Regional specialization within the intestinal immune system

Allan M. Mowat[1] *and William W. Agace*[2,3]

Abstract | The intestine represents the largest compartment of the immune system. It is continually exposed to antigens and immunomodulatory agents from the diet and the commensal microbiota, and it is the port of entry for many clinically important pathogens. Intestinal immune processes are also increasingly implicated in controlling disease development elsewhere in the body. In this Review, we detail the anatomical and physiological distinctions that are observed in the small and large intestines, and we suggest how these may account for the diversity in the immune apparatus that is seen throughout the intestine. We describe how the distribution of innate, adaptive and innate-like immune cells varies in different segments of the intestine and discuss the environmental factors that may influence this. Finally, we consider the implications of regional immune specialization for inflammatory disease in the intestine.

Paneth cells
Specialized epithelial cells located just below the epithelial stem cells in the small intestinal crypts of Lieberkühn. They are a rich source of antimicrobial peptides that preserve crypt sterility and protect the epithelial stem cell niche.

[1]*Centre for Immunobiology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Sir Graeme Davies Building, 120 University Avenue, Glasgow G12 8TA, Scotland, UK.*
[2]*Immunology Section, Department of Experimental Medical Science, Lund University, BMC D14, Sölvegatan 19, S-221 84 Lund, Sweden.*
[3]*Section of Immunology and Vaccinology, Danish Technical University Veterinary Institute, Bülowsvej 27, DK-1870 Copenhagen, Denmark.*
e-mails: Allan.Mowat@glasgow.ac.uk; William.agace@med.lu.se
doi:10.1038/nri3738
Published online 19 September 2014

The intestine contains the largest number of immune cells of any tissue in the body and it is continually exposed to a wide range of antigens and potential immune stimuli. There is an increasing awareness of how the contents of the intestine, such as the commensal bacteria and dietary constituents, influence physiological and pathological processes throughout the body. This has resulted in an explosion in the number of studies examining intestinal immune responses. However, many studies overlook the fact that the intestinal tract comprises different regions with distinct anatomical and physiological characteristics. In this Review, we discuss the nature of the adaptive and innate immune cells that are present in the small and large intestines, and we outline how their characteristics can be determined by their anatomical location. As the upper gastrointestinal tract has very little lymphoid tissue and it has not been studied in detail, we focus on intestinal segments from the stomach downwards. Unless specified otherwise, the factors that we discuss are similar in mice and humans.

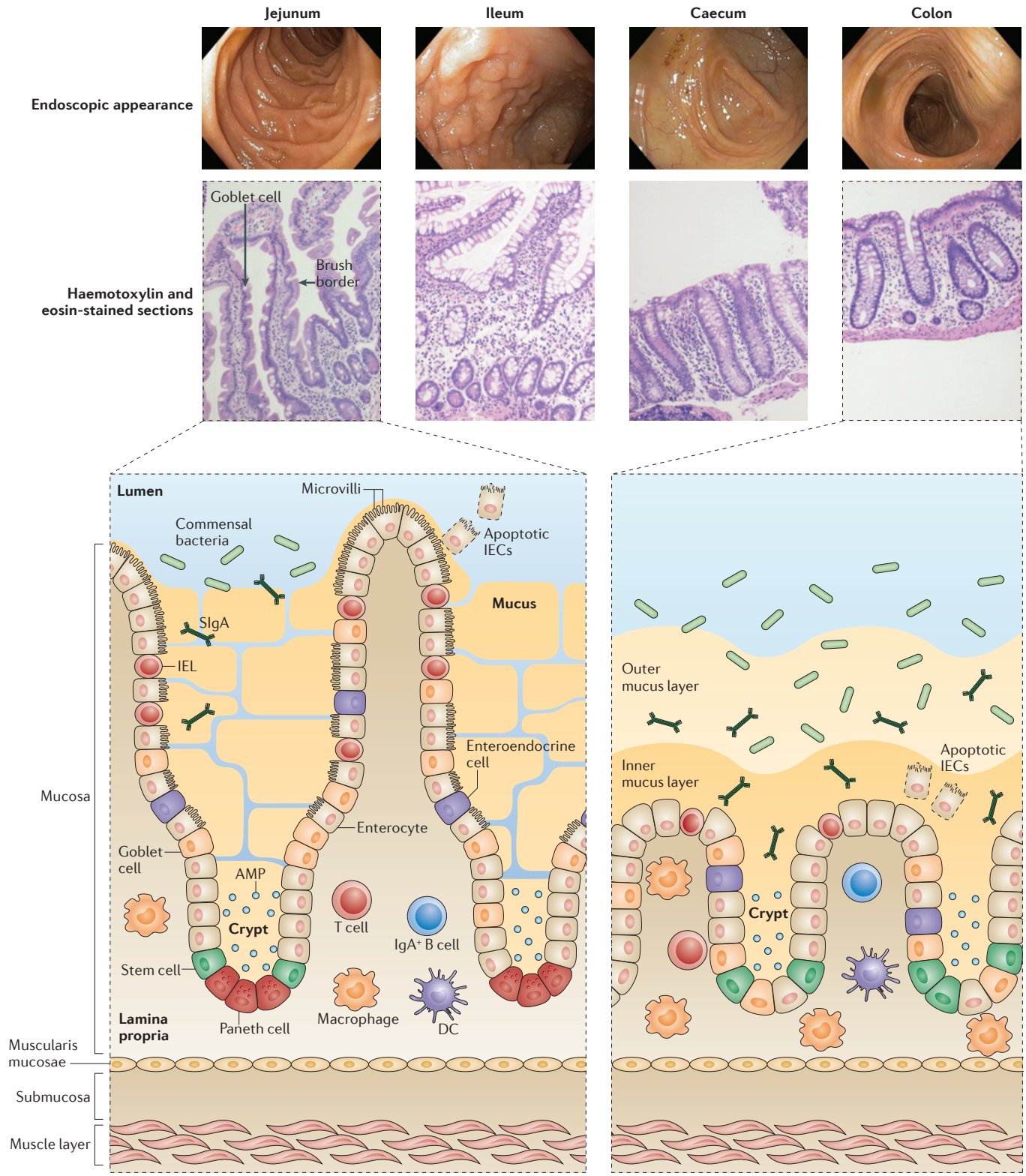
Anatomy and physiology of intestine

Structure of the intestine. The small and large intestines form a continuous tube that is lined internally by a single layer of columnar epithelium and stretches from the outlet of the stomach to the anus. The small intestine begins at the pylorus and ends at the ileocaecal valve, which is the entry point into the large intestine. It is divided into three main segments, with the duodenum being closest to the stomach, followed by the jejunum and then the ileum. The large intestine begins at the caecum, followed by the ascending (proximal) colon, the transverse colon, the descending (distal) colon and the rectum, terminating at the anus. The small and large intestines differ markedly in size, with the small intestine in humans consisting of multiple coils amounting to 6–7 m in length; the colon is wider in diameter and much shorter (~1.5 m) (FIG. 1).

The small intestine is characterized by finger-like projections known as villi, which extend into the lumen and increase the surface area of digestively active epithelium; by contrast, villi are absent from the caecum and the colon, where the surface is flat. In all parts of the intestine, the surface epithelium is continuously renewed by immature cells arising from invaginations known as the crypts of Lieberkühn, where multipotent stem cells give rise to several different types of mature epithelial cells. The vast majority of these cells are absorptive enterocytes but there are also Paneth cells, goblet cells and neuroendocrine cells. With the exception of Paneth cells, which move downwards to the base of the crypt, newly formed epithelial cells move along an 'escalator' from the bottom of the crypt to the tip of the villus, from where they are extruded after 4–5 days. During this journey, the epithelial cells mature, only acquiring the full range of enzymes and other properties that are needed for their digestive and absorptive functions as they reach the base of the villus. The local immune system must adapt to and function within this constantly changing environment.

Goblet cells
Specialized epithelial cells that produce mucus.

The majority of immunological processes take place in the mucosa, which comprises the epithelium, the underlying lamina propria and the muscularis mucosa, which is a thin muscle layer below the lamina propria (FIG. 1). The lamina propria consists of loosely packed connective tissue that forms the scaffolding for the villus, as well as containing the blood supply, lymph drainage and nervous supply for the mucosa. It also contains many cells of the innate and adaptive immune systems. Lymphocytes are also found in the epithelium and,



despite being separated by only a thin basement membrane, the lamina propria and epithelium form very distinct immunological compartments. Their composition and functions also vary considerably throughout the intestine.

Below the muscularis mucosae lies the area of connective tissue known as the submucosa, which is important for its plexus of parasympathetic nerves. The submucosa is lined by a thicker muscle layer and finally, the serosa provides the thick fibrous covering that separates the intestine from the surrounding peritoneal cavity (FIG. 1).

Functions of the intestine. The different regions of the intestine have distinct physiological functions. The surface of absorptive epithelial cells in the small intestine is covered by a layer of microvilli, in which are embedded the enzymes that are needed to digest dietary components, as well as nutrient transporters (FIG. 1). This 'brush border' increases the surface area available for digestion, which is already enhanced by the long villi in the duodenum and jejunum, where most digestion occurs. As a result, damage to the upper small intestine — such as that seen in coeliac disease — leads to severe malabsorption, protein leakage and malnutrition. Apart from the absorption of bile salts and vitamin B_{12}, the ileum contributes much less to nutrition, and it has markedly shorter villi and lower levels of brush border enzymes.

Submucosa
The layer of the intestine that is immediately below the mucosa and above the external muscle. Peyer's patches and colonic patches are located in the submucosa.

Coeliac disease
An inflammatory disorder of the duodenum and jejunum that is caused by immune responses to specific peptides that are found within the α-gliadin component of wheat gluten. Pathology includes the loss of villus architecture with reduced area of the surface epithelium, which leads to malabsorption.

◀ Figure 1 | **Anatomy of the intestinal mucosa and its immune apparatus.** The different segments of the intestine have quite distinct appearances when observed from the lumen using endoscopy (top panels). The upper small intestine, as exemplified by the jejunum (middle and lower panels), has long thin villi that are covered by a surface epithelium that has an extensive brush border (indicated by the arrow) comprising the microvilli that contain digestive enzymes. This provides an extensive surface area for the digestion and absorption of metabolites from the diet. Intestinal epithelial cells (IECs) are produced from stem cells near the bottom of the crypts. After a few days, IECs are lost from the tip of the villus and are replaced by new cells that are migrating upwards from the crypts. As well as the absorptive epithelial cells, stem cells in the crypts give rise to the mucus-secreting goblet cells found on the villus (indicated by the arrow), and to Paneth cells that migrate downwards to the bottom of the crypt. These Paneth cells are characterized by the presence of dense granules that contain antimicrobial peptides (AMPs). The central part of the villus comprises the lamina propria, where the majority of intestinal immune cells are found, whereas intraepithelial lymphocytes (IELs) are found lying between epithelial cells. The villi become progressively shorter and broader going down the length of the small intestine (the ileum is shown as an example), which is consistent with the lower rates of digestion and absorption that occur in these regions. In addition, goblet cells and Paneth cells become more numerous and IELs less frequent progressing down the length of the small intestine. The caecum is a blind-ended sac comprising the first part of the large intestine and it acts as a large reservoir for the commensal bacteria that are involved in the fermentative digestion of the complex carbohydrates that cannot be dealt with by small intestinal enzymes. The caecum has no villi and the mucosa consists mainly of crypts with only short regions of flat surface epithelium. Goblet cells are numerous and are found throughout the crypts. Paneth cells are rare. The caecum leads into the ascending (proximal) colon, then the transverse colon, descending (distal) colon and rectum. Villi are absent from all parts of the colon and crypts are smaller compared with those found in the small intestine; the main function of the surface epithelium is to reabsorb water from faeces and to act as a barrier to the commensal microbiota. This is assisted by the large number of goblet cells, which produce an extensive and thick layer of protective mucus (see text for details). Paneth cells are very rare in the colon and IELs are much rarer than in the small intestine. DC, dendritic cell; SIgA, secretory immunoglobulin A.

Lacking villi and a brush border, the large intestine has little or no intrinsic digestive function, with its main roles being the reabsorption of water and elimination of undigested foodstuffs. In addition, it is the main reservoir for the trillions of commensal bacteria which inhabit the intestine and which are essential for health (FIG. 2).

Regional differences in specialized epithelial cells. Paneth cells are only found in the small intestine, where they are particularly concentrated in the ileum. Unlike absorptive enterocytes, Paneth cells are long lived and they migrate downwards to the very base of the crypts after differentiating from stem cells. They have crucial antibacterial roles, producing antimicrobial peptides such as lysozyme, defensins and regenerating islet-derived protein IIIγ (REGIIIγ) in response to interleukin-22 (IL-22) or following stimulation of Toll-like receptors (TLRs), nucleotide-binding oligomerization domain 2 (NOD2) or cholinergic nerves[1,2]. Paneth cells also help to maintain normal crypt stem cell activity via their production of pro-epidermal growth factor (pro-EGF), WNT3 and Notch ligands[3], and they are crucial for small intestinal homeostasis. Indeed, dysregulation of Paneth cell function increases susceptibility to some forms of Crohn's disease (as discussed below). Paneth cells express many genes that have been associated with Crohn's disease, including ATG16-like 1 (*ATG16L1*), transcription factor 4 (*TCF4*), *NOD2* and immunity-related GTPase family M protein 1 (*IRGM1*)[2]. Knockout of many of these genes leads to impaired autophagy in Paneth cells and these functional defects render mice more susceptible to microbiota-dependent, spontaneous or experimentally induced intestinal inflammation[4–9,10].

