2015; Sano et al., 2015). SFB adherence to ileal epithelial cells is required for epithelial SAA and ROS production, and amplification of this epithelial SAA response through IL-23-dependent IL-22 production by ILC3 and IL-1β production from LP CD11c$^+$ cells is further required to drive local IL-17 production by infiltrating RORγt$^+$CD4$^+$ T cells (Atarashi et al., 2015; Sano et al., 2015). The ability to induce Th17 responses is not, however, unique to SFB but could be a general property of adherent commensal or pathogenic microbes (Atarashi et al., 2015; Tan et al., 2016b), whereby the site of intestinal IL-17$^+$ CD4$^+$ T cell accumulation is determined by the location of the adherent microbe. In this regard, it is interesting to speculate that easier access of adherent microbes to the small intestinal epithelium underlies the enhanced numbers of Th17 cells within this region of the intestine. Finally, although direct epithelial adherence appears key to the efficient generation of Th17 responses, the downstream molecular mechanisms driving this response are likely to be microbe specific (Atarashi et al., 2015; Tan et al., 2016b).

Given the key role of the microbiota in intestinal Th17 cell homeostasis, it is not surprising that dietary-induced changes in microbial composition can alter intestinal Th17 cell numbers. For example, alterations in microbial composition induced by a high-fat diet drive changes in small intestinal antigen-presenting cell functionality, resulting in reduced small intestinal Th17 cell numbers (Garidou et al., 2015), whereas dietary supplementation with long-chain fatty acids (LCFAs) alters the microbial composition by increasing levels of medium-chain fatty acids and LCFAs and reducing SCFAs to promote small intestinal Th17 development (Haghikia et al., 2015). Thus, bacterial communities generating high concentrations of SCFAs promote intestinal Treg cell development, whereas those generating LCFAs appear to favor Th17 cell development. Finally, luminal ATP also appears to promote intestinal Th17 development (Atarashi et al., 2008; Kusu et al., 2013).

Regional Compartmentalization of IgA$^+$ PCs

An estimated 80% of human and mouse PCs are located in the intestinal LP, and recent evidence suggests that they can be

extremely long lived (Landsverk et al., 2017). Their density is highest in the proximal and distal portions of the intestine, and the overwhelming majority of these produce IgA (approximately 75%–80% of PCs in duodenum and jejunum and 90% of PCs in the colon). In both mice and humans, intestinal IgA$^+$ PCs represent a collection of low-frequency and expanded clones (Lindner et al., 2012) that display specificity for the microbiota and self-antigens (Benckert et al., 2011). Intestinal IgA plays an essential role in establishing luminal microbial diversity and host-microbiota mutualism and contributes to preventing microbial access to systemic compartments (Fagarasan et al., 2002). In contrast to mice, which have one IgA subclass, humans have IgA1 and IgA2; IgA1-producing PCs dominate in the small intestine, and IgA2 PCs dominate in the colon (Crago et al., 1984; Kett et al., 1986; Lin et al., 2014). Because protein antigens tend to drive IgA1 responses and polysaccharides tend to drive the IgA2 subclass (Tarkowski et al., 1990), the differential distribution of these subclasses along the length of the intestine could reflect alterations in luminal content. Consistent with this, the dominance of IgA1 in the small intestine is reversed to IgA2 under conditions of bacterial outgrowth (Kett et al., 1995). IgA2 is also more resistant to bacterial proteases, suggesting adaptation to the colonic environment (Kilian et al., 1996; Plaut et al., 1974).

GALTs are considered the major sites of IgA class switching (Barone et al., 2011; Brandtzaeg, 2009), which (in mice) optimally requires M-cell-mediated uptake of luminal antigen (Rios et al., 2016). Whereas in mice large GALTs have been considered the primary site of T-cell-dependent IgA responses and ILFs the primary site of T-cell-independent IgA responses (Tsuji et al., 2008), it remains unclear whether this division of labor occurs in humans.

Several host-derived factors promote class switching to IgA in GALTs in vivo; the principal factor in mice is TGFβ (Cazac and Roes, 2000; van Ginkel et al., 1999). However, induction of IgA class switching and PC generation is also highly dependent on external diet- and microbial-derived signals (Figure 4). Germ-free mice display a dramatic reduction in intestinal IgA PCs (Crabbé et al., 1968). The mechanisms by which the microbiota promotes IgA class switching and IgA PC generation in GALTs are multifaceted and remain to be fully characterized. In addition to providing relevant antigenic material and promoting GALT development, the microbiota directly signals through MyD88 in T cells to drive germinal center (GC) T follicular helper (T_{FH}) cell generation in PPs and subsequent T-cell-dependent secretory IgA responses to the microbiota (Kubinak et al., 2015). Microbial-driven MyD88-dependent iNOS induction in CD11c$^+$ cells also promotes T-cell-dependent IgA switching through the induction of TGFβRII on B cells and T-cell-independent IgA responses through induction of a proliferation-inducing ligand (APRIL) and B-cell-activating factor of the TNF family (BAFF) (Tezuka et al., 2007). Additionally, SCFAs inhibit histone deacetylase (HDAC) in B cells to enhance glycolysis and fatty acid synthesis and support antibody production, and they appear to regulate the numbers of IgA$^+$ GC B cells in PPs and IgA$^+$ PCs in the small intestine and colon (Kim et al., 2016b). Finally, RA promotes IgA class switching and IgA production in vitro (Mora et al., 2006; Watanabe et al., 2010), and mice whose B cells are unresponsive to RA display a marked reduction in IgA$^+$ GC B cells in PPs and IgA$^+$ PCs in the small intestine (Pantazi et al., 2015). Notably, the SCFA acetate signals through GPR43 in DCs to enhance their ability to generate RA and promote IgA class-switch recombination and IgA production in vitro (Wu et al., 2016). Whether RA displays a more dominant role in driving IgA plasmablast generation in the small intestine than in the colon, as might be expected given the higher concentrations of retinol in the small intestine (Jaensson-Gyllenbäck et al., 2011), remains to be determined.

IgA plasma cell clones are not evenly distributed throughout the intestine; expanded clones detected throughout the small intestine are infrequently found in the colon of the same mouse and vice versa (Lindner et al., 2012). Such site-specific accumulation of IgA PCs within distinct intestinal segments, as with T cells, is likely to be regulated by differential expression of homing molecules (Figure 4). Consistent with this, RA-dependent CCR9 induction on IgA plasmablasts is required for optimal IgA PC accumulation in the small intestine, but not the colon (Mora et al., 2006; Pabst et al., 2004), and *Ccr9* deficiency results in an enhanced overlap of related expanded IgA clones between the small intestine and colon (Lindner et al., 2012). IgA PCs also express CCR10, whose ligand CCL28 is expressed by intestinal epithelial cells, particularly in the colon. CCR10 and CCL28 are required for optimal T-cell-dependent IgA plasmablast accumulation in both the small intestine and colon (Hieshima et al., 2004; Hu et al., 2011), as well as for the maintenance of long-lived intestinal IgA$^+$ PCs and IgA$^+$ memory B cells (Hu et al., 2011). Although the mechanisms involved in CCR10 induction remain to be determined, newly generated plasmablasts in caecal patches express CCR9 and CCR10, whereas those generated in PPs only express CCR9 (Masahata et al., 2014). As a result, newly generated IgA$^+$ plasmablasts in the caecal patch localize to both the small intestine and colon, whereas those generated in PPs migrate selectively to the small intestine (Masahata et al., 2014).

The titers of luminal IgA and the proportions of IgA-coated luminal bacteria in both humans and mice are far higher in the small intestine than in the colon (Bunker et al., 2015) (Figure 4). Under homeostasis, similar bacterial taxa are represented within IgA-bound and -unbound bacterial fractions in the small intestine, indicating that most small intestinal commensals can elicit an IgA response. In contrast, bacterial species that are selectively present in the colon have little IgA coating, suggesting that commensals in the colon for the most part do not elicit an IgA response. Interestingly, specific IgA coating of bacteria is preserved even when titers of unbound luminal IgA are dramatically reduced in the absence of T cells, suggesting that small intestinal commensals can efficiently induce T-cell-independent specific IgA responses and that most unbound IgA is dependent on T cells and specific to non-microbial antigens (Bunker et al., 2015). Nevertheless, there is clear evidence that certain commensals induce T-cell-dependent IgA (Palm et al., 2014) that can participate in bacterial coating and promote microbial diversity (for a review, see Pabst et al., 2016). Why certain commensals are capable of promoting T-cell-dependent IgA is currently unclear, although an ability to interact with the mucosal surface could be one contributing factor.