In contrast to Paneth cells, the frequency of mucus-producing goblet cells progressively increases going down the gastrointestinal tract. Goblet cells comprise at least 25% of all epithelial cells in the distal colon, compared with 10% or less in the upper small intestine. In parallel, the mucus layer that coats the mucosa — which is known as the glycocalyx — is at its thickest in the colon, where it is composed of two distinct layers: an inner, dense layer that is attached to the epithelial surface and an outer, loose layer which is similar to that found in the small intestine. In the colon, bacteria can be found in the outer mucus layer but they do not normally penetrate the inner layer[11,12]. The production of mucus is controlled by immune mediators including leukotrienes, interferon-γ (IFNγ), IL-9 and IL-13 (REFS 13–15). Mucus has antimicrobial roles, forming a highly charged gel that acts as a physical barrier, as well as being composed of mucin glycoproteins that are directly toxic to many bacteria. The looser glycocalyx of the small intestine also provides a matrix to which antibodies and antimicrobial peptides can adhere[16]. Defects in mucus synthesis lead to increased penetration of commensal bacteria into the epithelial surface of the colon[11], provoking enhanced susceptibility to colitis and colon cancer[17,18]. Thus, the small and large intestines seem to use different strategies to maintain a safe distance between functioning epithelial cells and their microbial neighbours.

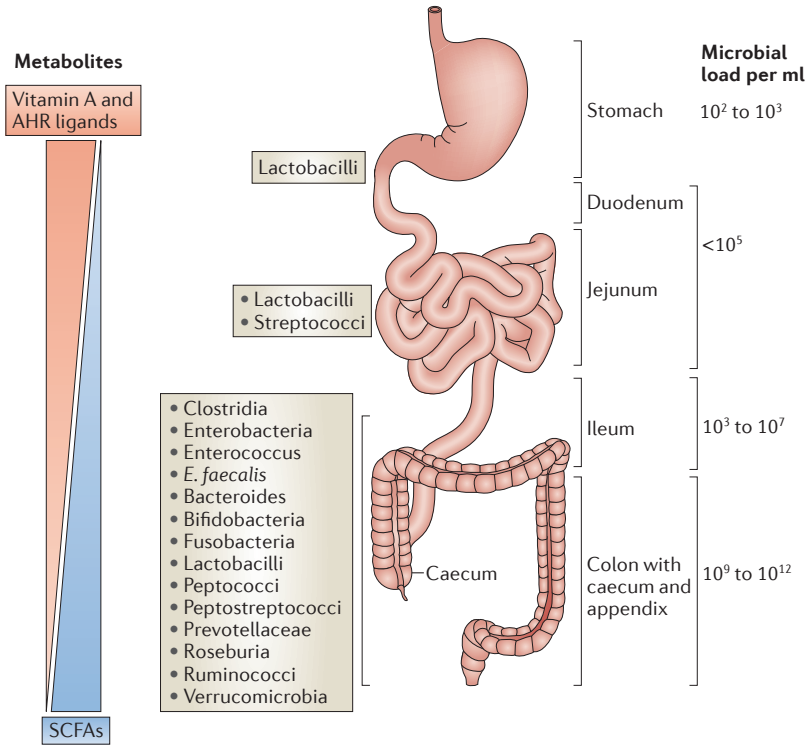


Figure 2 | **Distribution of environmental factors along the length of the intestine.** The intestine is the major source of commensal microbes containing 10^{14} microorganisms of more than 500 different species. The numbers of bacteria generally increase going down the gastrointestinal tract, ranging from 100–1,000 per ml in the highly acidic environment of the stomach to ~10^5 per ml in the upper small intestine and up to 10^{12} per ml in the colon. However, the terminal ileum may contain larger numbers of bacteria than in the colon[285]. These bacteria use complex polysaccharides and other components of mucus and undigested fibre as energy sources, producing essential metabolites such as biotin, short-chain fatty acids (SCFAs) and vitamin K. The composition and distribution of the populations are yet to be determined conclusively, as the majority of species are obligate anaerobes and are difficult to culture *in vitro*. However, molecular techniques reveal approximately 500–1000 species within several major phyla; the relative numbers of these vary between individuals but the Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria are the most prevalent. Members of the Archaea kingdom are also present. The caecum is the site of most species diversity and this is at its lowest in the distal colon[22,286], although there are marked differences between the species found in the proximal and distal colon. Aerobic species are prevalent in the upper small intestine, whereas anaerobic bacteria dominate in the colon, which is consistent with the low oxygen tension there. Alterations in the distribution of the species that make up the intestinal microbiota (dysbiosis) has been associated with many different diseases, ranging from psychiatric conditions to metabolic disease, allergy and autoimmunity[287]. The greater density of bacteria in the caecum has been suggested to explain the higher incidence of tumours that occur in this site compared with the adjacent small intestine[226]. There are also marked differences in the extent to which different regions of the intestinal tract are exposed to the various dietary constituents that can influence immune function (see the main text and BOX 4 for details). Vitamin A is found only in the diet and can also be delivered to the small intestine in the bile[186]; flavonoids and other ligands for the aryl hydrocarbon receptor (AHR) are also present at higher levels in the small intestine. *E. faecalis*, *Enterococcus faecalis*.

Regional differences in epithelial pattern recognition receptors. In addition to their barrier and absorptive functions, epithelial cells express pattern recognition receptors (PRRs), and they can be activated to produce mediators that recruit, activate and condition cells of the immune system[19–21]. To date, few studies have directly assessed variation in epithelial PRR expression along the length of the intestine but there is some relevant data on TLRs and their co-receptors. For example, TLR2 is most highly expressed by epithelial cells in the proximal colon, and its expression on the epithelium gradually decreases towards the distal colon[22]. By contrast, TLR4 and CD14 are expressed at higher levels in the colon than in the small intestine[22,23]. Although the functional implications of these differences remain obscure, they seem to be driven by the microbiota. Finally, the intracellular PRR NOD2 is mainly expressed by epithelial cells in the ileum, particularly by Paneth cells[24]. This pattern is consistent with the association of *NOD2* mutations with ileal Crohn's disease[25,26].

Defensins
Small cationic proteins with antimicrobial properties that are produced by leukocytes and epithelial cells, such as Paneth cells.

Lymphoid structures in the intestine

Organized lymphoid structures. The organized structures of the gut-associated lymphoid tissue (GALT) and the draining lymph nodes are the principal locations for priming adaptive immune cell responses in the intestine. Conversely, effector immune cells are diffusely distributed throughout the lamina propria and overlying epithelium.

The GALT comprises subepithelial lymphoid aggregates that lie in the mucosa and submucosa, and they are characterized by an overlying follicle-associated epithelium. This contains the microfold cells (M cells) that are specialized for the uptake and transport of particulate antigens from the lumen into an underlying dendritic cell (DC)-rich, subepithelial dome (SED) region, where they can be presented to adaptive immune cells. M cells are also major ports of entry for many intestinal pathogens (for a recent review, see REF. 27) (BOX 1). The best-characterized tissues of the GALT are the macroscopically visible Peyer's patches, which are located on the antimesenteric side of the small intestine. The size and density of Peyer's patches increases from the jejunum to the ileum; they are particularly concentrated in the distal ileum and are rare in the duodenum[28]. Peyer's patches consist of numerous B cell lymphoid follicles (up to ten in mice and several hundred in humans[28]), which are flanked by smaller T cell areas. In further contrast to lymph nodes, Peyer's patches are not encapsulated and always contain germinal centres, which is indicative of continual immune stimulation, presumably in response to luminal antigen. Equivalent M cell-containing macroscopic structures are found in the large intestine, in caecal patches around the ileocaecal valve and in colonic patches throughout the colon and the rectum[29,30]. As well as being important sites of T cell priming and immunoglobulin A production following intrarectal antigen administration[31], recent evidence indicates that caecal patches may have a crucial role in the generation of IgA-producing plasma cells that migrate to the colon in response to the local microbiota[32]. By contrast, Peyer's patches seem to be the main source of small intestine-homing IgA plasmablasts[32] (BOX 1). The development of Peyer's patches and colonic patches is initiated during embryonic life and completed after birth. However, the rapid segregation of B cells and T cells that normally occurs during the development of Peyer's patches is delayed in colonic patches, indicating differential regulation in some aspects of the development of these different organized tissues[33].

Box 1 | **Regionally specialized antigen uptake in the intestine**

Access of antigen from the lumen across the epithelium to underlying immune cells requires specialized transport mechanisms that vary depending on the region of the intestine. In the small intestine, bacteria, viruses and inert particles are taken up by microfold cells (M cells) in the follicle-associated epithelium of Peyer's patches and isolated lymphoid follicles (ILFs) to be transported to dendritic cells (DCs) in the adjacent subepithelial dome region. Similar mechanisms may occur in ILFs in the colon and in the caecal patches, which were recently suggested to be the main source of IgA-producing plasma cells in the colon[32]. Uptake of bacteria in the small intestine has also been reported to occur via M cells in the villus epithelium[229], or via DCs in the lamina propria sending transepithelial dendrites (TEDs) between epithelial cells[230–232] or into the lumen[233]. Although it now seems more likely that these TEDs come from CX_3C-chemokine receptor 1 (CX_3CR1)-expressing macrophages that cannot prime T cells[111], other studies have suggested that $CD103^+$ DCs may capture bacteria after crawling into the epithelium[234]. The small intestine also has several mechanisms for providing soluble antigens to the immune system. These may include the transfer of protein from the lumen to $CD103^+$ DCs in the lamina propria via channels formed by neighbouring goblet cells[235]; paracellular or transcytotic transport across the epithelium followed by uptake into DCs in the lamina propria; or carriage in lymph to DCs in the mesenteric lymph nodes. These processes may be facilitated in the presence of IgG antibody by the neonatal Fc receptor, which is expressed on epithelial cells[236]. CX_3CR1^+ macrophages may also contribute to antigen transport, passing it on to $CD103^+$ DCs via connexin 43 (REF. 237). Soluble antigen can also be taken up in the large intestine, but the routes are unknown.

The GALT also includes smaller lymphoid aggregates that are collectively termed solitary isolated lymphoid tissues (SILTs) that can only be detected microscopically. SILTs range in size from small cryptopatches to mature isolated lymphoid follicles (ILFs), which probably represent a continuum of maturational stages[34,35]. In contrast to Peyer's patches, larger ILFs primarily consist of B cells with no clear T cell zone, but similar to Peyer's patches, they contain germinal centres, which is indicative of ongoing humoral immune activation. Indeed, ILFs have been implicated as important sites of T cell-independent IgA class-switching in mice[36]. Mice have an estimated 1,000–1,500 evenly distributed SILTs within the small intestine[35,37], whereas colonic SILTs are concentrated in the distal colon[33]. Mature SILTs are more commonly observed in the ileum and colon, correlating with the increased bacterial burdens at these sites[38]. The human intestine contains an estimated 30,000 ILFs[39], the frequency of which increases 10-fold as one moves down from the jejunum, reaching an average density of one ILF per 28 villi in the ileum[40]. Colonic ILF frequencies also triple as one moves down from the ascending colon to the rectosigmoid colon, reaching a maximum density of 0.6 follicles per cm of tissue section[41].

In mice, cryptopatches in the small intestine and colon develop postnatally within the first 2 weeks of life[33,37,42]. Notably, cryptopatches have yet to be identified in humans, potentially because most intestinal material that has been examined is derived from older patients in whom most SILT structures are likely to have matured into ILFs. Recognition of bacterial peptidoglycans by NOD1 in stromal cells contributes to small intestinal SILT maturation[38]; NOD1 signalling induces the upregulation of CC-chemokine ligand 20 (CCL20) expression by intestinal stromal cells, which promotes CC-chemokine receptor 6 (CCR6)-dependent B cell entry[38] (BOX 2). DCs within small intestinal SILTs express CXC-chemokine ligand 13 (CXCL13)[43], and the expression of the CXCL13 receptor CXC-chemokine receptor 5 (CXCR5) by B cells is also required for B cell localization to small intestinal SILTs[44]. By contrast, colonic SILT maturation seems to be independent of the intestinal microbiota — although it is partially dependent on signalling via myeloid differentiation primary response protein 88 (MYD88) — and it occurs independently of the CCR6–CCL20 axis[33]. B cell recruitment to colonic SILTs is also not perturbed in CXCL13-deficient mice, although these structures fail to form B cell follicles[33]. Another notable distinction between small intestinal and colonic SILT development is the differential requirement for receptor activator of nuclear factor-κB (RANK; also known as TNFRSF11A) signalling. Thus, mice that are deficient for RANK ligand (RANKL; also known as TNFSF11) have fewer small intestinal SILTs and these structures do not contain B cells, whereas colonic SILTs are found in normal numbers and contain B cell follicles in these animals[45].

Lymph drainage along the length of the intestine. The lymph nodes draining the intestine are the largest in the body, reflecting the constant exposure of the intestine to environmental materials. A common misconception is that the mesenteric lymph nodes (MLNs) in rodents drain the entire small and large intestine; it has long been known that separate nodes drain different segments of the intestine[46–48]. By injecting Chicago blue dye into the mouse intestine, Carter and Collins[47] made several important observations: first, lymph from the duodenum primarily drains to a small lymph node that is embedded in the pancreatic tissue; second, the jejunum drains to the middle MLNs; third, the distal ileum, caecum and ascending colon drain to the distal segments of the MLNs; fourth, two small lymph nodes that are buried in the pancreas drain the transverse colon; and finally, lymph from the descending colon and rectum primarily drains to the caudal lymph node (FIG. 3). Similar regional differences in lymph drainage apply to other species including humans, in which the duodenum drains to the duodenopancreatic lymph nodes, the descending colon and rectum to the para-aortic lymph nodes, and the rest of the intestine to the MLNs.