Development and Maturation of Adaptive Immune Cells in the Intestine

Postnatal development of adaptive immune cells occurs rapidly after birth. Very few αβTCR$^+$ T cells are present in the mouse intestine

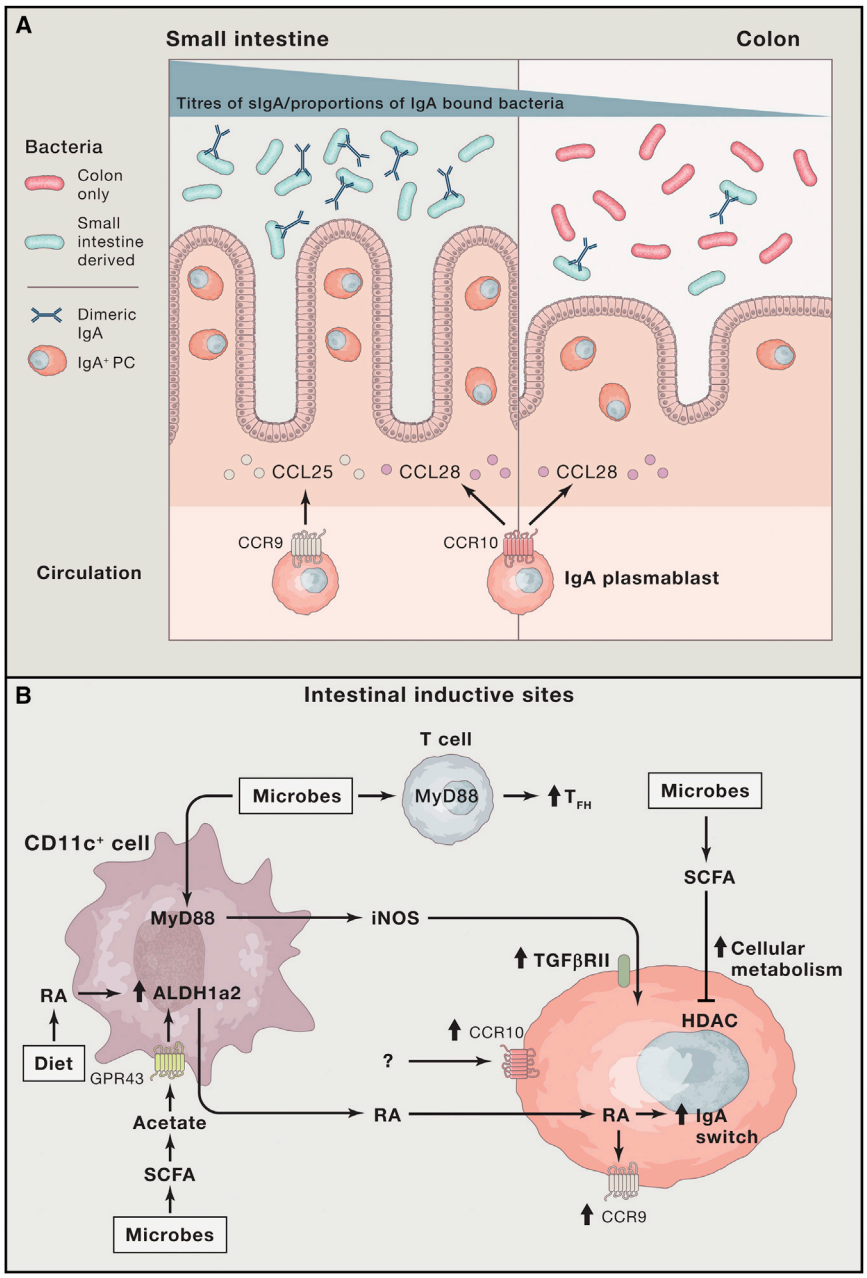

Figure 4. Impact of Environmental Signals on the Regionalized Generation and Distribution of IgA PCs

(A) Luminal concentrations of SIgA and the proportions of IgA-bound bacteria are higher in the small intestine than in the colon. Bacteria that are selectively present in the colon show little IgA coating, suggesting that most commensal-specific IgA responses take place in the small intestine. IgA plasmablast recruitment to the small intestine is mediated by CCR9 and CCR10 and that to the colon is mediated by CCR10. The CCR9-CCL25 axis appears to play a dominant role in IgA^+ plasmablast accumulation to the small intestine and most likely underlies the largely non-overlapping distribution of dominant IgA plasma cell clones between the small intestine and colon.

(B) Under homeostasis, B cells activated in intestinal inductive sites preferentially differentiate into IgA plasmablasts. TGFβ signaling in B cells is essential for IgA class switching in vivo. Bacterial-dependent MyD88 signaling in $CD11c^+$ cells induces iNOS to promote TGFβRII expression on B cells. Bacterial-dependent MyD88 signaling in T cells promotes T_{FH} generation in GALTs, enhancing T-cell-dependent IgA responses. SCFAs act directly on B cells to enhance their cellular metabolism and support B cell differentiation and antibody production. GALT DCs are imprinted with the ability to generate RA via direct RA signaling and potentially through acetate-induced GPR43-dependent signals. RA from DCs, and potentially other cells, acts directly on B cells to promote IgA class switching and induce CCR9 expression on newly generated plasmablasts. IgA^+ plasmablasts generated in PPs are induced to express CCR9, whereas those generated in the caecal patch appear to express both CCR10 and CCR9. As a result, PP-derived plasmablasts localize to the small intestine, whereas caecal-patch-derived plasmablasts can gain access to both the small intestinal and colon. The mechanisms underlying CCR10 induction on IgA^+ plasmablasts remain unclear.

before birth, but they increase massively within 2 days (Torow et al., 2015b). The majority of the T cells that appear in the early phase are thymic-derived $CD4^+\alpha\beta TCR^+$ T cells that home to early PPs, whereas $CD4^+$ T cell recruitment to the LP takes place between days 11 and 28. Another wave of T cells arrives after weaning and is mostly composed of $CD8\alpha\beta^+TCR\alpha\beta^+$ T cells, although there is some contribution from $CD8\alpha\alpha^+TCR\alpha\beta^+$ and $CD4^+CD8\alpha\alpha^+TCR\alpha\beta^+$ T cells. The very early influx of $CD4^+$ T cells to the neonatal gut is independent of microbial and TLR signals but requires β7-integrin-dependent pathways. Despite ongoing microbial colonization, these early $CD4^+\alpha\beta TCR^+$ T cells retain a naive phenotype through a pathway involving both maternal SIgA and Treg cells (Torow et al., 2015a; Torow et al., 2015b).

After birth, B cell numbers increase steadily until 3 weeks, when they stabilize (Torow et al., 2015b). Although the bone marrow is the main site for postnatal B cell development, the small intestinal LP of young mice harbors *Rag*-expressing immature B cells that perform V(D)J recombination and receptor editing locally in response to microbial colonization (Wesemann et al., 2013). The colon also harbors small numbers of these cells, but they are absent from PPs. These cells could help to shape the pre-immune repertoire, potentially to negatively (or positively) select against bacterial reactivity, and the dramatic decrease in their presence in the LP after weaning would suggest a limited window for this to occur. It is unclear whether an equivalent population exists in the neonatal human gut.

Microbial colonization of the intestine stimulates B cell class-switch recombination, primarily to IgA, in the PPs and ILFs. In mice, maturation of the mucosal IgA response is inhibited by the presence of maternal SIgA, and IgA^+ PCs do not appear in the LP until after weaning (Harris et al., 2006). Maternal

anti-commensal IgG (and IgA) in the milk also dampens development of a mucosal T_{FH} cell response in neonatal mice, which limits GC B cell responses in early life (Koch et al., 2016). In humans, despite the presence of ILFs at birth and a rapid influx of B cells after birth, the development of a mucosal IgA response is also delayed until after 1 month, and this delay correlates with reduced expression of APRIL and its receptors TACI and BCMA, but not AID, in the GALTs (Gustafson et al., 2014).

Relevance for Disease Susceptibility Later in Life

Hippocrates once stated that "all disease begins in the gut." Indeed, the intestinal microbiota contributes to host immunity, and proper development of the immune landscape within the intestine is of critical importance to decreasing susceptibility to diseases that affect both the gastrointestinal tract and systemic sites. As discussed above, the immune landscape within the intestine starts to take shape during fetal life but reaches full maturity only after birth with guidance by exogenous signals from the microbiota and diet. This dynamic phase in early life represents a critical window whereby proper or improper immune development can affect resistance or susceptibility, respectively, to diseases later in life. The development of intestinal lymphoid tissues and innate and adaptive immunity early in life defines this neonatal window.