Given the alterations in dietary and bacterial metabolite concentrations along the length of the intestine (BOXES 3,4), lymph that drains from different intestinal segments probably contains very different constituents that may confer specialized immunological characteristics to the various lymph nodes. Clearly, these issues need to be taken into account when selecting lymph nodes to assess adaptive immune responses in different models of infection and inflammation.

Distribution of effector cells along the intestine

The lamina propria and epithelium are the effector sites of the intestinal immune system and they comprise quite distinct compartments (FIG. 4). The lamina propria contains B cells, T cells and numerous innate immune cell

Crohn's disease
An inflammatory bowel disease that is characterized by chronic transmural inflammation and associated granuloma formation. Pathology can affect all parts of the digestive tract, in particular, the colon and terminal ileum.

Follicle-associated epithelium
(FAE). A layer of columnar epithelial cells covering the surface of gut-associated lymphoid tissues such as Peyer's patches, the appendix and isolated lymphoid follicles. The FAE contains several immune cell populations and microfold cells.

Microfold cells
(M cells). Specialized epithelial cells found within the follicle-associated epithelium that covers gut-associated lymphoid tissues. M cells lack microvilli and an overlying glycocalyx, and they are specialized in the uptake of bacteria and other particulate antigens, transporting them to neighbouring antigen-presenting cells. They represent a major site of entry for many viral and bacterial intestinal pathogens.

Box 2 | **Site-specific expression of G protein-coupled receptor ligands**

Several G protein-coupled receptor (GPCR)–GPCR ligand pairs have been suggested to contribute to regionalized immune cell localization within the intestine.

CC-chemokine ligand 25 and CC-chemokine receptor 9. CC-chemokine ligand 25 (CCL25) is expressed by epithelial cells in the small intestine[177,179,238,239], and its interaction with CC-chemokine receptor 9 (CCR9) has an important role in directing the migration of T cells, IgA^+ plasmablasts and plasmacytoid dendritic cells (pDCs) to the small intestine, but not to the colon[149,178,179,240–242]. Epithelial expression of CCL25 decreases from the proximal to the distal murine small intestine[242], and CCL25 seems to have a more important role in the recruitment of T cells to the small intestinal epithelium compared with the lamina propria, and to the duodenum compared with the ileum[242].

CCL28 and CCR10. CCL28 is constitutively expressed by intestinal epithelial cells, particularly in the colon and rectum[243], and its receptor CCR10 is expressed by IgA^+ plasmablasts[244]. Whereas CCL25 cooperates with CCL28 in the recruitment of IgA^+ plasmablasts to the small intestinal mucosa, the CCR10–CCL28 axis alone is involved in T cell-dependent IgA^+ plasmablast recruitment into the colon[32,180–182].

CCL20 and CCR6. CCL20 is constitutively expressed by the follicle-associated epithelium (FAE) overlying Peyer's patches in the small intestine and at lower levels in colonic patches[245–247]. The CCL20–CCR6 axis is required for efficient recruitment of T helper 17 cells and forkhead box P3 (FOXP3)-expressing regulatory T (T_{Reg}) cells into Peyer's patches[247,248], and it has been implicated in the recruitment of DCs into the subepithelial dome (SED) of Peyer's patches[245,246,249]. Consistent with this, CCR6-deficient mice display reduced Peyer's patch size and they lack isolated lymphoid follicles in the small intestine but not in the colon[33,250,251]. CCR6-deficient T_{Reg} cells also display a reduced ability to enter the inflamed colon and, as a result, naive CCR6-deficient T cells induce more severe colitis when transferred into immunodeficient recipient mice[247,248].

CCL9 and CCR1. CCL9 is produced by the FAE of the Peyer's patches and seems to be important for the recruitment of $CD11b^+$ DCs into the SED[252].

GPR15. This orphan GPCR has recently been implicated in the recruitment of T_{Reg} cells to the colon but not to the small intestine[183].

Subepithelial dome
(SED). The area directly beneath the follicular-associated epithelium of gut-associated lymphoid tissues. It is rich in antigen-presenting cells and B cells.

Caecal patches
Areas of organized lymphoid tissue found in the submucosa of the caecum in mice that are thought to be equivalent to the human appendix and to be responsible for generating colon-homing IgA^+ plasma cells. The removal of caecal patches can prevent experimental inflammatory bowel disease.

Colonic patches
Organized lymphoid tissues that are found in the submucosa of the colon. They contain both B cell and T cell areas, and develop before birth.

Solitary isolated lymphoid tissues
(SILTs). The collective term for small lymphoid follicle aggregates that are found in the mucosa of the intestine. They include both cryptopatches and more mature isolated lymphoid follicles.

Cryptopatches
Small organized areas of lymphoid tissue that are found in the wall of the intestine. Cryptopatches contain dendritic cells and lymphoid tissue inducer cells, and they are thought to be immature forms of isolated lymphoid follicles.

Isolated lymphoid follicles
(ILFs). Mature lymphoid aggregates that are found in the mucosa. They primarily consist of B cells, dendritic cells and innate lymphoid cells, and they are covered by a follicle-associated epithelium. ILFs are thought to have a role in initiating local IgA responses and they are found throughout the intestine.

populations — including DCs, macrophages, eosinophils and mast cells — whereas the epithelium primarily contains T cells. Collectively, the intestinal lamina propria and epithelium contain the largest population of T cells, plasma cells and macrophages in the body. There are, however, important regional differences in the distribution of immune cell populations along the length of the intestine, as we discuss below.

Lymphocyte distribution in the intestine

Intraepithelial lymphocytes. The intestinal epithelium of all mammals contains numerous T cells that are located at the basement membrane between enterocytes at a frequency of up to 10–15 intraepithelial lymphocytes (IELs) per 100 epithelial cells[49]. IELs have a wide range of regulatory and effector activities, which are not covered here owing to space constraints (for recent reviews, see REFS 50,51).

In mice, IELs can broadly be divided into two major subsets. 'Conventional' or 'type a' IELs express an αβ T cell receptor (αβTCR) and the CD8αβ heterodimer or CD4, and they are thought to primarily derive from naive T cells that have been activated in secondary lymph nodes. 'Unconventional' or 'type b' IELs do not express CD8β, but most express a CD8αα homodimer and either a γδTCR or an αβTCR. The ontogeny of type b IELs has been the subject of considerable debate (for reviews, see REFS 51,52), but prevailing evidence suggests that the majority of $\alpha\beta TCR^+CD8\alpha\alpha^+$ IELs are thymically derived and originate from agonist-selected autoreactive double-positive thymocytes that migrate as $CD8\alpha^-CD8\beta^-CD4^-$ triple-negative cells to the intestine where they undergo further differentiation[52–55].

The density and composition of the IEL compartment varies greatly with age, antigen exposure and species, as well as along the length of the intestine. In mice, 10-fold to 20-fold more IELs can be isolated from the small intestine compared with the colon, with a gradual decrease in number as one moves from the proximal to distal small intestine[49,56–58]. Analyses of intestinal tissue sections also revealed reduced proportions of IELs in the epithelium of the distal compared with the proximal small intestine[56,59]. Although type b IELs are the dominant T cell population along the length of the small intestine, their proportions are higher in the proximal compared with distal small intestine[59]. By contrast, the murine colonic epithelium contains fewer IELs compared with the small intestine, but has proportionally more $CD4^+$ and $CD8\alpha\beta^+$ type a IELs[56–58,60]. Although germ-free mice have dramatically reduced numbers of IELs, particularly of type a IELs[61], the regional variations in subset composition are largely maintained in these mice[62]. Notably, the survival and proliferation of IELs, in particular that of type b IELs, in both the small intestine and colon was recently suggested to require NOD2-dependent IL-15 production by intestinal mononuclear phagocytes[63].

The majority of human IELs are also T cells, although higher proportions of non-T cells are found within the human colonic epithelium[64]. IEL numbers are also several-fold higher in the proximal compared with the distal small intestine, decreasing even further in the colon[65]. Compared to the mouse, there are fewer type b IELs in the human intestine. Most IELs in the adult human jejunum are $\alpha\beta TCR^+CD8\alpha\beta^+$ type a IELs[65], representing tissue-resident effector memory $CD8^+$ T cells (for a review, see REF. 66), whereas the ileum and colon contain

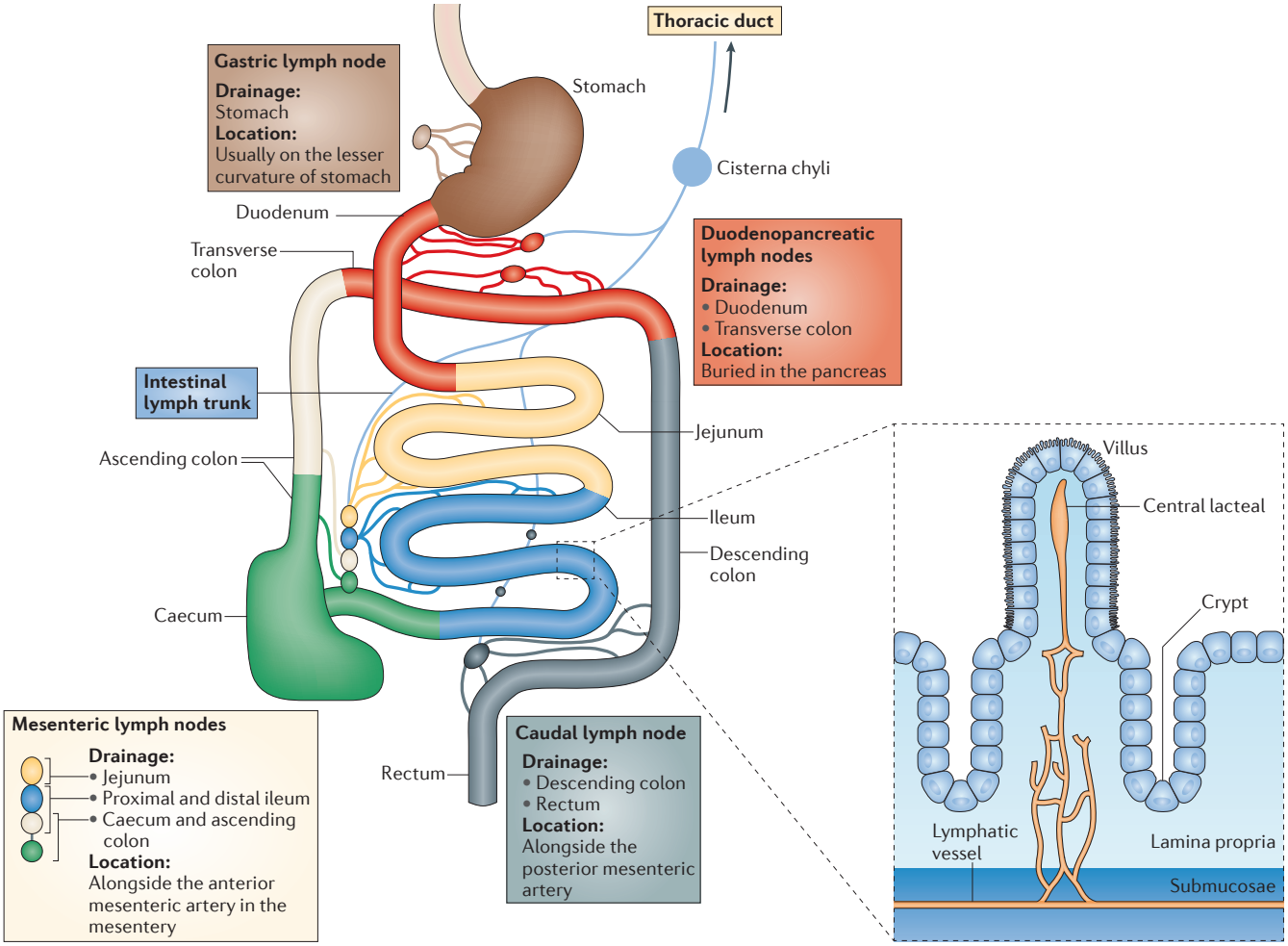


Figure 3 | **Lymph drainage along the length of the mucosa.** The intestinal lymphatic system consists of two distinct compartments — one which drains the muscle layer and the other which drains the mucosa itself[288]. Tissue channels in the intestinal villi deliver luminal and interstitial lymph to lymphatic capillaries, termed lacteals (see inset)[289], which connect to a submucosal lymphatic network. Lymph from the mucosal and muscle lymphatics accumulates in collecting lymphatics for transport to the draining lymph nodes. The figure provides a consensus for where the key intestinal draining lymph nodes are located in the mouse and indicates the intestinal segments that they drain[46,48] (S. A. Houston, V. Cerovic and S. W. F. Milling, unpublished observations). Note that there may be differences in the location, size and distribution of these lymph nodes depending on the genetic background of the mouse and environmental factors, such as the microbiota. Efferent lymph from the caudal lymph node flows into the ileac lymph node and subsequently to the lumbar nodes[290]. The thoracic duct originates in the cisterna chyli — a dilated sac that collects lymph from the intestinal trunk and lumbar nodes — and drains into the blood circulation at the junction of the left subclavian vein and left jugular vein.

Mesenteric lymph nodes (MLNs). The series of lymph nodes draining the small intestine and upper colon. Human MLNs are found throughout the intestinal mesentery, whereas in the mouse, they consist of a string of four or five lymph nodes.

Caudal lymph node
A lymph node found in the abdomen near the bifurcation of the aorta that is responsible for draining lymph from the descending colon and rectum.

proportionally more αβTCR$^+$CD4$^-$CD8$^-$ IELs. Although the frequency of γδTCR$^+$ T cells is relatively higher in the epithelium than in other lymphoid compartments in humans, they remain a rather minor proportion of IELs and this does not change along the length of the intestine. This is also the case for the small population of αβTCR$^+$CD4$^+$ IELs[65]. The relevance of such regional variation in size and composition of the IEL population for intestinal immune homeostasis remains unclear.

Lamina propria T cells. Both CD4$^+$ T cells and CD8$^+$ T cells are found in the lamina propria, at an approximate ratio of 2/1. They are thought to derive from conventional T cells that have been primed in secondary lymphoid organs. Consistent with this, most lamina propria CD4$^+$ T cells and CD8$^+$ T cells in the human and rodent intestine display an effector memory phenotype. The CD4$^+$ T cell compartment of the lamina propria is highly diverse, containing IL-2$^+$, IL-2$^+$IFNγ$^+$, IFNγ$^+$ and IL-17$^+$ subsets, as well as IL-10-producing forkhead box P3 (FOXP3)-expressing regulatory T (T_{Reg}) cells and FOXP3$^-$ T regulatory type 1 (T_R1) cells[67–70]. However, the distribution and function of these subsets differs markedly along the length of the intestine due, at least in part, to variations in luminal content (BOXES 3,4).

An inverse correlation between the number of T helper 17 (T_H17) and T_{Reg} cells has been reported along the length of the intestine in mice, with T_H17 cell numbers progressively decreasing from the duodenum to the colon and T_{Reg} cell numbers being highest in the colon[71].

Box 3 | **Impact of diet on immune cell distribution along the intestine**

Given that the digestion of foodstuffs primarily occurs in the small intestine, any immune-modulating effects of dietary constituents are likely to show a regionalized effect. The best examples of such agents are vitamin A (retinol) and ligands of the aryl hydrocarbon receptor (AHR). The only source of vitamin A in mammals is through the diet in the form of plant carotenoids or retinol from animal material, and it is found at higher concentrations in the small intestine and mesenteric lymph nodes (MLNs) compared with the colon[186]. Although retinoic acid — the major active metabolite of vitamin A — has a plethora of effects on immune cell development and function[253–263], these are most evident in the small intestine. Thus, small intestinal dendritic cells (DCs) receive enhanced retinoic acid signals in the steady state compared with their colonic counterparts[186], becoming imprinted with the ability to generate retinoic acid themselves, which in turn induces gut-homing properties in B cells and T cells (for a review, see REF. 184). Furthermore, the efficient generation of gut-homing T cells in the MLNs *in vivo* is dependent on retinoic acid that is potentially derived from small intestinal migratory DCs, as well as local stromal cells[186,256,264,265]. Retinoic acid also has a direct role in innate lymphoid cell (ILC) homeostasis in the small intestine, supporting the maintenance of interleukin-17 (IL-17)- and IL-22-producing retinoic acid receptor-related orphan receptor-γt (RORγt)-expressing ILC3s, while suppressing the maturation and expansion of IL-13-producing ILC2s[185]. Although it was originally thought to have a selective role in the induction of tolerance, it is now clear that the effects of retinoic acid on adaptive immunity are context dependent and that it can tune multiple aspects of the immune response[184,193].

The diet contains a number of ligands for the AHR, including the phytochemical indole-3-carbinol, which is found in cruciferous vegetables such as broccoli and cabbage; flavonoids such as quercetin, which is found in apples; and resveratrol, which is found in red wine (for recent reviews, see REFS 266,267). AHR signalling is required for the postnatal expansion and function of small intestinal $CD4^-RORγt^+$ ILCs, in part, through the induction of the stem cell factor receptor KIT[266]. As a consequence, deletion of the gene encoding AHR in $RORγt^+$ ILCs leads to delayed postnatal development of cryptopatches and isolated lymphoid follicles, but does not affect the prenatal development of Peyer's patches or caecal patches[266,268]. This results in reduced levels of ILC-derived IL-22, causing outgrowth of segmented filamentous bacteria, and a corresponding increase in $IL\text{-}17^+CD4^+$ T cells in the small intestine but not in the colon[269]. Dietary AHR ligands also have a direct role in the maintenance of small intestinal type b intraepithelial lymphocytes that express CD8αα and either an αβ or a γδ T cell receptor (TCR), but not that of $αβTCR^+CD8αβ^+$ type a intraepithelial lymphocytes[270].

Although IL-10-producing $CD4^+$ T cells are present in large numbers throughout the intestinal mucosa, they seem to be more dominant in the colon[68]. Furthermore, in the small intestine, these cells consist of equal proportions of $FOXP3^+$ and $FOXP3^-$ $CD4^+$ T cells, whereas most IL-10-producing cells in the colon are $FOXP3^+$ T_{Reg} cells[68,70]. Conversely, in humans, higher proportions of IL-17-producing $CD4^+$ T cells have been reported in the lamina propria of the colon and the ileum compared with the jejunum[69]. Other studies suggest that the caecum has higher proportions of T_H17 cells, T_H22 cells and $FOXP3^+$ T_{Reg} cells compared with the terminal ileum and more distal regions of the large intestine. By contrast, the frequencies of T_H1 cells and T_H2 cells do not seem to vary significantly along the human intestine[72].

Intestinal B cells. Unlike most healthy tissues, the normal intestinal lamina propria contains large numbers of plasma cells, the density of which is highest at the most proximal and distal ends of the tract[73,74]. IgA-producing plasma cells predominate throughout the length of the intestine, with the bias towards IgA production progressively increasing from ~75% of the plasma cells in the duodenum to ~90% in the colon; most of the other plasma cells secrete IgM[75,76].

Secretory IgA (SIgA) production is almost completely dependent on the presence of the microbiota, and the polymeric Ig receptor (pIgR; which is necessary for transporting IgA into the lumen) shows a similar microbiota-dependent pattern of enhanced expression in the large intestine[74]. In humans, but not in mice, there are two IgA isotypes and their relative proportions vary along the intestine, with most plasma cells in the duodenum and jejunum producing IgA1. The proportion of IgA2-producing cells then progressively increases from ~25% in the small intestine to >60% in the distal colon[77–80]. Notably, the usual IgA1 dominance in the small intestine can be reversed by bacterial overgrowth[81]. The short hinge-region of IgA2 makes it more resistant to bacterial proteases than IgA1 (REFS 82,83) and gives it a more rigid structure that may favour binding to large particles[84]. Thus, IgA2 may be specially adapted to the bacteria-rich environment of the colon, and it has been suggested that the colonic antibody response may focus on bacterial polysaccharides, such as lipopolysaccharide[85]. This suggests that the B cell-priming environment of colonic follicles and patches may differ from that of small intestinal Peyer's patches in humans. An alternative explanation is that IgA1-committed and IgA2-committed B cell lymphoblasts may express different homing receptors[86].

Innate lymphoid cells. Innate lymphoid cells (ILCs) are a recently described cell population that are currently the focus of intense research interest. They are believed to have important roles in intestinal immunity, inflammation and GALT development (for a review, see REF. 87).

In both mice and humans, type 1 ILCs (ILC1s) are found in approximately equal numbers in the small and large intestines[87,88], with few anatomical differences being described. ILC2s are present in the small intestine of mice and humans, and seem to be present from before birth onwards[89,90]; whether they are also found in the colon has not been examined. Consistent with their small intestinal location, ILC2 function is strongly influenced by feeding activity and circadian rhythm-dependent feeding cycles, which are controlled by the neuroendocrine hormone vasoactive intestinal peptide[89].

ILC3s and their lymphoid tissue inducer (LTi) cell-like precursors are relatively more abundant in the small intestine compared with in the colon[88]. Furthermore, a higher proportion of small intestinal ILC3s belongs to the IL-22-producing subset that expresses NKp46 (also known as NCR1) compared with colonic ILC3s[88]. These differences seem to reflect imprinting effects of the local environment, as adoptively transferred retinoic acid receptor-related orphan receptor-γt (RORγt)-expressing LTi cell-like precursors of ILCs lose RORγt expression preferentially in the colon and give rise to distinct ILC3 subsets in the small intestine compared to the large intestine[88]. ILC3 numbers also seem to increase from the proximal to the distal small intestine, which is consistent with the differences in bacterial density along this axis and with the fact that many of their functions seem to be directed at bacterial defence[15].

Innate lymphoid cells (ILCs). A relatively recently identified population of non-T, non-B lymphocytes that are believed to have a central role in early innate immune responses in the intestinal mucosa. They have been subdivided into three main groups according to whether they express T helper 1 (T_H1)-type, T_H2-type or T_H17-type transcription factors and cytokines.

Box 4 | **Impact of microorganisms and their products on immune cell distribution along the intestine**

Germ-free mice are severely compromised in terms of immune function and lymphoid organ development[271], and it is now clear that individual members of the commensal microbiota can influence the regional specialization of the immune system along the length of the intestine. Colonization of mice with segmented filamentous bacteria (SFB) drives enhanced IgA production and increases intestinal effector $CD4^+$ T cell numbers, especially those producing interleukin-17 (IL-17). These changes occur specifically in the terminal ileum, a site that SFB preferentially colonizes, and they are thought to explain the differences in T helper 17 (T_H17) cell numbers that are found in mouse strains from different suppliers[272,273]. The generation of T_H17 cells requires the presentation of SFB antigens by intestinal dendritic cells (DCs)[274], and SFB may activate these DCs by stimulating serum amyloid A production by epithelial cells[273]. The effects of SFB on the production of IgA and IL-17 are assisted by its ability to drive the development of isolated lymphoid follicles and germinal centres in Peyer's patches[96]. Tryptophan metabolism by *Lactobacillus* species may be a further mechanism underpinning the production of the T_H17 cell product IL-22 in the small intestine, via the generation of the aryl hydrocarbon receptor ligand indole-3-aldehyde[275]. Conversely, certain anaerobic Clostridia species, including some that are present in humans, drive the preferential generation of regulatory T (T_{Reg}) cells in the colon, apparently by promoting a transforming growth factor-β (TGFβ)-rich environment[276,277]. Another mechanism that may account for the enhanced generation of T_{Reg} cells in the colon is the production of polysaccharide A by *Bacteroides fragilis*, which drives the generation of IL-10-producing T_{Reg} cells owing to its ability to stimulate tolerogenic plasmacytoid DCs via Toll-like receptor 2 (REFS 156,278). Short-chain fatty acids (SCFAs) — such as butyrate, acetate and propionate — that are produced by colonic bacteria can influence immune function in this part of the intestine. In particular, the numbers and function of peripherally induced forkhead box P3 (FOXP3)-expressing T_{Reg} cells in the colon are enhanced by butyrate, an effect which may reflect the ability of SCFAs to induce histone H3 acetylation of the *Foxp3* promoter and the conserved non-coding sequence 1 in the *Foxp3* enhancer[279,280]. The SCFAs propionate, butyrate and acetate can also drive the expansion of IL-10-producing $FOXP3^+$ T_{Reg} cells in the colon. This requires ligation of the SCFA receptor GPCR43 and may also reflect histone acetylation within T_{Reg} cells[281], as well as increased expression of GPR15, which has been found to selectively recruit T_{Reg} cells to the colon[183]. Butyrate can also regulate antigen-presenting cell function in the colon through GPR109A (also known as HCAR2)[282]. The microbiome of the intestine also contains large numbers of commensal viruses, bacteriophages and fungi. Although much remains to be discovered about the identity, location and properties of these microorganisms, recent work indicates that at least one viral species, norovirus, can interact with ATG16-like 1 (ATG16L1) in determining susceptibility to Crohn's disease in the small intestine[283,284].

Segmented filamentous bacteria
(SFB). Commensal bacteria that are found as part of the normal microbiota in certain mouse facilities. Currently unculturable, these anaerobes colonize the terminal ileum of rodents, adhering to ileal enterocytes, and they have a major impact on the development and composition of immune cell populations in the small intestine.