The impact of improper immune development during this critical window and the elucidation of underlying mechanisms have best been illustrated in mouse models together with the use of gnotobiotics. Microbial colonization of adult germ-free mice does not lead to the same level of immune maturation as colonization of neonates, suggesting that exposure to the microbiota must occur within a neonatal window in order to properly imprint host immunity and homeostasis (El Aidy et al., 2013). However, germ-free mice do not just have an immature immune system—they also display signs of immune dysregulation. Germ-free mice harbor increased numbers of iNKT cells in both the lung and colon, leading to increased susceptibility to inflammation in both tissues (Olszak et al., 2012). Importantly, colonization before weaning is necessary for inhibiting the accumulation of these cells and protecting from inflammatory disease later in life, illustrating the need for microbial education during this critical window (Olszak et al., 2012). Germ-free mice also display increased serum IgE levels and increased susceptibility to anaphylaxis (Cahenzli et al., 2013). Neonatal colonization with a relatively diverse microbiota suppresses IgE induction and protects from allergy (Cahenzli et al., 2013). The requirement for a diverse microbiota suggests either that multiple microbes are necessary for the provision of a full range of immune educational cues or that a keystone microbial species normally present in a diverse microbial community is missing. Further evidence for a critical window is illustrated by the increased susceptibility to a variety of diseases in mice that received antibiotics in early life. Administration of clinical doses of select antibiotics to neonatal, but not adult, mice led to enhanced susceptibility to experimental allergic asthma, which was linked to a reduction in the number of colonic Treg cells and an increase in IgE (Russell et al., 2012; Russell et al., 2013). In another study, antibiotic treatment during early life led to an increased sensitivity to food allergy via depletion of beneficial *Clostridia spp*., and supplementation stimulated IL-22 production, leading to an enhanced epithelial barrier and reduced bacterial translocation (Stefka et al., 2014).

The impact of antibiotic use is not limited to Th2-cell-mediated immunity. In the non-obese diabetic (NOD) mouse model of type 1 diabetes (T1D), pulses of antibiotics administered in early life altered the composition and metabolism of the gut microbiome and led to changes in T cell populations and gene expression in the small intestine, which accelerated the onset of T1D (Livanos et al., 2016). Antibiotic treatment during pregnancy can also alter the development of T1D in the offspring by changing the intestinal immune landscape (Hu et al., 2015; Tormo-Badia et al., 2014), such that different antibiotic treatments give different effects (Hu et al., 2016). Prenatal antibiotic delivery of vancomycin accelerated T1D and increased the numbers of Th17 and $IFN\gamma^+$ $CD8^+$ T cells in the PPs, whereas neomycin treatment protected from T1D and induced tolerogenic antigen-presenting cells (Hu et al., 2016).

Early-life antibiotic use can also affect host metabolism, leading to alterations in adiposity, hepatic metabolism, metabolic hormones, and long-term development of adipose tissue, lean muscle, and bone (Cho et al., 2012; Cox et al., 2014) or accelerating body weight and bone growth (Nobel et al., 2015). However, in these studies, it is not clear whether this was mediated through alterations in the intestinal immune landscape or directly through altered microbial metabolites.

Although changes during the neonatal window are induced most profoundly by microbial changes, alterations in diet during this critical window can also change the intestinal immune landscape and affect health. Vitamin A supplementation in neonatal mice can promote early induction of oral tolerance by decreasing gut permeability and increasing Th1 cell responses (Turfkruyer et al., 2016). Given that maternal retinoid supplementation can also increase GALT induction in utero (van de Pavert et al., 2014) and maternal dietary supplementation with AhR ligands can shape intestinal ILC3 populations (Gomez de Agüero et al., 2016), the maternal diet could play a strong role in setting the stage for proper development of the intestinal immune landscape.

A similar critical window in early life in humans is also likely, although identifying the mechanisms involved is more difficult. The intestinal immune landscape in humans also undergoes dynamic developmental changes in the first few months after birth and could reflect a critical window for immune imprinting that has life-long effects. Although the intestinal microbial community does not stabilize in humans until about 3 years of age (Yassour et al., 2016; Yatsunenko et al., 2012), the precise age of the critical window in infants is not yet clear. Antibiotic use during the prenatal period or the first years of life is associated with an increased risk of asthma, allergic disease, and atopic dermatitis (Marra et al., 2009; Martel et al., 2009; Murk et al., 2011; Raciborski et al., 2012; Risnes et al., 2011; Stensballe et al., 2013). The microbiome is also implicated in susceptibility to T1D (for a review, see Paun et al., 2017). Changes in the microbiome in T1D can precede the onset of T1D (Kostic et al., 2015), and infants at risk of developing T1D have been found to harbor a distinct LPS that inhibits immune responsiveness (Vatanen et al., 2016), highlighting a potential role for the intestinal immune landscape in early life. Whether these altered disease

susceptibilities were due to changes in the intestinal immune landscape that then affected disease phenotypes at systemic sites is difficult to assess in humans. It is tempting to speculate that alterations in intestinal T cell phenotypes through antigen-specific recognition of bacterial components might play a role in setting the threshold for disease susceptibility (Tai et al., 2016).

Concluding Remarks

The intestinal immune system shows remarkable heterogeneity along the length of the gut, reflecting regional differences in intestinal function and site-specific intrinsic and environmental factors. Alterations in the development of the intestinal immune landscape, especially during a neonatal window, can have far-reaching effects and could affect susceptibility to multiple diseases later in life. Such changes can be mediated by antibiotics in early life, which alters the intestinal microbiota and leads to improper or insufficient immune development, or through dietary changes that act either indirectly through alterations in the microbiota or microbial metabolism (Tan et al., 2016a; Trompette et al., 2014) or directly through an impact on immune cells. Collectively, the findings described above suggest that specific alterations in diet and/or antibiotic usage could provide novel therapeutic opportunities for reducing disease susceptibility or progression. Before such possibilities can be realized, further mechanistic studies are required for understanding the full impact of environmental conditioning on mucosal and systemic immune system development and functionality. For example, a direct role for RA or AhR ligands on different cells types and at different intestinal sites requires clarification. Additionally, state-of-the-art mapping of human intestinal immune compartments under different conditions and ages is necessary for fully realizing the translational possibilities of these findings.

REFERENCES

Abeler-Dörner, L., Swamy, M., Williams, G., Hayday, A.C., and Bas, A. (2012). Butyrophilins: an emerging family of immune regulators. Trends Immunol. *33*, 34–41.

Adachi, S., Yoshida, H., Honda, K., Maki, K., Saijo, K., Ikuta, K., Saito, T., and Nishikawa, S.I. (1998). Essential role of IL-7 receptor alpha in the formation of Peyer's patch anlage. Int. Immunol. *10*, 1–6.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., and Rudensky, A.Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature *504*, 451–455.

Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., Yagita, H., Ishii, N., Evans, R., Honda, K., and Takeda, K. (2008). ATP drives lamina propria T(H)17 cell differentiation. Nature *455*, 808–812.

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., et al. (2011). Induction of colonic regulatory T cells by indigenous Clostridium species. Science *331*, 337–341.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., et al. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature *500*, 232–236.

Atarashi, K., Tanoue, T., Ando, M., Kamada, N., Nagano, Y., Narushima, S., Suda, W., Imaoka, A., Setoyama, H., Nagamori, T., et al. (2015). Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. Cell *163*, 367–380.

Bandeira, A., Mota-Santos, T., Itohara, S., Degermann, S., Heusser, C., Tonegawa, S., and Coutinho, A. (1990). Localization of gamma/delta T cells to the intestinal epithelium is independent of normal microbial colonization. J. Exp. Med. *172*, 239–244.

Baptista, A.P., Olivier, B.J., Goverse, G., Greuter, M., Knippenberg, M., Kusser, K., Domingues, R.G., Veiga-Fernandes, H., Luster, A.D., Lugering, A., et al. (2013). Colonic patch and colonic SILT development are independent and differentially regulated events. Mucosal Immunol. *6*, 511–521.

Barone, F., Vossenkamper, A., Boursier, L., Su, W., Watson, A., John, S., Dunn-Walters, D.K., Fields, P., Wijetilleka, S., Edgeworth, J.D., and Spencer, J. (2011). IgA-producing plasma cells originate from germinal centers that are induced by B-cell receptor engagement in humans. Gastroenterology *140*, 947–956.

Bas, A., Swamy, M., Abeler-Dörner, L., Williams, G., Pang, D.J., Barbee, S.D., and Hayday, A.C. (2011). Butyrophilin-like 1 encodes an enterocyte protein that selectively regulates functional interactions with T lymphocytes. Proc. Natl. Acad. Sci. USA *108*, 4376–4381.

Beagley, K.W., Fujihashi, K., Lagoo, A.S., Lagoo-Deenadaylan, S., Black, C.A., Murray, A.M., Sharmanov, A.T., Yamamoto, M., McGhee, J.R., Elson, C.O., et al. (1995). Differences in intraepithelial lymphocyte T cell subsets isolated from murine small versus large intestine. J. Immunol. *154*, 5611–5619.

Benckert, J., Schmolka, N., Kreschel, C., Zoller, M.J., Sturm, A., Wiedenmann, B., and Wardemann, H. (2011). The majority of intestinal IgA+ and IgG+ plasmablasts in the human gut are antigen-specific. J. Clin. Invest. *121*, 1946–1955.