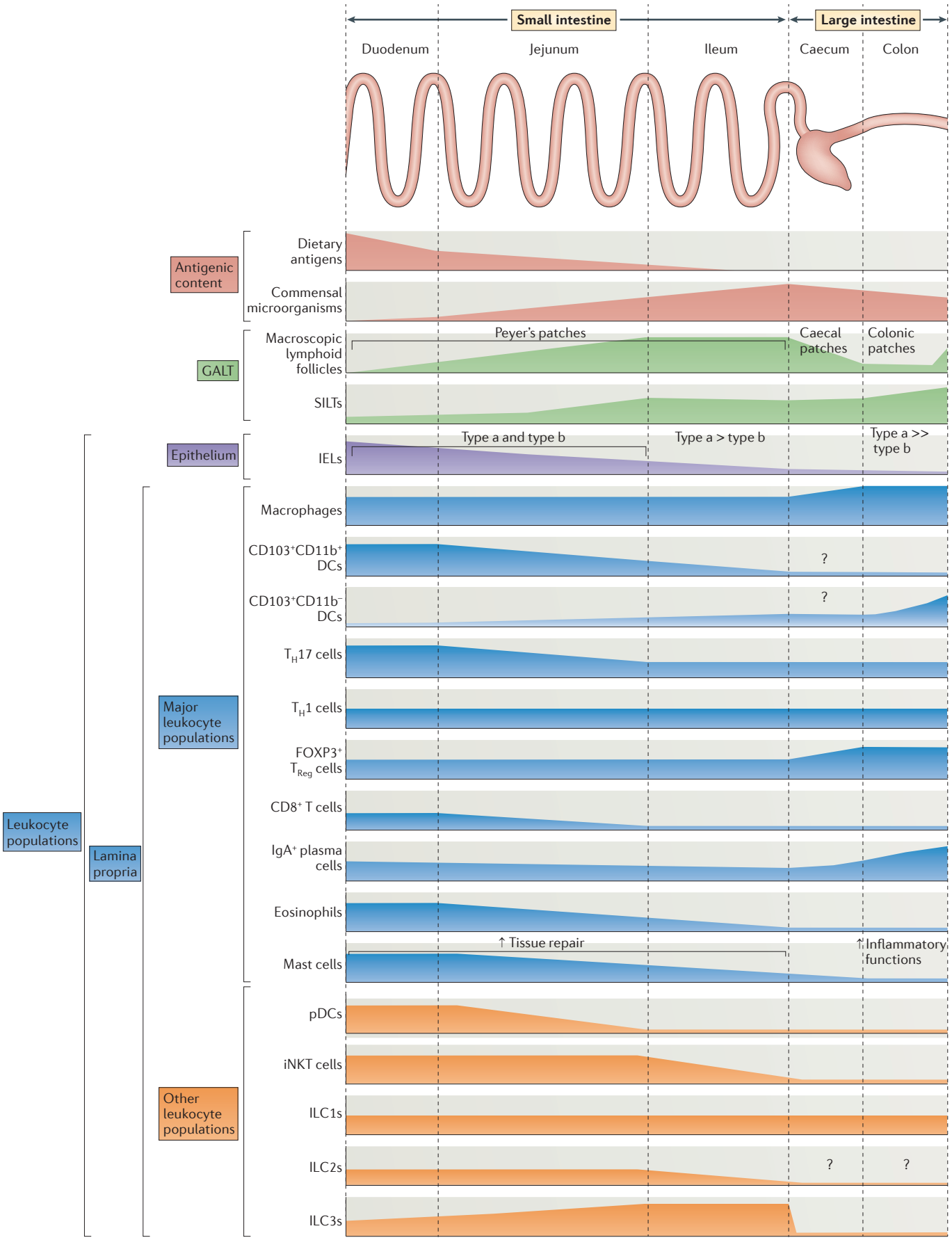
Mucosal-associated invariant T cells
(MAIT cells). Cells that express a semi-invariant T cell receptor (TCR) comprising the canonical TCR Vα7.2 and Jα33 in humans, and Vα19 and Jα33 in mice. They are predominantly found in the human jejunum and are believed to recognize vitamin B metabolites presented by MHC class I-related protein.

Invariant natural killer T cell
(iNKT cell). T cells that express an invariant form of the αβ T cell receptor that recognizes glycolipid antigens presented by the CD1d molecule. They produce cytokines at an early stage in immune responses and may contribute to intestinal inflammation.

However, the exact role of the microbiota in the development of intestinal $NKp46^+$ ILC3 development remains controversial, with some studies finding increased numbers of these cells in germ-free mice and others reporting fewer ILC3s under germ-free conditions[15,91,92]. A dependence on the microbiota would seem to contradict the relative paucity of $NKp46^+$ ILC3s in the colon (which has a higher bacterial load than the small intestine), but this could reflect the anatomical differences in lymphoid organization in the small and large intestines. $NKp46^+$ ILC3s seem to mostly be found in organized lymphoid tissues of the colon, such as ILFs and colonic patches, but they also populate the lamina propria of the small intestine[93–95]. In addition to their production of effector cytokines, recent studies indicate that ILC3s can express MHC class II molecules and that they may contribute to immune regulation in the intestine by suppressing T_H17 cell responses to segmented filamentous bacteria (SFB)[96].

Invariant T cells. The intestine contains minor subsets of T cells that express invariant forms of the TCR and that may have innate immune functions. These subsets include $CD3^+CD161^{hi}CD8\alpha\alpha^+$ (or $CD4^-CD8^-$) mucosal-associated invariant T cells (MAIT cells), as well as invariant natural killer T cells (iNKT cells). MAIT cells have only been described in the human jejunum, where they account for 2–3% of IELs and lamina propria T cells; they are even rarer in mouse intestine[97,98]. Human MAIT cells express a single TCRα chain containing Vα7.2 and Jα33, paired with a limited range of TCRβ chains (usually Vβ6 or Vβ20), and they recognize metabolites of vitamin B presented by the highly conserved MHC class I-related protein (MR1)[97,99–102]. These metabolites are mainly derived from the riboflavin metabolism pathway in microorganisms and MAIT cells are absent from germ-free mice[97]. MAIT cells are selectively activated by cells infected with bacteria, including several enteric species, and they rapidly produce cytokines and exert cytolytic activity[97,99,103,104].

Intestinal iNKT cells display an effector memory phenotype, and they produce large amounts of IL-4 and IFNγ within hours of iNKT cell TCR-mediated recognition of conserved glycolipids that are presented by the MHC class I-like molecule CD1d. Their natural ligands include self and bacterial lipids[105]. Recent studies using α-galactosylceramide-loaded CD1d tetramers have shown that iNKT cells account for ~0.5% of small intestinal lamina propria lymphocytes in mice[106], and similar numbers have been reported in human jejunum[98]. In the mouse, there are more iNKT cells in the small intestine than in the colon, and there are fewer in the IEL compartment than in the lamina propria. In the intestine of germ-free mice, iNKT cells are actually increased in number, possibly owing to enhanced mucosal expression of the chemokine CXCL16 (REFS 107,108), and they are already present in the human fetal intestine[109]. A recent study has suggested that the relative lack of iNKT cells in the mouse colon may reflect the inhibitory effects of sphingolipids that are produced by the anaerobic bacteria *Bacteroides fragilis*[110].



Distribution of non-lymphoid innate immune cells

Mononuclear phagocytes. Mononuclear phagocytes consist of macrophages and DCs, both of which have been implicated in the uptake and presentation of antigens in the intestine (BOX 1). The distinction of these cells from one another has been the source of much controversy in recent years, as they share expression of several markers such as CD11c (also known as integrin αX), MHC class II, CD11b (also known as integrin αM) and CX_3C-chemokine receptor 1 (CX_3CR1)[111,112]. These confounding issues have only recently been appreciated and thus caution has to be exercised in interpreting many studies of the relative distribution of these cell types in the intestine.

Intestinal macrophages. Macrophages are the most abundant leukocytes in the healthy intestinal lamina propria. They have several important functions in intestinal homeostasis, including the phagocytosis and degradation of microorganisms and dead tissue cells, as well as the production of mediators that drive epithelial cell renewal. They also produce large amounts of IL-10, which not only prevents inflammation by blocking pro-inflammatory responses to stimuli such as TLR ligation[113–115], but also promotes the survival and functions of local $FOXP3^+$ T_{Reg} cells in the mucosa[116,117]. The production of IL-1β by resident macrophages in response to the microbiota may have an analogous ability to maintain T_H17 cell activity in the steady-state small intestine[118]. The ability of intestinal macrophages to respond to IL-10 is also a crucial factor in maintaining local homeostasis, as specific knockout of IL-10 receptor signalling in these cells leads to spontaneous colitis in mice[119,120]. These properties are rather specific to the intestine and, unlike many other tissue macrophages, those in the mucosa of both the small intestine and the colon are derived by continuous replenishment from blood monocytes that differentiate locally under the control of the mucosal environment[121] (for a review, see REF. 122).

Fortunately, the earliest reports of intestinal macrophages in mice and humans used what are now known to be specific markers, such as F4/80 (also known as EMR4), CD68 (also known as macrosialin) or CD163. They showed that macrophages are present in both the colon and small intestine, where they represent the largest pool of mononuclear phagocytes in the body[123]. These and most subsequent studies agree that the absolute numbers and relative frequencies of macrophages are higher in the colon than in the small intestine[71,124–126] (C. C. Bain and A.M.M, unpublished observations). Although it has been proposed that there is an increasing proximal-to-distal gradient in macrophage numbers throughout the length of the mouse gastrointestinal tract[71], their numbers do not seem to vary between different parts of the human colon[124]. In all parts of the intestine, macrophages are found relatively close to the epithelial surface, but this seems to be more prominent in the colon[127–130].

Both small intestinal and colonic macrophages show high expression of MHC class II, the scavenger receptor CD163 and, in mice, CX_3CR1. However, colonic macrophages display higher levels of the activation markers acid phosphatase, nonspecific esterase and CD40, as well as CCR5 and formyl peptide receptor[128,131,132]. Despite this, no marked functional differences have been reported between small intestinal and colonic macrophages. Thus, similar processes seem to control the development and functions of macrophages throughout the intestine[113,121,133–136].

Intestinal DCs. Bona fide DCs in the intestine need to be identified using a combination of approaches. In addition to being $CD11c^+$ and MHC class II^+, they lack expression of the macrophage-associated markers CD64 and F4/80, but they express the DC-specific transcription factor zinc finger and BTB domain-containing protein 46 (ZBTB46), they depend on FMS-like tyrosine kinase 3 (FLT3) ligand for their development and they migrate in afferent lymph to draining lymph nodes[69,137–140]. Four main subsets of DCs have been identified in mouse intestinal lamina propria cell preparations and they are classified on the basis of their expression of CD103 (also known as integrin αE) and CD11b. $CD103^+CD11b^-$ and $CD103^+CD11b^+$ intestinal DCs differ in their transcription factor requirements and recent studies suggest that these subsets may have distinct roles in intestinal immune homeostasis (for recent reviews, see REFS 112,140). Similarly to cross-presenting lymph node-resident $CD8^+$ DCs and $CD103^+$ DCs in other tissues, $CD103^+CD11b^-$ DCs in the intestine co-express CD8α, XC-chemokine receptor 1 (XCR1) and DC natural killer lectin group receptor 1 (DNGR1; also known as CLEC9A), and their development requires the transcription factors IFN-regulatory factor 8 (IRF8), inhibitor of DNA binding 2 (ID2) and basic leucine zipper transcriptional factor ATF-like 3 (BATF3)[71,141,142]. By contrast, $CD103^+CD11b^+$ lamina propria DCs are similar to lymph node-resident $CD11b^+$ DCs, as they express signal-regulatory protein-α (SIRPα; also known as SHPS1 and CD172a) and require IRF4 for their survival[143,144]. We and others have recently identified equivalent subsets of $CD103^+CD11b^+SIRPα^+$ and $CD103^+CD11b^-DNGR1^+$ DCs in the human small intestine[144–146], but it remains to be determined whether these subsets are present in the human colon. The ontogeny and functions of $CD103^-$ DCs in the lamina propria, and their relationship to $CD103^+$ DCs, are currently unclear. In particular, the small group of $CD103^-CD11b^-$ DCs seems to be heterogeneous and at least some may be contaminants from secondary lymphoid tissues (see below)[112,139].

There are marked differences in the ratio of $CD103^+CD11b^+$ and $CD103^+CD11b^-$ DCs along the length of the mouse intestine, with $CD103^+CD11b^+$ DCs making up the majority of DCs in the small intestine,

◀ Figure 4 | **The immune apparatus varies along the intestinal tract.** The figure highlights regional specialization in the mouse intestine, indicating how antigenic content (red graphs), gut-associated lymphoid tissue (GALT; green graphs) and various leukocyte populations (blue and orange graphs) change in frequency along the length of the intestinal tract. The question marks indicate regions for which the regional specialization has not been characterized. Similar immune cell subsets are observed in the human intestine, although here their relative proportions in different intestinal segments remain less clear (see the main text for details). DC, dendritic cell; FOXP3, forkhead box P3; IEL, intraepithelial lymphocyte; ILC, innate lymphoid cell; iNKT, invariant natural killer T; pDC, plasmacytoid DC; SILT, solitary isolated lymphoid tissue; T_H, T helper.

but being rare in the colon and mostly absent from other tissues[71,140]. It seems likely that factors within the small intestine are responsible for inducing the expression of CD103 on CD11b$^+$ DCs, although this remains to be demonstrated. The numbers of CD103$^+$CD11b$^+$ DCs in different segments of the intestine correlate with the presence of T_H17 cells[71], indicating a potential role for these DCs in intestinal T_H17 cell homeostasis. Consistent with this possibility, selective reduction of this intestinal DC subset in mice results in reduced numbers of intestinal T_H17 cells[140,143,147,148] and inefficient T_H17 cell differentiation in MLNs[140]. The mechanistic basis for this association is still unclear, but may relate to reduced production of the T_H17 cell-polarizing cytokine IL-6 secondary to the absence of CD103$^+$CD11b$^+$ DCs[140].