Bénézech, C., White, A., Mader, E., Serre, K., Parnell, S., Pfeffer, K., Ware, C.F., Anderson, G., and Caamaño, J.H. (2010). Ontogeny of stromal organizer cells during lymph node development. J. Immunol. *184*, 4521–4530.

Boll, G., Rudolphi, A., Spiess, S., and Reimann, J. (1995). Regional specialization of intraepithelial T cells in the murine small and large intestine. Scand. J. Immunol. *41*, 103–113.

Bouskra, D., Brézillon, C., Bérard, M., Werts, C., Varona, R., Boneca, I.G., and Eberl, G. (2008). Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature *456*, 507–510.

Brandtzaeg, P. (2009). Mucosal immunity: induction, dissemination, and effector functions. Scand. J. Immunol. *70*, 505–515.

Brown, C.C., and Noelle, R.J. (2015). Seeing through the dark: New insights into the immune regulatory functions of vitamin A. Eur. J. Immunol. *45*, 1287–1295.

Bunker, J.J., Flynn, T.M., Koval, J.C., Shaw, D.G., Meisel, M., McDonald, B.D., Ishizuka, I.E., Dent, A.L., Wilson, P.C., Jabri, B., et al. (2015). Innate and Adaptive Humoral Responses Coat Distinct Commensal Bacteria with Immunoglobulin A. Immunity *43*, 541–553.

Cahenzli, J., Köller, Y., Wyss, M., Geuking, M.B., and McCoy, K.D. (2013). Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. Cell Host Microbe *14*, 559–570.

Camerini, V., Panwala, C., and Kronenberg, M. (1993). Regional specialization of the mucosal immune system. Intraepithelial lymphocytes of the large intestine have a different phenotype and function than those of the small intestine. J. Immunol. *151*, 1765–1776.

Campbell, D.J., and Butcher, E.C. (2002). Rapid acquisition of tissue-specific homing phenotypes by CD4(+) T cells activated in cutaneous or mucosal lymphoid tissues. J. Exp. Med. *195*, 135–141.

Cassani, B., Villablanca, E.J., Quintana, F.J., Love, P.E., Lacy-Hulbert, A., Blaner, W.S., Sparwasser, T., Snapper, S.B., Weiner, H.L., and Mora, J.R. (2011). Gut-tropic T cells that express integrin α4β7 and CCR9 are required for induction of oral immune tolerance in mice. Gastroenterology *141*, 2109–2118.

Cazac, B.B., and Roes, J. (2000). TGF-beta receptor controls B cell responsiveness and induction of IgA in vivo. Immunity *13*, 443–451.

Cella, M., and Colonna, M. (2015). Aryl hydrocarbon receptor: Linking environment to immunity. Semin. Immunol. *27*, 310–314.

Cheroutre, H., and Madakamutil, L. (2004). Acquired and natural memory T cells join forces at the mucosal front line. Nat. Rev. Immunol. *4*, 290–300.

Cho, I., Yamanishi, S., Cox, L., Methé, B.A., Zavadil, J., Li, K., Gao, Z., Mahana, D., Raju, K., Teitler, I., et al. (2012). Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature *488*, 621–626.

Cornes, J.S. (1965). Number, size, and distribution of Peyer's patches in the human small intestine: Part I The development of Peyer's patches. Gut *6*, 225–229.

Cox, L.M., Yamanishi, S., Sohn, J., Alekseyenko, A.V., Leung, J.M., Cho, I., Kim, S.G., Li, H., Gao, Z., Mahana, D., et al. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell *158*, 705–721.

Crabbé, P.A., Bazin, H., Eyssen, H., and Heremans, J.F. (1968). The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. The germ-free intestinal tract. Int. Arch. Allergy Appl. Immunol. *34*, 362–375.

Crago, S.S., Kutteh, W.H., Moro, I., Allansmith, M.R., Radl, J., Haaijman, J.J., and Mestecky, J. (1984). Distribution of IgA1-, IgA2-, and J chain-containing cells in human tissues. J. Immunol. *132*, 16–18.

Cummings, J.H., Pomare, E.W., Branch, W.J., Naylor, C.P., and Macfarlane, G.T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut *28*, 1221–1227.

Cupedo, T., Lund, F.E., Ngo, V.N., Randall, T.D., Jansen, W., Greuter, M.J., de Waal-Malefyt, R., Kraal, G., Cyster, J.G., and Mebius, R.E. (2004). Initiation of cellular organization in lymph nodes is regulated by non-B cell-derived signals and is not dependent on CXC chemokine ligand 13. J. Immunol. *173*, 4889–4896.

Dasgupta, S., Erturk-Hasdemir, D., Ochoa-Reparaz, J., Reinecker, H.C., and Kasper, D.L. (2014). Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. Cell Host Microbe *15*, 413–423.

Dejardin, E., Droin, N.M., Delhase, M., Haas, E., Cao, Y., Makris, C., Li, Z.W., Karin, M., Ware, C.F., and Green, D.R. (2002). The lymphotoxin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. Immunity *17*, 525–535.

Denning, T.L., Norris, B.A., Medina-Contreras, O., Manicassamy, S., Geem, D., Madan, R., Karp, C.L., and Pulendran, B. (2011). Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. J. Immunol. *187*, 733–747.

Di Marco Barros, R., Roberts, N.A., Dart, R.J., Vantourout, P., Jandke, A., Nussbaumer, O., Deban, L., Cipolat, S., Hart, R., Iannitto, M.L., et al. (2016). Epithelia Use Butyrophilin-like Molecules to Shape Organ-Specific γδ T Cell Compartments. Cell *167*, 203–218.e17.

Donaldson, D.S., Bradford, B.M., Artis, D., and Mabbott, N.A. (2015). Reciprocal regulation of lymphoid tissue development in the large intestine by IL-25 and IL-23. Mucosal Immunol. *8*, 582–595.

El Aidy, S., Hooiveld, G., Tremaroli, V., Bäckhed, F., and Kleerebezem, M. (2013). The gut microbiota and mucosal homeostasis: colonized at birth or at adulthood, does it matter? Gut Microbes *4*, 118–124.

Ericsson, A., Arya, A., and Agace, W. (2004). CCL25 enhances CD103-mediated lymphocyte adhesion to E-cadherin. Ann. N Y Acad. Sci. *1029*, 334–336.

Fagarasan, S., Muramatsu, M., Suzuki, K., Nagaoka, H., Hiai, H., and Honjo, T. (2002). Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. Science *298*, 1424–1427.

Farache, J., Koren, I., Milo, I., Gurevich, I., Kim, K.W., Zigmond, E., Furtado, G.C., Lira, S.A., and Shakhar, G. (2013). Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. Immunity *38*, 581–595.

Fischer, A., Zundler, S., Atreya, R., Rath, T., Voskens, C., Hirschmann, S., López-Posadas, R., Watson, A., Becker, C., Schuler, G., et al. (2016). Differential effects of α4β7 and GPR15 on homing of effector and regulatory T cells from patients with UC to the inflamed gut in vivo. Gut *65*, 1642–1664.

Fukuyama, S., and Kiyono, H. (2007). Neuroregulator RET initiates Peyer's-patch tissue genesis. Immunity *26*, 393–395.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature *504*, 446–450.

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi, G., et al. (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity *31*, 677–689.

Gangadharan, D., Lambolez, F., Attinger, A., Wang-Zhu, Y., Sullivan, B.A., and Cheroutre, H. (2006). Identification of pre- and postselection TCRalphabeta+ intraepithelial lymphocyte precursors in the thymus. Immunity *25*, 631–641.

Garidou, L., Pomié, C., Klopp, P., Waget, A., Charpentier, J., Aloulou, M., Giry, A., Serino, M., Stenman, L., Lahtinen, S., et al. (2015). The Gut Microbiota Regulates Intestinal CD4 T Cells Expressing RORγt and Controls Metabolic Disease. Cell Metab. *22*, 100–112.

Geem, D., Medina-Contreras, O., McBride, M., Newberry, R.D., Koni, P.A., and Denning, T.L. (2014). Specific microbiota-induced intestinal Th17 differentiation requires MHC class II but not GALT and mesenteric lymph nodes. J. Immunol. *193*, 431–438.

Geuking, M.B., Cahenzli, J., Lawson, M.A., Ng, D.C., Slack, E., Hapfelmeier, S., McCoy, K.D., and Macpherson, A.J. (2011). Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity *34*, 794–806.

Gomez de Agüero, M., Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., et al. (2016). The maternal microbiota drives early postnatal innate immune development. Science *351*, 1296–1302.

Goto, Y., Panea, C., Nakato, G., Cebula, A., Lee, C., Diez, M.G., Laufer, T.M., Ignatowicz, L., and Ivanov, I.I. (2014). Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. Immunity *40*, 594–607.

Gustafson, C.E., Higbee, D., Yeckes, A.R., Wilson, C.C., De Zoeten, E.F., Jedlicka, P., and Janoff, E.N. (2014). Limited expression of APRIL and its receptors prior to intestinal IgA plasma cell development during human infancy. Mucosal Immunol. *7*, 467–477.

Guy-Grand, D., Vassalli, P., Eberl, G., Pereira, P., Burlen-Defranoux, O., Lemaitre, F., Di Santo, J.P., Freitas, A.A., Cumano, A., and Bandeira, A. (2013). Origin, trafficking, and intraepithelial fate of gut-tropic T cells. J. Exp. Med. *210*, 1839–1854.

Haghikia, A., Jörg, S., Duscha, A., Berg, J., Manzel, A., Waschbisch, A., Hammer, A., Lee, D.H., May, C., Wilck, N., et al. (2015). Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. Immunity *43*, 817–829.

Hamada, H., Hiroi, T., Nishiyama, Y., Takahashi, H., Masunaga, Y., Hachimura, S., Kaminogawa, S., Takahashi-Iwanaga, H., Iwanaga, T., Kiyono, H., et al. (2002). Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. J. Immunol. *168*, 57–64.

Harris, N.L., Spoerri, I., Schopfer, J.F., Nembrini, C., Merky, P., Massacand, J., Urban, J.F., Jr., Lamarre, A., Burki, K., Odermatt, B., et al. (2006). Mechanisms of neonatal mucosal antibody protection. J. Immunol. *177*, 6256–6262.

Herbrand, H., Bernhardt, G., Förster, R., and Pabst, O. (2008). Dynamics and function of solitary intestinal lymphoid tissue. Crit. Rev. Immunol. *28*, 1–13.

Hieshima, K., Kawasaki, Y., Hanamoto, H., Nakayama, T., Nagakubo, D., Kanamaru, A., and Yoshie, O. (2004). CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. J. Immunol. *173*, 3668–3675.

Hoorweg, K., and Cupedo, T. (2008). Development of human lymph nodes and Peyer's patches. Semin. Immunol. *20*, 164–170.

Hu, S., Yang, K., Yang, J., Li, M., and Xiong, N. (2011). Critical roles of chemokine receptor CCR10 in regulating memory IgA responses in intestines. Proc. Natl. Acad. Sci. USA *108*, E1035–E1044.

Hu, Y., Peng, J., Tai, N., Hu, C., Zhang, X., Wong, F.S., and Wen, L. (2015). Maternal Antibiotic Treatment Protects Offspring from Diabetes Development in Nonobese Diabetic Mice by Generation of Tolerogenic APCs. J. Immunol. *195*, 4176–4184.

Hu, Y., Jin, P., Peng, J., Zhang, X., Wong, F.S., and Wen, L. (2016). Different immunological responses to early-life antibiotic exposure affecting autoimmune diabetes development in NOD mice. J. Autoimmun. *72*, 47–56.

Huang, F.P., Platt, N., Wykes, M., Major, J.R., Powell, T.J., Jenkins, C.D., and MacPherson, G.G. (2000). A discrete subpopulation of dendritic cells

transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J. Exp. Med. *191*, 435–444.

Ibraghimov, A.R., and Lynch, R.G. (1994). Heterogeneity and biased T cell receptor alpha/beta repertoire of mucosal CD8+ cells from murine large intestine: implications for functional state. J. Exp. Med. *180*, 433–444.

Iiyama, R., Kanai, T., Uraushihara, K., Ishikura, T., Makita, S., Totsuka, T., Yamazaki, M., Nakamura, T., Miyata, T., Yoshida, H., et al. (2003). Normal development of the gut-associated lymphoid tissue except Peyer's patch in MyD88-deficient mice. Scand. J. Immunol. *58*, 620–627.

Ismail, A.S., Severson, K.M., Vaishnava, S., Behrendt, C.L., Yu, X., Benjamin, J.L., Ruhn, K.A., Hou, B., DeFranco, A.L., Yarovinsky, F., and Hooper, L.V. (2011). Gammadelta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. Proc. Natl. Acad. Sci. USA *108*, 8743–8748.

Ivanov, I.I., Frutos, Rde.L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B., and Littman, D.R. (2008). Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe *4*, 337–349.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485–498.

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., and Song, S.Y. (2004). Retinoic acid imprints gut-homing specificity on T cells. Immunity *21*, 527–538.

Jaensson-Gyllenbäck, E., Kotarsky, K., Zapata, F., Persson, E.K., Gundersen, T.E., Blomhoff, R., and Agace, W.W. (2011). Bile retinoids imprint intestinal CD103+ dendritic cells with the ability to generate gut-tropic T cells. Mucosal Immunol. *4*, 438–447.

Jang, M.H., Kweon, M.N., Iwatani, K., Yamamoto, M., Terahara, K., Sasakawa, C., Suzuki, T., Nochi, T., Yokota, Y., Rennert, P.D., et al. (2004). Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc. Natl. Acad. Sci. USA *101*, 6110–6115.

Jiang, W., Wang, X., Zeng, B., Liu, L., Tardivel, A., Wei, H., Han, J., MacDonald, H.R., Tschopp, J., Tian, Z., and Zhou, R. (2013). Recognition of gut microbiota by NOD2 is essential for the homeostasis of intestinal intraepithelial lymphocytes. J. Exp. Med. *210*, 2465–2476.

Johansson-Lindbom, B., Svensson, M., Wurbel, M.A., Malissen, B., Márquez, G., and Agace, W. (2003). Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant. J. Exp. Med. *198*, 963–969.

Josefowicz, S.Z., Niec, R.E., Kim, H.Y., Treuting, P., Chinen, T., Zheng, Y., Umetsu, D.T., and Rudensky, A.Y. (2012). Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature *482*, 395–399.

Kanamori, Y., Ishimaru, K., Nanno, M., Maki, K., Ikuta, K., Nariuchi, H., and Ishikawa, H. (1996). Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit+ IL-7R+ Thy1+ lympho-hemopoietic progenitors develop. J. Exp. Med. *184*, 1449–1459.

Kang, S.G., Lim, H.W., Andrisani, O.M., Broxmeyer, H.E., and Kim, C.H. (2007). Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J. Immunol. *179*, 3724–3733.

Kang, S.W., Kim, S.H., Lee, N., Lee, W.W., Hwang, K.A., Shin, M.S., Lee, S.H., Kim, W.U., and Kang, I. (2012). 1,25-Dihyroxyvitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved noncoding sequence region. J. Immunol. *188*, 5276–5282.

Kawaguchi, M., Nanno, M., Umesaki, Y., Matsumoto, S., Okada, Y., Cai, Z., Shimamura, T., Matsuoka, Y., Ohwaki, M., and Ishikawa, H. (1993). Cytolytic activity of intestinal intraepithelial lymphocytes in germ-free mice is strain dependent and determined by T cells expressing gamma delta T-cell antigen receptors. Proc. Natl. Acad. Sci. USA *90*, 8591–8594.

Kett, K., Brandtzaeg, P., Radl, J., and Haaijman, J.J. (1986). Different subclass distribution of IgA-producing cells in human lymphoid organs and various secretory tissues. J. Immunol. *136*, 3631–3635.

Kett, K., Baklien, K., Bakken, A., Kral, J.G., Fausa, O., and Brandtzaeg, P. (1995). Intestinal B-cell isotype response in relation to local bacterial load: evidence for immunoglobulin A subclass adaptation. Gastroenterology *109*, 819–825.

Kilian, M., Reinholdt, J., Lomholt, H., Poulsen, K., and Frandsen, E.V. (1996). Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. APMIS *104*, 321–338.

Kim, S.V., Xiang, W.V., Kwak, C., Yang, Y., Lin, X.W., Ota, M., Sarpel, U., Rifkin, D.B., Xu, R., and Littman, D.R. (2013). GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. Science *340*, 1456–1459.

Kim, K.S., Hong, S.W., Han, D., Yi, J., Jung, J., Yang, B.G., Lee, J.Y., Lee, M., and Surh, C.D. (2016a). Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. Science *351*, 858–863.

Kim, M., Qie, Y., Park, J., and Kim, C.H. (2016b). Gut Microbial Metabolites Fuel Host Antibody Responses. Cell Host Microbe *20*, 202–214.

Kinoshita, M., Kayama, H., Kusu, T., Yamaguchi, T., Kunisawa, J., Kiyono, H., Sakaguchi, S., and Takeda, K. (2012). Dietary folic acid promotes survival of Foxp3+ regulatory T cells in the colon. J. Immunol. *189*, 2869–2878.

Kiss, E.A., Vonarbourg, C., Kopfmann, S., Hobeika, E., Finke, D., Esser, C., and Diefenbach, A. (2011). Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science *334*, 1561–1565.

Knoop, K.A., Butler, B.R., Kumar, N., Newberry, R.D., and Williams, I.R. (2011). Distinct developmental requirements for isolated lymphoid follicle formation in the small and large intestine: RANKL is essential only in the small intestine. Am. J. Pathol. *179*, 1861–1871.