By contrast, CD103$^+$CD11b$^-$ DCs are the major CD103$^+$ DC subset in the colon, and they are also enriched in small intestinal GALT compared with their CD103$^+$CD11b$^+$ counterparts[71,137] (C. L. Scott and A.M.M., unpublished observations). As colonic lamina propria isolates inevitably include cells that are derived from colonic GALT, the increased proportions of CD103$^+$CD11b$^-$ DCs in the colon may partially reflect GALT contamination. However, CD103$^+$CD11b$^-$ DCs are also readily identified in the lamina propria of the human small intestine by immunohistochemistry[145,146]. Recent studies have also demonstrated that CD103$^+$CD11b$^-$ DCs are present in normal numbers in the small intestinal lymph of RORγt-deficient mice or mice treated with a lymphotoxin-β receptor–Fc fusion protein (all of which lack GALT), indicating that these cells do not require lymphoid environments for their development[139]. What role CD103$^+$CD11b$^-$ DCs have in mucosal immune homeostasis, and whether their differential localization contributes to regional immune specialization, remains to be assessed. In contrast to lamina propria-derived DCs, it is not known if the composition of DC subsets varies in GALT compartments along the length of the intestine.

Plasmacytoid DCs. Plasmacytoid DCs (pDCs) are also present in the intestinal mucosa but in lower numbers than conventional DCs (cDCs). In addition, they seem to mainly be restricted to the small intestine in mice and primates, with very few being found in the colon[149–153]. As pDCs are mostly CCR9$^+$, this may reflect CCR9-dependent recruitment to the small intestine[149,153] (BOX 2). However, pDCs can be recruited to the colonic mucosa in response to inflammation or virus infection[150,151,154]. The exact functions of pDCs in the intestine remain to be determined and have only been studied in mice. Unlike cDCs, they do not migrate to the MLNs, but they have been implicated in driving the mobilization of cDCs into lymph in response to TLR7 and/or TLR8 ligands, by mechanisms that may include the production of tumour necrosis factor (TNF) and type I IFNs[139,149,155]. Similarly to pDCs in other tissues, intestinal pDCs have been associated with the induction of tolerance and they have protective roles in experimental models of small intestinal inflammation and food allergy[152,153]. In this respect, recent studies indicate that the immunomodulatory properties of the polysaccharide A derived from *Bacteroides fragilis* (BOX 4) may reflect a selective ability to ligate TLR2 on pDCs, leading to the generation of IL-10-producing CD4$^+$ T cells[156].

Other innate cells in the intestine. Although usually associated with allergic reactions and responses to parasitic worms, eosinophils and mast cells are surprisingly abundant in the normal intestinal mucosa of most species, including humans, indicating that they probably have important physiological roles[157–162].

Eosinophils. Although not always immediately obvious on histological examination, eosinophils can be identified in intestinal cell preparations by their high side-scatter profile, and their expression of CCR3 and Siglec-F[163,164]. Indeed, they can account for up to 30% of myeloid cells isolated from samples of normal mucosa[160]. The recruitment of eosinophils to the intestine involves the CCR3 ligand CCL11 (also known as eotaxin 1)[165–167] and α4β7 integrin[168]. In mice, eosinophil numbers are highest in the duodenum and decrease along the entire length of the intestine[170]. Moreover, a greater proportion of eosinophils in the small intestine express IL-33 receptor ST2 (also known as IL-1RL1), CD69 and LY6C, as well as high levels of CD11c and the inhibitory molecule CD22, and mice lacking CD22 have increased numbers of eosinophils in this tissue[169,170]. Thus, different mechanisms may control the homeostasis of eosinophils in different parts of the intestine.

Eosinophils are present in the fetal intestine and their numbers are normal or increased in germ-free mice[165,169]. As a result, they are thought to be important for tissue repair both in the steady state and during inflammation in both the small and large intestines[159,166,167]. Recent studies also indicate that mucosal eosinophils may be important for IgA class-switching in Peyer's patches and for maintaining the numbers of IgA$^+$ plasma cells, CD103$^+$ DCs and FOXP3$^+$ T_{Reg} cells in the small intestinal lamina propria; these homeostatic effects seem to reflect the ability of eosinophils to produce transforming growth factor-β (TGFβ)-activating metalloproteinases[170].

Mast cells. Mast cells are found throughout the healthy gastrointestinal tract, mostly in the lamina propria and submucosa, although there are also a few in the epithelium[162,171–173]. They produce mediators that regulate epithelial barrier integrity, peristalsis, vascular tone and permeability, and there are important two-way interactions between mast cells and the local nervous system[161,162]. They can also detect microorganisms using TLRs. An early study suggested that mast cell numbers progressively decreased from the ileum to the rectum[174]. In addition, mast cells may be functionally distinct at different intestinal sites; mast cells in the small intestine produce a unique TGFβ-dependent protease that is thought to be involved in tissue remodelling[175], whereas the colon contains more pro-inflammatory connective tissue mast cells[176].

Lymphotoxin-β receptor–Fc fusion protein
A fusion protein comprising the lymphotoxin-β receptor linked to the Fc portion of an immunoglobulin heavy chain. When given to pregnant mice, it prevents the development of Peyer's patches and other components of the gut-associated lymphoid tissue in the offspring by specifically blocking access of the $\alpha_1\beta_2$ isoform of lymphotoxin to the LTβ receptor.

Plasmacytoid DCs
(Plasmacytoid dendritic cells; pDCs). A population of dendritic cells with the appearance of plasma cells and a specialized ability to produce type I interferons in response to viruses and Toll-like receptor ligation, but little or no antigen presentation activity.

What shapes regional immunity in the intestine?

Tissue-specific homing molecules. Immune cell entry into lymphoid and extralymphoid tissues is a highly coordinated process that is controlled by the selective expression of cell adhesion receptors on circulating immune cell subsets and their respective ligands within distinct vascular beds. There is evidence that different intestinal sites show distinct patterns of constitutive chemokine expression, and that this favours the recruitment of different types of immune cells (BOX 2). For example, CCL25 is expressed by small intestinal but not colonic epithelium and it has been shown to specifically promote T cell and plasmablast homing to the small intestine[177–179]. By contrast, CCR10 and its ligand CCL28 (REFS 32,180–182) are involved in recruiting IgA^+ plasmablasts to the colon, whereas G protein-coupled receptor 15 (GPR15) has emerged as a novel regulator of T cell homeostasis in the colon[183]. GPR15-deficient mice have reduced numbers of $FOXP3^+$ T_{Reg} cells in the colon and GPR15-deficient T cells show reduced migration to the colonic but not small intestinal lamina propria[183]. The ligands for GPR15 remain to be identified but experiments with broad-spectrum antibiotics suggest that they are not of bacterial origin[183]. Collectively, these findings suggest that differences in the expression levels of luminal and host-derived G protein-coupled receptor ligands in the different parts of the intestine may have a central role in shaping regional differences in immune composition within and along the length of the intestine.

Environmental influences. Different regions of the intestine are continuously exposed to distinct environmental factors, and it is likely that this markedly affects immune cell composition (BOX 3). Retinoic acid, which is derived from dietary vitamin A, has many immunological functions including 'imprinting' DCs with the ability to induce gut-homing receptors on T cells (for a review, see REF. 184) and governing ILC homeostasis in the intestine[185]. As the concentrations of vitamin A are markedly higher in the small intestine and MLNs compared with the colon[186], the immunomodulatory effects of retinoic acid are probably most prominent at these intestinal sites. Other dietary components, such as indole-3-carbinol, flavonoids and resveratrol, regulate ILC and T cell responses by signalling via the aryl hydrocarbon receptor (AHR); again, these are likely to be present at the highest levels in the upper intestinal tract (BOX 3).

By contrast, immunomodulatory short-chain fatty acids (SCFAs) — such as butyrate, acetate and propionate — are generated by anaerobic digestion of oligosaccharides derived from mucus and dietary fibre by commensal Firmicutes and Bacteroidetes species in the colon (BOX 4). SCFAs are found at higher concentrations in the colon and thus probably have a more predominant role in shaping immune responses at this site[187]. Because the composition and density of the microbiota vary greatly between different intestinal sites (FIG. 2), these differences are also likely to have specific effects in shaping regional variation in immune cell populations (BOX 4).

Ulcerative colitis
One of the two major forms of human inflammatory bowel disease. It presents as a continuous area of inflammation that is restricted to the large intestine and is characterized by erythema, superficial ulceration and pseudopolyps.

Implications of regional specialization

Most intestinal diseases show anatomically restricted patterns of distribution. Although this is obviously the case for pathogenic infections, which all have their own special niche, it also applies to inflammatory conditions and malignant disease. Nevertheless, it remains unclear how the aforementioned differences in the proportions of effector T cells, T_{Reg} cells and other immune cell subsets in the small intestine and colon influence disease location.

Inflammatory diseases of the intestine. Of the inflammatory disorders, coeliac disease is restricted to the duodenum and upper small intestine, as would be expected from the fact that it occurs as a result of a T cell response directed at dietary gluten[188]. Conversely, inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis, are more prevalent in the distal small intestine and colon, respectively, consistent with the idea that they reflect aberrant inflammatory responses to commensal bacteria[189]. Indeed, ulcerative colitis is entirely restricted to the colon, with a predilection for the rectum and distal colon, and it usually begins in these most distal sites. Although Crohn's disease is also frequent in the colon, it is more common in the ileocaecal region and can affect all regions of the gastrointestinal tract from the mouth to the anus[190]. Anatomical differences in the distribution of Crohn's disease may reflect true heterogeneity within the disorder.

Regional inflammation in IBD. Many of the genetic factors underpinning susceptibility to Crohn's disease determine innate and adaptive immune responses against bacteria and, as noted earlier, are expressed by Paneth cells of the small intestine. These include *NOD2*, *ATG16L1* and *IRGM1* (REFS 25,189,191–193). The production of antimicrobial peptides by Paneth cells is decreased in patients with ileal Crohn's disease[189,194,195]. The most frequent susceptibility gene is a non-functional mutation in *NOD2*, which is found in 25–30% of patients with terminal ileal disease[25,26]. Interestingly, the terminal ileum is also a site where IL-23-producing DCs and macrophages accumulate[196], which is consistent with the idea that the tissue damage in this form of disease is driven by T_H17 cell responses to the local microbiota[189,197]. Indeed, the gene encoding the IL-23 receptor is a further susceptibility factor in Crohn's disease[189]. Although many microorganisms have been implicated in the pathogenesis of IBD, most have not proved to be causal agents or to be restricted to specific parts of the intestine; however, there is some evidence that the microbiome may have a different composition in patients with ileal and colonic Crohn's disease[198]. In addition, one interesting specific example is adherent-invasive *Escherichia coli* (AIEC), which is mostly found in the small intestine and is often found to be associated with the mucosa of patients with ileal Crohn's disease[199]. It remains to be determined whether this is a primary feature of the disease or whether it is secondary to the presence of inflammation.

Studies of genetic susceptibility, the microbiome and immune function in ulcerative colitis have provided fewer clues about its pathogenesis and why the pathology is so anatomically restricted. As in Crohn's disease, many of the genes that are associated with ulcerative colitis encode proteins that are involved in immune responsiveness against infection[192], and dysbiosis of the microbiota has also been found in ulcerative colitis[198]. However, despite the fact that there is some evidence that different microorganisms may be involved in the two conditions, no specific associations have been established that might account for where the diseases are distributed along the gastrointestinal tract[200]. Similarly, although the genes that are associated with ileal Crohn's disease, unsurprisingly, show little or no association with susceptibility to ulcerative colitis, there is considerable overlap between many of the other genes that are associated with these diseases in general, including the *IL10* locus[192,201]. Interestingly, however, ulcerative colitis shows some unique linkages to genes that control epithelial barrier function and to the MHC locus, the latter being shared by colonic but not small intestinal Crohn's disease[201]. Finally, initial ideas that ulcerative colitis was a T_H2-type disorder[202] — as opposed to the T_H1 cell- and T_H17 cell-dependent pathology that seems to dominate in Crohn's disease — have proved difficult to confirm and the immunological basis of ulcerative colitis remains unclear[203].

Experimental models of spontaneous IBD in gene-deficient mice, or mice in which disease is induced by the transfer of T cells or the administration of chemical agents such as dextran sodium sulphate (DSS), are usually restricted to the colon, often in the distal colon and caecum[23,190,204–206]. Almost all of these models are also dependent on the presence of the microbiota, and involve $CD4^+$ T cells producing IL-17 and/or IFNγ[207]. One exception to this is the experimental colitis that can be induced in rodents by rectal instillation of the contact-sensitizing agent oxazolone, which is thought to be driven by IL-13 generated by iNKT cells[208,209]. Similar IL-13-producing iNKT cells have been reported in humans with ulcerative colitis[190,208,209], but whether they have a direct role in this disease has been challenged[203].

There are no spontaneous models of inflammation of the upper small intestine, but pathology in the distal small intestine is occasionally seen in IL-10-deficient mice that develop spontaneous colitis[210]. A non-functional mutation in the *IL10R* (which encodes the IL-10 receptor) also leads to early onset IBD in children, affecting both the colon and the small intestine[211]. The only other experimental models of IBD that reproducibly cause small intestinal pathology are $TNF^{\Delta ARE}$ mice, in which TNF is overproduced as a result of the deletion of a regulatory element in the *TNF* locus[212], and the SAMP1/Yit mouse strain, the genetic and immunological basis of which remains obscure[213–215]. Both of these models display *trans*-mural terminal ileitis similar to that seen in patients with Crohn's disease.