Koch, M.A., Reiner, G.L., Lugo, K.A., Kreuk, L.S., Stanbery, A.G., Ansaldo, E., Seher, T.D., Ludington, W.B., and Barton, G.M. (2016). Maternal IgG and IgA Antibodies Dampen Mucosal T Helper Cell Responses in Early Life. Cell *165*, 827–841.

Korn, L.L., Hubbeling, H.G., Porrett, P.M., Yang, Q., Barnett, L.G., and Laufer, T.M. (2014). Regulatory T cells occupy an isolated niche in the intestine that is antigen independent. Cell Rep. *9*, 1567–1573.

Kostic, A.D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A.M., Peet, A., Tillmann, V., Pöhö, P., Mattila, I., et al.; DIABIMMUNE Study Group (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe *17*, 260–273.

Kubinak, J.L., Petersen, C., Stephens, W.Z., Soto, R., Bake, E., O'Connell, R.M., and Round, J.L. (2015). MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. Cell Host Microbe *17*, 153–163.

Kunisawa, J., Hashimoto, E., Ishikawa, I., and Kiyono, H. (2012). A pivotal role of vitamin B9 in the maintenance of regulatory T cells in vitro and in vivo. PLoS ONE *7*, e32094.

Kunkel, E.J., Campbell, J.J., Haraldsen, G., Pan, J., Boisvert, J., Roberts, A.I., Ebert, E.C., Vierra, M.A., Goodman, S.B., Genovese, M.C., et al. (2000). Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: Epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity. J. Exp. Med. *192*, 761–768.

Kusu, T., Kayama, H., Kinoshita, M., Jeon, S.G., Ueda, Y., Goto, Y., Okumura, R., Saiga, H., Kurakawa, T., Ikeda, K., et al. (2013). Ecto-nucleoside triphosphate diphosphohydrolase 7 controls Th17 cell responses through regulation of luminal ATP in the small intestine. J. Immunol. *190*, 774–783.

Landsverk, O.J., Snir, O., Casado, R.B., Richter, L., Mold, J.E., Réu, P., Horneland, R., Paulsen, V., Yaqub, S., Aandahl, E.M., et al. (2017). Antibody-secreting plasma cells persist for decades in human intestine. J. Exp. Med. *214*, 309–317.

Lécuyer, E., Rakotobe, S., Lengliné-Garnier, H., Lebreton, C., Picard, M., Juste, C., Fritzen, R., Eberl, G., McCoy, K.D., Macpherson, A.J., et al. (2014). Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. Immunity *40*, 608–620.

Lee, J.S., Cella, M., McDonald, K.G., Garlanda, C., Kennedy, G.D., Nukaya, M., Mantovani, A., Kopan, R., Bradfield, C.A., Newberry, R.D., and Colonna, M. (2011). AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. Nat. Immunol. *13*, 144–151.

Leishman, A.J., Gapin, L., Capone, M., Palmer, E., MacDonald, H.R., Kronenberg, M., and Cheroutre, H. (2002). Precursors of functional MHC class I- or class II-restricted CD8alphaalpha(+) T cells are positively selected in the thymus by agonist self-peptides. Immunity *16*, 355–364.

Li, Y., Innocentin, S., Withers, D.R., Roberts, N.A., Gallagher, A.R., Grigorieva, E.F., Wilhelm, C., and Veldhoen, M. (2011). Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell *147*, 629–640.

Lin, M., Du, L., Brandtzaeg, P., and Pan-Hammarström, Q. (2014). IgA subclass switch recombination in human mucosal and systemic immune compartments. Mucosal Immunol. *7*, 511–520.

Lindner, C., Wahl, B., Föhse, L., Suerbaum, S., Macpherson, A.J., Prinz, I., and Pabst, O. (2012). Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. J. Exp. Med. *209*, 365–377.

Livanos, A.E., Greiner, T.U., Vangay, P., Pathmasiri, W., Stewart, D., McRitchie, S., Li, H., Chung, J., Sohn, J., Kim, S., et al. (2016). Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. Nat. Microbiol. *1*, 16140.

Lochner, M., Peduto, L., Cherrier, M., Sawa, S., Langa, F., Varona, R., Riethmacher, D., Si-Tahar, M., Di Santo, J.P., and Eberl, G. (2008). In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. J. Exp. Med. *205*, 1381–1393.

Lundqvist, C., Baranov, V., Hammarström, S., Athlin, L., and Hammarström, M.L. (1995). Intra-epithelial lymphocytes. Evidence for regional specialization and extrathymic T cell maturation in the human gut epithelium. Int. Immunol. *7*, 1473–1487.

Luther, S.A., Ansel, K.M., and Cyster, J.G. (2003). Overlapping roles of CXCL13, interleukin 7 receptor alpha, and CCR7 ligands in lymph node development. J. Exp. Med. *197*, 1191–1198.

Macpherson, A.J., and Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science *303*, 1662–1665.

Marra, F., Marra, C.A., Richardson, K., Lynd, L.D., Kozyrskyj, A., Patrick, D.M., Bowie, W.R., and Fitzgerald, J.M. (2009). Antibiotic use in children is associated with increased risk of asthma. Pediatrics *123*, 1003–1010.

Martel, M.J., Rey, E., Malo, J.L., Perreault, S., Beauchesne, M.F., Forget, A., and Blais, L. (2009). Determinants of the incidence of childhood asthma: a two-stage case-control study. Am. J. Epidemiol. *169*, 195–205.

Masahata, K., Umemoto, E., Kayama, H., Kotani, M., Nakamura, S., Kurakawa, T., Kikuta, J., Gotoh, K., Motooka, D., Sato, S., et al. (2014). Generation of colonic IgA-secreting cells in the caecal patch. Nat. Commun. *5*, 3704.

Mazzini, E., Massimiliano, L., Penna, G., and Rescigno, M. (2014). Oral tolerance can be established via gap junction transfer of fed antigens from $CX3CR1^{+}$ macrophages to $CD103^{+}$ dendritic cells. Immunity *40*, 248–261.

McDole, J.R., Wheeler, L.W., McDonald, K.G., Wang, B., Konjufca, V., Knoop, K.A., Newberry, R.D., and Miller, M.J. (2012). Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature *483*, 345–349.

McDonald, K.G., McDonough, J.S., Dieckgraefe, B.K., and Newberry, R.D. (2010). Dendritic cells produce CXCL13 and participate in the development of murine small intestine lymphoid tissues. Am. J. Pathol. *176*, 2367–2377.

McDonald, K.G., Leach, M.R., Brooke, K.W., Wang, C., Wheeler, L.W., Hanly, E.K., Rowley, C.W., Levin, M.S., Wagner, M., Li, E., and Newberry, R.D. (2012). Epithelial expression of the cytosolic retinoid chaperone cellular retinol binding protein II is essential for in vivo imprinting of local gut dendritic cells by lumenal retinoids. Am. J. Pathol. *180*, 984–997.

Mezrich, J.D., Fechner, J.H., Zhang, X., Johnson, B.P., Burlingham, W.J., and Bradfield, C.A. (2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J. Immunol. *185*, 3190–3198.

Moghaddami, M., Cummins, A., and Mayrhofer, G. (1998). Lymphocyte-filled villi: comparison with other lymphoid aggregations in the mucosa of the human small intestine. Gastroenterology *115*, 1414–1425.

Mora, J.R., Iwata, M., Eksteen, B., Song, S.Y., Junt, T., Senman, B., Otipoby, K.L., Yokota, A., Takeuchi, H., Ricciardi-Castagnoli, P., et al. (2006). Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science *314*, 1157–1160.

Mowat, A.M., and Agace, W.W. (2014). Regional specialization within the intestinal immune system. Nat. Rev. Immunol. *14*, 667–685.

Murk, W., Risnes, K.R., and Bracken, M.B. (2011). Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. Pediatrics *127*, 1125–1138.

Nguyen, L.P., Pan, J., Dinh, T.T., Hadeiba, H., O'Hara, E., 3rd, Ebtikar, A., Hertweck, A., Gökmen, M.R., Lord, G.M., Jenner, R.G., et al. (2015). Role and species-specific expression of colon T cell homing receptor GPR15 in colitis. Nat. Immunol. *16*, 207–213.

Nobel, Y.R., Cox, L.M., Kirigin, F.F., Bokulich, N.A., Yamanishi, S., Teitler, I., Chung, J., Sohn, J., Barber, C.M., Goldfarb, D.S., et al. (2015). Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment. Nat. Commun. *6*, 7486.

O'Leary, A.D., and Sweeney, E.C. (1986). Lymphoglandular complexes of the colon: structure and distribution. Histopathology *10*, 267–283.

Ohnmacht, C., Park, J.H., Cording, S., Wing, J.B., Atarashi, K., Obata, Y., Gaboriau-Routhiau, V., Marques, R., Dulauroy, S., Fedoseeva, M., et al. (2015). MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through $ROR\gamma t^{+}$ T cells. Science *349*, 989–993.

Olszak, T., An, D., Zeissig, S., Vera, M.P., Richter, J., Franke, A., Glickman, J.N., Siebert, R., Baron, R.M., Kasper, D.L., and Blumberg, R.S. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. Science *336*, 489–493.

Pabst, O., Ohl, L., Wendland, M., Wurbel, M.A., Kremmer, E., Malissen, B., and Förster, R. (2004). Chemokine receptor CCR9 contributes to the localization of plasma cells to the small intestine. J. Exp. Med. *199*, 411–416.

Pabst, O., Herbrand, H., Worbs, T., Friedrichsen, M., Yan, S., Hoffmann, M.W., Körner, H., Bernhardt, G., Pabst, R., and Förster, R. (2005). Cryptopatches and isolated lymphoid follicles: dynamic lymphoid tissues dispensable for the generation of intraepithelial lymphocytes. Eur. J. Immunol. *35*, 98–107.

Pabst, O., Cerovic, V., and Hornef, M. (2016). Secretory IgA in the Coordination of Establishment and Maintenance of the Microbiota. Trends Immunol. *37*, 287–296.

Palm, N.W., de Zoete, M.R., Cullen, T.W., Barry, N.A., Stefanowski, J., Hao, L., Degnan, P.H., Hu, J., Peter, I., Zhang, W., et al. (2014). Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell *158*, 1000–1010.

Panea, C., Farkas, A.M., Goto, Y., Abdollahi-Roodsaz, S., Lee, C., Koscsó, B., Gowda, K., Hohl, T.M., Bogunovic, M., and Ivanov, I.I. (2015). Intestinal Monocyte-Derived Macrophages Control Commensal-Specific Th17 Responses. Cell Rep. *12*, 1314–1324.

Pantazi, E., Marks, E., Stolarczyk, E., Lycke, N., Noelle, R.J., and Elgueta, R. (2015). Cutting Edge: Retinoic Acid Signaling in B Cells Is Essential for Oral Immunization and Microflora Composition. J. Immunol. *195*, 1368–1371.

Patel, A., Harker, N., Moreira-Santos, L., Ferreira, M., Alden, K., Timmis, J., Foster, K., Garefalaki, A., Pachnis, P., Andrews, P., et al. (2012). Differential RET signaling pathways drive development of the enteric lymphoid and nervous systems. Sci. Signal. *5*, ra55.

Paun, A., Yau, C., and Danska, J.S. (2017). The Influence of the Microbiome on Type 1 Diabetes. J. Immunol. *198*, 590–595.

Plaut, A.G., Wistar, R., Jr., and Capra, J.D. (1974). Differential susceptibility of human IgA immunoglobulins to streptococcal IgA protease. J. Clin. Invest. *54*, 1295–1300.

Pobezinsky, L.A., Angelov, G.S., Tai, X., Jeurling, S., Van Laethem, F., Feigenbaum, L., Park, J.H., and Singer, A. (2012). Clonal deletion and the fate of autoreactive thymocytes that survive negative selection. Nat. Immunol. *13*, 569–578.

Qiu, Y., Pu, A., Zheng, H., Liu, M., Chen, W., Wang, W., Xiao, W., and Yang, H. (2016). TLR2-Dependent Signaling for IL-15 Production Is Essential for the Homeostasis of Intestinal Intraepithelial Lymphocytes. Mediators Inflamm. *2016*, 4281865.

Raciborski, F., Tomaszewska, A., Komorowski, J., Samel-Kowalik, P., Białoszewski, A.Z., Walkiewicz, A., Lusawa, A., Szymański, J., Opoczyńska, D., Drużba, M., et al. (2012). The relationship between antibiotic therapy in early childhood and the symptoms of allergy in children aged 6-8 years - the questionnaire study results. Int. J. Occup. Med. Environ. Health *25*, 470–480.

Reis, B.S., Hoytema van Konijnenburg, D.P., Grivennikov, S.I., and Mucida, D. (2014). Transcription factor T-bet regulates intraepithelial lymphocyte functional maturation. Immunity *41*, 244–256.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., and Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. *2*, 361–367.

Rios, D., Wood, M.B., Li, J., Chassaing, B., Gewirtz, A.T., and Williams, I.R. (2016). Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. Mucosal Immunol. *9*, 907–916.

Risnes, K.R., Belanger, K., Murk, W., and Bracken, M.B. (2011). Antibiotic exposure by 6 months and asthma and allergy at 6 years: Findings in a cohort of 1,401 US children. Am. J. Epidemiol. *173*, 310–318.

Round, J.L., and Mazmanian, S.K. (2010). Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc. Natl. Acad. Sci. USA *107*, 12204–12209.

Russell, S.L., Gold, M.J., Hartmann, M., Willing, B.P., Thorson, L., Wlodarska, M., Gill, N., Blanchet, M.R., Mohn, W.W., McNagny, K.M., and Finlay, B.B. (2012). Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep. *13*, 440–447.

Russell, S.L., Gold, M.J., Willing, B.P., Thorson, L., McNagny, K.M., and Finlay, B.B. (2013). Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. Gut Microbes *4*, 158–164.

Sano, T., Huang, W., Hall, J.A., Yang, Y., Chen, A., Gavzy, S.J., Lee, J.Y., Ziel, J.W., Miraldi, E.R., Domingos, A.I., et al. (2015). An IL-23R/IL-22 Circuit Regulates Epithelial Serum Amyloid A to Promote Local Effector Th17 Responses. Cell *163*, 381–393.

Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A.M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., et al. (2015). MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORγ$^+$ regulatory T cells. Science *349*, 993–997.

Shen, Y., Giardino Torchia, M.L., Lawson, G.W., Karp, C.L., Ashwell, J.D., and Mazmanian, S.K. (2012). Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe *12*, 509–520.

Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., Thangaraju, M., Prasad, P.D., Manicassamy, S., Munn, D.H., et al. (2014). Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity *40*, 128–139.

Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly-Y, M., Glickman, J.N., and Garrett, W.S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science *341*, 569–573.

Stefka, A.T., Feehley, T., Tripathi, P., Qiu, J., McCoy, K., Mazmanian, S.K., Tjota, M.Y., Seo, G.Y., Cao, S., Theriault, B.R., et al. (2014). Commensal bacteria protect against food allergen sensitization. Proc. Natl. Acad. Sci. USA *111*, 13145–13150.

Stensballe, L.G., Simonsen, J., Jensen, S.M., Bønnelykke, K., and Bisgaard, H. (2013). Use of antibiotics sduring pregnancy increases the risk of asthma in early childhood. J. Pediatr. *162*, 832–838.e3.

Stenstad, H., Ericsson, A., Johansson-Lindbom, B., Svensson, M., Marsal, J., Mack, M., Picarella, D., Soler, D., Marquez, G., Briskin, M., and Agace, W.W. (2006). Gut-associated lymphoid tissue-primed CD4+ T cells display CCR9-dependent and -independent homing to the small intestine. Blood *107*, 3447–3454.

Stenstad, H., Svensson, M., Cucak, H., Kotarsky, K., and Agace, W.W. (2007). Differential homing mechanisms regulate regionalized effector CD8alphabeta+ T cell accumulation within the small intestine. Proc. Natl. Acad. Sci. USA *104*, 10122–10127.

Sun, C.M., Hall, J.A., Blank, R.B., Bouladoux, N., Oukka, M., Mora, J.R., and Belkaid, Y. (2007). Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J. Exp. Med. *204*, 1775–1785.

Suzuki, H., Jeong, K.I., Itoh, K., and Doi, K. (2002). Regional variations in the distributions of small intestinal intraepithelial lymphocytes in germ-free and specific pathogen-free mice. Exp. Mol. Pathol. *72*, 230–235.

Svensson, M., Marsal, J., Ericsson, A., Carramolino, L., Brodén, T., Márquez, G., and Agace, W.W. (2002). CCL25 mediates the localization of recently activated CD8alphabeta(+) lymphocytes to the small-intestinal mucosa. J. Clin. Invest. *110*, 1113–1121.

Svensson, M., Johansson-Lindbom, B., Zapata, F., Jaensson, E., Austenaa, L.M., Blomhoff, R., and Agace, W.W. (2008). Retinoic acid receptor signaling levels and antigen dose regulate gut homing receptor expression on CD8+ T cells. Mucosal Immunol. *1*, 38–48.

Tai, N., Peng, J., Liu, F., Gulden, E., Hu, Y., Zhang, X., Chen, L., Wong, F.S., and Wen, L. (2016). Microbial antigen mimics activate diabetogenic CD8 T cells in NOD mice. J. Exp. Med. *213*, 2129–2146.

Tan, J., McKenzie, C., Vuillermin, P.J., Goverse, G., Vinuesa, C.G., Mebius, R.E., Macia, L., and Mackay, C.R. (2016a). Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways. Cell Rep. *15*, 2809–2824.