Interestingly, gene deficiencies that are restricted to $CD11c^+$ cells have been shown to have site-specific effects in the intestine. Mice with a $CD11c^+$ cell-restricted deficiency of TNF receptor-associated factor 6 (TRAF6) have reduced numbers of $FOXP3^+$ T_{Reg} cells and develop a microbiota-driven, T_H2 cell-mediated inflammatory disease in the small intestine but not in the colon[216]. Conversely, $CD11c^+$ cell-restricted deficiency of the TGFβ-activating αVβ8 integrin leads to decreased T_{Reg} cell numbers and to inflammation of the colon, but does not seem to affect the small intestine[217,218]. Macrophage-specific knockout of IL-10 receptor signalling also leads to colitis but not small intestinal disease[119,120].

Coeliac disease. Although coeliac disease is clearly caused by an immune response against dietary gluten, recent studies have suggested that the nature of the local microbiota may also influence the inflammatory response that is seen in this condition. Children who are healthy but are genetically predisposed to develop coeliac disease also have different faecal bacteria compared with those lacking the HLA-DQ2 susceptibility allele[219,220]. The changes included lower numbers of *Bifidobacterium* species and an increased prevalence of *Clostridium leptum*[219,220]. In addition, the so-called coeliac disease 'epidemic' that occurred in Sweden during the 1980s and 1990s has subsequently been associated with a permanent microbial dysbiosis in the small intestine, with relative increases in the prevalence of *Lachnoanaerobaculum*, *Prevotella* and *Actinomyces* genera, even when the tissue pathology has resolved following the introduction of a gluten-free diet[221,222]. Some of the microorganisms involved include adherent spore-forming bacteria that are similar to SFB and, in T cells from patients with coeliac disease, these bacteria have been found to modulate IL-17A production induced by gluten peptides *in vitro*[189,222].

Together, these results highlight the need to more clearly define the microbial species that are present in each anatomical compartment of the intestine and to understand their influence on susceptibility to disease.

Colon cancer. Adenocarcinoma arising from absorptive enterocytes is the third most common cause of death due to malignant disease in developed countries, and disease is almost entirely restricted to the large intestine, especially the distal colon. By contrast, cancer of the small intestine is extremely rare and usually affects cells other than enterocytes, including lymphoid cells. Lymphomas are particularly prevalent in the distal small intestine compared with the jejunum, which is consistent with the relative abundance of secondary lymphoid organs in these regions. The difference in frequency of carcinoma is thought to reflect variations in the local inflammatory responses induced by the widely different numbers and diversity of the microbiota in the caecum and colon compared with the small intestine[223–226] (also see Further information).

A recent study has suggested a specific mechanism by which dysregulated inflammation might account for the heightened susceptibility of the colon to malignant disease. Normally, this tissue contains high levels of IL-22-binding protein (IL-22BP; also known as IL-22RA2), which inhibits the proliferative effects of IL-22 on epithelial cells[227], and is produced

Dysbiosis
An imbalance in the composition of the microbial species that are normally found in the intestine. It is associated with alterations in immune function and susceptibility to inflammatory diseases, allergies and metabolic conditions.

Dextran sodium sulphate
(DSS). Sodium salt of dextran that causes an acute colitis in rodents when administered orally.

Box 5 | **Unresolved questions**

- What are the relative contributions of intrinsic and environmental factors in determining immune apparatus development and function along the length of the intestine?
- How do regionalized differences in microbial and dietary composition contribute to immune apparatus specialization along the length of the intestine? What are the underlying molecular mechanisms?
- How do regionalized differences in the immunological landscape relate to the physiological functions of different parts of the intestine?
- How do stromal elements and immune cells interact to define the distinctive development, composition and functions of immune cell subsets along the length of the intestine?
- Are different routes and mechanisms of antigen uptake used in the small intestine and colon? Do these relate to the nature of the antigen or the anatomical environment?
- Do alterations in the composition of dendritic cell subsets and macrophages along the length of the intestine determine regional differences in adaptive immune cell proportions and function and, if so, how?
- What are the common and distinct molecular mechanisms regulating immune cell homing and maintenance within different intestinal segments?
- Do anatomical differences in immune cell composition and function contribute to disease distribution along the intestine and, if so, how?

by $CD103^+CD11b^+$ lamina propria DCs in the mouse small intestine and immature DCs in humans[228]. IL-22BP-deficient mice readily develop cancer that is restricted to the colon when they also have a mutation in the tumour-suppressor gene adenomatous polyposis coli (*Apc*), or when they are subjected to chronic inflammatory stimuli. This novel model seems to be independent of the presence of the microbiota.

Conclusions

The function of the intestinal immune system in the steady state is to maintain physiological function in the face of constant bombardment by environmental materials. There are, however, marked differences in luminal content, function and anatomy along the length of the intestine, which present distinct challenges to the immune system. As a result, although the various segments of the gastrointestinal tract can deploy a similar battery of immune functions, the exact balance between individual cell types and mechanisms varies considerably along the length of the intestine. Unsurprisingly, the focus of immune responses in the small intestine is to preserve the sterility and barrier function of an epithelium that has the principal function of digesting and absorbing nutrients. The small intestine shows a bias towards processes that are associated with defence against extracellular infections, including the production of IL-17, IL-22 and antimicrobial peptides. Furthermore, although the regulation of inappropriate immune responses is important along the length of the intestine, in the small intestine this is directed mostly at food proteins that can disseminate widely throughout the body, requiring tolerance mechanisms at both the local and systemic level. Conversely, the colon acts as the main reservoir for the commensal microbiota that are essential for life, and its immune system seems to be geared to keep these organisms at bay and prevent inflammatory responses against them. Thus, the colon contains a particularly thick layer of mucus that is produced by goblet cells, together with large numbers of mucosal IgA-producing plasma cells, IL-10-producing macrophages and $FOXP3^+$ T_{Reg} cells.

Although there seem to be key differences in antigen uptake mechanisms and antigen-presenting cell subset composition along the length of the intestine, the importance of these variations in regulating adaptive immune cell composition within different gastrointestinal segments remains unclear and warrants further study (BOX 5). By contrast, it is becoming increasingly clear that variations in the concentrations of dietary and bacterial metabolites, as well as the wide diversity of individual bacterial species that inhabit distinct niches within the intestine, have crucial roles in regionalized immune specialization. For example, dietary metabolites such as retinoic acid and AHR ligands, as well as SFB, have a major impact on both innate and adaptive immunity in the small intestine, whereas certain Clostridia species and SCFA production by colonic anaerobes dramatically influence colonic immune homeostasis. These findings are likely to represent the tip of the iceberg and they highlight a future potential for novel dietary intervention strategies for the treatment of inflammatory disease within distinct intestinal segments.

1. Ouellette, A. J. Paneth cells and innate mucosal immunity. *Curr. Opin. Gastroenterol.* **26**, 547–553 (2010).
2. Clevers, H. C. & Bevins, C. L. Paneth cells: maestros of the small intestinal crypts. *Annu. Rev. Physiol.* **75**, 289–311 (2013).
This review covers the physiology and functions of Paneth cells.
3. Sato, T. *et al.* Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415–418 (2011).
4. Adolph, T. E. *et al.* Paneth cells as a site of origin for intestinal inflammation. *Nature* **503**, 272–276 (2013).
5. Cadwell, K. *et al.* A key role for autophagy and the autophagy gene *Atg16l1* in mouse and human intestinal Paneth cells. *Nature* **456**, 259–263 (2008).
6. Gunther, C. *et al.* Caspase-8 regulates TNF-α-induced epithelial necroptosis and terminal ileitis. *Nature* **477**, 335–339 (2011).
7. Wittkopf, N. *et al.* Lack of intestinal epithelial Atg7 affects Paneth cell granule formation but does not compromise immune homeostasis in the gut. *Clin. Dev. Immunol.* **2012**, 278059 (2012).
8. Kaser, A. & Blumberg, R. S. Autophagy, microbial sensing, endoplasmic reticulum stress, and epithelial function in inflammatory bowel disease. *Gastroenterology* **140**, 1738–1747 (2011).
This is an accessible review which places the intestinal epithelial cell in an immunological and anatomical context.
9. Vandussen, K. L. *et al.* Genetic variants synthesize to produce Paneth cell phenotypes that define subtypes of Crohn's disease. *Gastroenterology* **146**, 200–209 (2014).
10. Liu, B. *et al.* Irgm1-deficient mice exhibit Paneth cell abnormalities and increased susceptibility to acute intestinal inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **305**, G573–G584 (2013).
11. Johansson, M. E. *et al.* The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl Acad. Sci. USA* **105**, 15064–15069 (2008).
This study describes the structure and properties of the mucus layer in different parts of the intestine.
12. Hansson, G. C. Role of mucus layers in gut infection and inflammation. *Curr. Opin. Microbiol.* **15**, 57–62 (2012).
13. Bancroft, A. J., McKenzie, A. N. & Grencis, R. K. A critical role for IL-13 in resistance to intestinal nematode infection. *J. Immunol.* **160**, 3453–3461 (1998).
14. Steenwinckel, V. *et al.* IL-9 promotes IL-13-dependent Paneth cell hyperplasia and up-regulation of innate immunity mediators in intestinal mucosa. *J. Immunol.* **182**, 4737–4743 (2009).
15. Klose, C. S. *et al.* A T-bet gradient controls the fate and function of $CCR6^-RORγt^+$ innate lymphoid cells. *Nature* **494**, 261–265 (2013).
16. Johansson, M. E. *et al.* Composition and functional role of the mucus layers in the intestine. *Cell. Mol. Life Sci.* **68**, 3635–3641 (2011).
17. Velcich, A. *et al.* Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science* **295**, 1726–1729 (2002).
18. Van der Sluis, M. *et al.* Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* **131**, 117–129 (2006).
19. Zaph, C. *et al.* Epithelial-cell-intrinsic IKK-β expression regulates intestinal immune homeostasis. *Nature* **446**, 552–556 (2007).