Tan, T.G., Sefik, E., Geva-Zatorsky, N., Kua, L., Naskar, D., Teng, F., Pasman, L., Ortiz-Lopez, A., Jupp, R., Wu, H.J., et al. (2016b). Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. Proc. Natl. Acad. Sci. USA *113*, E8141–E8150.

Tanoue, T., Atarashi, K., and Honda, K. (2016). Development and maintenance of intestinal regulatory T cells. Nat. Rev. Immunol. *16*, 295–309.

Tarkowski, A., Kjellson, B., Carlsten, H., Holmdahl, R., Josefsson, E., and Trollmo, C. (1990). Frequency and phenotypic feature of autoantibody-producing cell precursors in the preclinical stage of murine lupus. Immunology *71*, 335–340.

Tezuka, H., Abe, Y., Iwata, M., Takeuchi, H., Ishikawa, H., Matsushita, M., Shiohara, T., Akira, S., and Ohteki, T. (2007). Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. Nature *448*, 929–933.

Tormo-Badia, N., Håkansson, Å., Vasudevan, K., Molin, G., Ahrné, S., and Cilio, C.M. (2014). Antibiotic treatment of pregnant non-obese diabetic mice leads to altered gut microbiota and intestinal immunological changes in the offspring. Scand. J. Immunol. *80*, 250–260.

Torow, N., Dittrich-Breiholz, O., and Hornef, M.W. (2015a). Transcriptional profiling of intestinal CD4(+) T cells in the neonatal and adult mice. Genom. Data *5*, 371–374.

Torow, N., Yu, K., Hassani, K., Freitag, J., Schulz, O., Basic, M., Brennecke, A., Sparwasser, T., Wagner, N., Bleich, A., et al. (2015b). Active suppression of intestinal CD4(+)TCRαβ(+) T-lymphocyte maturation during the postnatal period. Nat. Commun. *6*, 7725.

Trompette, A., Gollwitzer, E.S., Yadava, K., Sichelstiel, A.K., Sprenger, N., Ngom-Bru, C., Blanchard, C., Junt, T., Nicod, L.P., Harris, N.L., and Marsland, B.J. (2014). Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat. Med. *20*, 159–166.

Tsuji, M., Suzuki, K., Kitamura, H., Maruya, M., Kinoshita, K., Ivanov, I.I., Itoh, K., Littman, D.R., and Fagarasan, S. (2008). Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. Immunity *29*, 261–271.

Turfkruyer, M., Rekima, A., Macchiaverni, P., Le Bourhis, L., Muncan, V., van den Brink, G.R., Tulic, M.K., and Verhasselt, V. (2016). Oral tolerance is inefficient in neonatal mice due to a physiological vitamin A deficiency. Mucosal Immunol. *9*, 479–491.

van de Pavert, S.A., Ferreira, M., Domingues, R.G., Ribeiro, H., Molenaar, R., Moreira-Santos, L., Almeida, F.F., Ibiza, S., Barbosa, I., Goverse, G., et al. (2014). Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. Nature *508*, 123–127.

van Ginkel, F.W., Wahl, S.M., Kearney, J.F., Kweon, M.N., Fujihashi, K., Burrows, P.D., Kiyono, H., and McGhee, J.R. (1999). Partial IgA-deficiency with increased Th2-type cytokines in TGF-beta 1 knockout mice. J. Immunol. *163*, 1951–1957.

Vatanen, T., Kostic, A.D., d'Hennezel, E., Siljander, H., Franzosa, E.A., Yassour, M., Kolde, R., Vlamakis, H., Arthur, T.D., Hämäläinen, A.M., et al.; DIABIMMUNE Study Group (2016). Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. Cell *165*, 842–853.

Veiga-Fernandes, H., Coles, M.C., Foster, K.E., Patel, A., Williams, A., Natarajan, D., Barlow, A., Pachnis, V., and Kioussis, D. (2007). Tyrosine kinase receptor RET is a key regulator of Peyer's patch organogenesis. Nature *446*, 547–551.

Velaga, S., Herbrand, H., Friedrichsen, M., Jiong, T., Dorsch, M., Hoffmann, M.W., Förster, R., and Pabst, O. (2009). Chemokine receptor CXCR5 supports solitary intestinal lymphoid tissue formation, B cell homing, and induction of intestinal IgA responses. J. Immunol. *182*, 2610–2619.

Vicente-Suarez, I., Larange, A., Reardon, C., Matho, M., Feau, S., Chodaczek, G., Park, Y., Obata, Y., Gold, R., Wang-Zhu, Y., et al. (2015). Unique lamina propria stromal cells imprint the functional phenotype of mucosal dendritic cells. Mucosal Immunol. *8*, 141–151.

Vondenhoff, M.F., Greuter, M., Goverse, G., Elewaut, D., Dewint, P., Ware, C.F., Hoorweg, K., Kraal, G., and Mebius, R.E. (2009). LTbetaR signaling induces cytokine expression and up-regulates lymphangiogenic factors in lymph node anlagen. J. Immunol. *182*, 5439–5445.

Wang, X., Sumida, H., and Cyster, J.G. (2014). GPR18 is required for a normal CD8$\alpha\alpha$ intestinal intraepithelial lymphocyte compartment. J. Exp. Med. *211*, 2351–2359.

Wang, S., Charbonnier, L.M., Noval Rivas, M., Georgiev, P., Li, N., Gerber, G., Bry, L., and Chatila, T.A. (2015). MyD88 Adaptor-Dependent Microbial Sensing by Regulatory T Cells Promotes Mucosal Tolerance and Enforces Commensalism. Immunity *43*, 289–303.

Watanabe, K., Sugai, M., Nambu, Y., Osato, M., Hayashi, T., Kawaguchi, M., Komori, T., Ito, Y., and Shimizu, A. (2010). Requirement for Runx proteins in IgA class switching acting downstream of TGF-beta 1 and retinoic acid signaling. J. Immunol. *184*, 2785–2792.

Wesemann, D.R., Portuguese, A.J., Meyers, R.M., Gallagher, M.P., Cluff-Jones, K., Magee, J.M., Panchakshari, R.A., Rodig, S.J., Kepler, T.B., and Alt, F.W. (2013). Microbial colonization influences early B-lineage development in the gut lamina propria. Nature *501*, 112–115.

Wu, W., Sun, M., Chen, F., Cao, A.T., Liu, H., Zhao, Y., Huang, X., Xiao, Y., Yao, S., Zhao, Q., et al. (2016). Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. Mucosal Immunol.

Wurbel, M.A., Malissen, M., Guy-Grand, D., Meffre, E., Nussenzweig, M.C., Richelme, M., Carrier, A., and Malissen, B. (2001). Mice lacking the CCR9 CC-chemokine receptor show a mild impairment of early T- and B-cell development and a reduction in T-cell receptor gammadelta(+) gut intraepithelial lymphocytes. Blood *98*, 2626–2632.

Wurbel, M.A., Malissen, M., Guy-Grand, D., Malissen, B., and Campbell, J.J. (2007). Impaired accumulation of antigen-specific CD8 lymphocytes in chemokine CCL25-deficient intestinal epithelium and lamina propria. J. Immunol. *178*, 7598–7606.

Yamaguchi, T., Hirota, K., Nagahama, K., Ohkawa, K., Takahashi, T., Nomura, T., and Sakaguchi, S. (2007). Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. Immunity *27*, 145–159.

Yang, Y., Torchinsky, M.B., Gobert, M., Xiong, H., Xu, M., Linehan, J.L., Alonzo, F., Ng, C., Chen, A., Lin, X., et al. (2014). Focused specificity of intestinal TH17 cells towards commensal bacterial antigens. Nature *510*, 152–156.

Yang, B.H., Hagemann, S., Mamareli, P., Lauer, U., Hoffmann, U., Beckstette, M., Föhse, L., Prinz, I., Pezoldt, J., Suerbaum, S., et al. (2016). Foxp3(+) T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. Mucosal Immunol. *9*, 444–457.

Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A.M., Härkönen, T., Ryhänen, S.J., Franzosa, E.A., Vlamakis, H., Huttenhower, C., Gevers, D., et al.; DIABIMMUNE Study Group (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Sci. Transl. Med. *8*, 343ra81.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., et al. (2012). Human gut microbiome viewed across age and geography. Nature *486*, 222–227.

Yoshida, H., Honda, K., Shinkura, R., Adachi, S., Nishikawa, S., Maki, K., Ikuta, K., and Nishikawa, S.I. (1999). IL-7 receptor alpha+ CD3(-) cells in the embryonic intestine induces the organizing center of Peyer's patches. Int. Immunol. *11*, 643–655.

Yu, Q., Tang, C., Xun, S., Yajima, T., Takeda, K., and Yoshikai, Y. (2006). MyD88-dependent signaling for IL-15 production plays an important role in maintenance of CD8 alpha alpha TCR alpha beta and TCR gamma delta intestinal intraepithelial lymphocytes. J. Immunol. *176*, 6180–6185.