20. Rescigno, M. The intestinal epithelial barrier in the control of homeostasis and immunity. *Trends Immunol.* **32**, 256–264 (2011).
21. Maldonado-Contreras, A. L. & McCormick, B. A. Intestinal epithelial cells and their role in innate mucosal immunity. *Cell Tissue Res.* **343**, 5–12 (2011).
22. Wang, Y. *et al.* Regional mucosa-associated microbiota determine physiological expression of TLR2 and TLR4 in murine colon. *PLoS ONE* **5**, e13607 (2010).
23. Ortega-Cava, C. F. *et al.* Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. *J. Immunol.* **170**, 3977–3985 (2003).
24. Lala, S. *et al.* Crohn's disease and the NOD2 gene: a role for paneth cells. *Gastroenterology* **125**, 47–57 (2003).
25. Barrett, J. C. *et al.* Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nature Genet.* **40**, 955–962 (2008).
26. Hugot, J. P. *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**, 599–603 (2001).
27. Mabbott, N. A., Donaldson, D. S., Ohno, H., Williams, I. R. & Mahajan, A. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunol.* **6**, 666–677 (2013).
This review describes the location, functions and development of M cells in different sites.
28. Cornes, J. S. 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 (1965).
29. Owen, R. L., Piazza, A. J. & Ermak, T. H. Ultrastructural and cytoarchitectural features of lymphoreticular organs in the colon and rectum of adult BALB/c mice. *Am. J. Anat.* **190**, 10–18 (1991).
30. Perry, G. A. & Sharp, J. G. Characterization of proximal colonic lymphoid tissue in the mouse. *Anat. Rec.* **220**, 305–312 (1988).
31. Lee, A. Y. *et al.* Dendritic cells in colonic patches and iliac lymph nodes are essential in mucosal IgA induction following intrarectal administration via CCR7 interaction. *Eur. J. Immunol.* **38**, 1127–1137 (2008).
32. Masahata, K. *et al.* Generation of colonic IgA-secreting cells in the caecal patch. *Nature Commun.* **5**, 3704 (2014).
This novel study indicates that the recognition of the intestinal microbiota in the Peyer's patches and caecal patches leads to the generation of IgA-producing plasma cells in the small intestine and colon, respectively.
33. Baptista, A. P. *et al.* Colonic patch and colonic SILT development are independent and differentially regulated events. *Mucosal Immunol.* **6**, 511–521 (2013).
34. Herbrand, H., Bernhardt, G., Forster, R. & Pabst, O. Dynamics and function of solitary intestinal lymphoid tissue. *Crit. Rev. Immunol.* **28**, 1–13 (2008).
This review discusses the ILFs that are found in different regions of the intestine.
35. Pabst, O. *et al.* Cryptopatches and isolated lymphoid follicles: dynamic lymphoid tissues dispensable for the generation of intraepithelial lymphocytes. *Eur. J. Immunol.* **35**, 98–107 (2005).
36. Tsuji, M. *et al.* Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. *Immunity* **29**, 261–271 (2008).
37. Kanamori, Y. *et al.* 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 (1996).
38. Bouskra, D. *et al.* Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* **456**, 507–510 (2008).
39. Trepel, F. Number and distribution of lymphocytes in man. A critical analysis. *Klin. Wochenschr.* **52**, 511–515 (1974).
40. Moghaddami, M., Cummins, A. & Mayrhofer, G. Lymphocyte-filled villi: comparison with other lymphoid aggregations in the mucosa of the human small intestine. *Gastroenterology* **115**, 1414–1425 (1998).
41. O'Leary, A. D. & Sweeney, E. C. Lymphoglandular complexes of the colon: structure and distribution. *Histopathology* **10**, 267–283 (1986).
42. Hamada, H. *et al.* Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J. Immunol.* **168**, 57–64 (2002).
43. McDonald, K. G., McDonough, J. S., Dieckgraefe, B. K. & Newberry, R. D. Dendritic cells produce CXCL13 and participate in the development of murine small intestine lymphoid tissues. *Am. J. Pathol.* **176**, 2367–2377 (2010).
44. Velaga, S. *et al.* Chemokine receptor CXCR5 supports solitary intestinal lymphoid tissue formation, B cell homing, and induction of intestinal IgA responses. *J. Immunol.* **182**, 2610–2619 (2009).
45. Knoop, K. A., Butler, B. R., Kumar, N., Newberry, R. D. & Williams, I. R. 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 (2011).
This is one of the few studies that directly compares the development of secondary lymphoid organs in the small and large intestines.
46. Tilney, N. L. Patterns of lymphatic drainage in the adult laboratory rat. *J. Anat.* **109**, 369–383 (1971).
47. Carter, P. B. & Collins, F. M. The route of enteric infection in normal mice. *J. Exp. Med.* **139**, 1189–1203 (1974).
48. Van den Broeck, W., Derore, A. & Simoens, P. Anatomy and nomenclature of murine lymph nodes: Descriptive study and nomenclatory standardization in BALB/cAnNCrl mice. *J. Immunol. Methods* **312**, 12–19 (2006).
49. Ferguson, A. Intraepithelial lymphocytes of the small intestine. *Gut* **18**, 921–937 (1977).
50. Sheridan, B. S. & Lefrancois, L. Intraepithelial lymphocytes: to serve and protect. *Curr. Gastroenterol. Rep.* **12**, 513–521 (2010).
51. Cheroutre, H., Lambolez, F. & Mucida, D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nature Rev. Immunol.* **11**, 445–456 (2011).
52. Hayday, A. & Gibbons, D. Brokering the peace: the origin of intestinal T cells. *Mucosal Immunol.* **1**, 172–174 (2008).
In this review, the concept of distinct ontogenic and functional subsets of IELs is defined.
53. Gangadharan, D. *et al.* Identification of pre- and postselection TCRαβ$^+$ intraepithelial lymphocyte precursors in the thymus. *Immunity* **25**, 631–641 (2006).
54. Pobezinsky, L. A. *et al.* Clonal deletion and the fate of autoreactive thymocytes that survive negative selection. *Nature Immunol.* **13**, 569–578 (2012).
55. Leishman, A. J. *et al.* Precursors of functional MHC class I- or class II-restricted CD8αα$^+$ T cells are positively selected in the thymus by agonist self-peptides. *Immunity* **16**, 355–364 (2002).
56. Beagley, K. W. *et al.* Differences in intraepithelial lymphocyte T cell subsets isolated from murine small versus large intestine. *J. Immunol.* **154**, 5611–5619 (1995).
57. Boll, G., Rudolphi, A., Spiess, S. & Reimann, J. Regional specialization of intraepithelial T cells in the murine small and large intestine. *Scand. J. Immunol.* **41**, 103–113 (1995).
58. Camerini, V., Panwala, C. & Kronenberg, M. 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 (1993).
This study is a direct comparison of intraepithelial lymphocytes in the small and large intestines.
59. Suzuki, K. *et al.* Gut cryptopatches: direct evidence of extrathymic anatomical sites for intestinal T lymphopoiesis. *Immunity* **13**, 691–702 (2000).
60. Ibraghimov, A. R. & Lynch, R. G. 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 (1994).
61. Bandeira, A. *et al.* Localization of γ/δ T cells to the intestinal epithelium is independent of normal microbial colonization. *J. Exp. Med.* **172**, 239–244 (1990).
62. Suzuki, H., Jeong, K. I., Itoh, K. & Doi, K. Regional variations in the distributions of small intestinal intraepithelial lymphocytes in germ-free and specific pathogen-free mice. *Exp. Mol. Pathol.* **72**, 230–235 (2002).
63. Jiang, W. *et al.* Recognition of gut microbiota by NOD2 is essential for the homeostasis of intestinal intraepithelial lymphocytes. *J. Exp. Med.* **210**, 2465–2476 (2013).
64. Selby, W. S., Janossy, G. & Jewell, D. P. Immunohistological characterisation of intraepithelial lymphocytes of the human gastrointestinal tract. *Gut* **22**, 169–176 (1981).
65. Lundqvist, C., Baranov, V., Hammarstrom, S., Athlin, L. & Hammarstrom, M. L. Intra-epithelial lymphocytes. Evidence for regional specialization and extrathymic T cell maturation in the human gut epithelium. *Int. Immunol.* **7**, 1473–1487 (1995).
66. Jabri, B. & Ebert, E. Human CD8$^+$ intraepithelial lymphocytes: a unique model to study the regulation of effector cytotoxic T lymphocytes in tissue. *Immunol. Rev.* **215**, 202–214 (2007).
67. Ivanov, I. I. *et al.* The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17$^+$ T helper cells. *Cell* **126**, 1121–1133 (2006).
68. Maynard, C. L. *et al.* Regulatory T cells expressing interleukin 10 develop from Foxp3$^+$ and Foxp3$^-$ precursor cells in the absence of interleukin 10. *Nature Immunol.* **8**, 931–941 (2007).
69. Sathaliyawala, T. *et al.* Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity* **38**, 187–197 (2013).
70. Veenbergen, S. & Samsom, J. N. Maintenance of small intestinal and colonic tolerance by IL-10-producing regulatory T cell subsets. *Curr. Opin. Immunol.* **24**, 269–276 (2012).
This is a review of the mechanisms that may control tolerance in the different segments of the intestine.
71. Denning, T. L. *et al.* 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 (2011).
This detailed study describes gradients of DCs, macrophages, effector T cells and T_{Reg} cells along the length of the intestine.
72. Wolff, M. J. *et al.* T_H17, T_H22 and T_{Reg} cells are enriched in the healthy human cecum. *PLoS ONE* **7**, e41373 (2012).
73. Brandtzaeg, P. Function of mucosa-associated lymphoid tissue in antibody formation. *Immunol. Invest.* **39**, 303–355 (2010).
74. Brandtzaeg, P., Carlsen, H. & Farstad, I. N. in *Mucosal Immunology, 3rd edition* (eds Mestecky, J. *et al.*) 617–665 (Academic Press, 2005).
75. Brandtzaeg, P. & Johansen, F. E. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. *Immunol. Rev.* **206**, 32–63 (2005).
76. Smith, P. D., MacDonald, T. T. & Blumberg, R. S. in *Principles of Mucosal Immunology* (eds Smith, P. D., MacDonald, T. T. & Blumberg, R. S.) 103–119 (Garland Science, 2013).
77. Kett, K., Brandtzaeg, P., Radl, J. & Haaijman, J. J. Different subclass distribution of IgA-producing cells in human lymphoid organs and various secretory tissues. *J. Immunol.* **136**, 3631–3635 (1986).
78. Crago, S. S. *et al.* Distribution of IgA1-, IgA2-, and J chain-containing cells in human tissues. *J. Immunol.* **132**, 16–18 (1984).
79. He, B. *et al.* Intestinal bacteria trigger T cell-independent immunoglobulin A_2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity* **26**, 812–826 (2007).
80. Lin, M., Du, L., Brandtzaeg, P. & Pan-Hammarstrom, Q. IgA subclass switch recombination in human mucosal and systemic immune compartments. *Mucosal Immunol.* **7**, 511–520 (2014).
This study provides a definitive description of IgA isotype specialization in different segments of the intestine.
81. Kett, K. *et al.* Intestinal B-cell isotype response in relation to local bacterial load: evidence for immunoglobulin A subclass adaptation. *Gastroenterology* **109**, 819–825 (1995).
82. Plaut, A. G., Wistar, R. Jr & Capra, J. D. Differential susceptibility of human IgA immunoglobulins to streptococcal IgA protease. *J. Clin. Invest.* **54**, 1295–1300 (1974).
83. Kilian, M., Reinholdt, J., Lomholt, H., Poulsen, K. & Frandsen, E. V. Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *APMIS* **104**, 321–338 (1996).
84. Bonner, A., Almogren, A., Furtado, P. B., Kerr, M. A. & Perkins, S. J. The nonplanar secretory IgA2 and near planar secretory IgA1 solution structures rationalize their different mucosal immune responses. *J. Biol. Chem.* **284**, 5077–5087 (2009).
85. Tarkowski, A. *et al.* Immunization of humans with polysaccharide vaccines induces systemic, predominantly polymeric IgA2-subclass antibody responses. *J. Immunol.* **144**, 3770–3778 (1990).
86. Pakkanen, S. H. *et al.* Expression of homing receptors on IgA1 and IgA2 plasmablasts in blood reflects differential distribution of IgA1 and IgA2 in various body fluids. *Clin. Vaccine Immunol.* **17**, 393–401 (2010).

87. Spits, H. *et al.* Innate lymphoid cells — a proposal for uniform nomenclature. *Nature Rev. Immunol.* **13**, 145–149 (2013).
This is an important review which defines the current classification of ILCs.
88. Vonarbourg, C. *et al.* Regulated expression of nuclear receptor RORγt confers distinct functional fates to NK cell receptor-expressing RORγt$^+$ innate lymphocytes. *Immunity* **33**, 736–751 (2010).
89. Nussbaum, J. C. *et al.* Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* **502**, 245–248 (2013).
90. Mjosberg, J. M. *et al.* Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nature Immunol.* **12**, 1055–1062 (2011).
91. Sawa, S. *et al.* Lineage relationship analysis of RORγt$^+$ innate lymphoid cells. *Science* **330**, 665–669 (2010).
92. Chen, V. L., Surana, N. K., Duan, J. & Kasper, D. L. Role of murine intestinal interleukin-1 receptor 1-expressing lymphoid tissue inducer-like cells in *Salmonella* infection. *PLoS ONE* **8**, e65405 (2013).
93. Tumanov, A. V. *et al.* Lymphotoxin controls the IL-22 protection pathway in gut innate lymphoid cells during mucosal pathogen challenge. *Cell Host Microbe* **10**, 44–53 (2011).
94. Satoh-Takayama, N. *et al.* Lymphotoxin-β receptor-independent development of intestinal IL-22-producing NKp46$^+$ innate lymphoid cells. *Eur. J. Immunol.* **41**, 780–786 (2011).
95. Reynders, A. *et al.* Identity, regulation and *in vivo* function of gut NKp46$^+$RORγt$^+$ and NKp46$^+$RORγt$^-$ lymphoid cells. *EMBO J.* **30**, 2934–2947 (2011).
96. Lecuyer, E. *et al.* Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. *Immunity* **40**, 608–620 (2014).
This very recent report describes how SFB interacts with secondary lymphoid tissues in the intestine to shape local immune cell functions.
97. Le Bourhis, L. *et al.* Mucosal-associated invariant T cells: unconventional development and function. *Trends Immunol.* **32**, 212–218 (2011).
98. Dunne, M. R. *et al.* Persistent changes in circulating and intestinal γδ T cell subsets, invariant natural killer T cells and mucosal-associated invariant T cells in children and adults with coeliac disease. *PLoS ONE* **8**, e76008 (2013).
99. Gold, M. C. & Lewinsohn, D. M. Co-dependents: MR1-restricted MAIT cells and their antimicrobial function. *Nature Rev. Microbiol.* **11**, 14–19 (2013).
100. Kjer-Nielsen, L. *et al.* MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491**, 717–723 (2012).
This paper provides the first description of how the preferential location of MAIT cells reflects their recognition of microbial products.
101. Patel, O. *et al.* Recognition of vitamin B metabolites by mucosal-associated invariant T cells. *Nature Commun.* **4**, 2142 (2013).
102. Reantragoon, R. *et al.* Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J. Exp. Med.* **210**, 2305–2320 (2013).
103. Dusseaux, M. *et al.* Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161^{hi} IL-17-secreting T cells. *Blood* **117**, 1250–1259 (2011).
104. Le Bourhis, L. *et al.* MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. *PLoS Pathog.* **9**, e1003681 (2013).
105. Zeissig, S. & Blumberg, R. S. Commensal microbiota and NKT cells in the control of inflammatory diseases at mucosal surfaces. *Curr. Opin. Immunol.* **25**, 690–696 (2013).
106. Wingender, G. & Kronenberg, M. Role of NKT cells in the digestive system. IV. The role of canonical natural killer T cells in mucosal immunity and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **294**, G1–G8 (2008).
107. Wingender, G. *et al.* Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. *Gastroenterology* **143**, 418–428 (2012).
108. Olszak, T. *et al.* Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* **336**, 489–493 (2012).
109. Loh, L., Ivarsson, M. A., Michaelsson, J., Sandberg, J. K. & Nixon, D. F. Invariant natural killer T cells developing in the human fetus accumulate and mature in the small intestine. *Mucosal Immunol.* **7**, 1233–1243 (2014).
110. An, D. *et al.* Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* **156**, 123–133 (2014).
111. Persson, E. K., Jaensson, E. & Agace, W. W. The diverse ontogeny and function of murine small intestinal dendritic cell/macrophage subsets. *Immunobiology* **215**, 692–697 (2010).
112. Cerovic, V., Bain, C. C., Mowat, A. M. & Milling, S. W. F. Intestinal macrophages and dendritic cells: what's the difference? *Trends Immunol.* **35**, 270–277 (2014).
This review highlights the distinct properties and ontogeny of intestinal DCs and macrophages.
113. Ueda, Y. *et al.* Commensal microbiota induce LPS hyporesponsiveness in colonic macrophages via the production of IL-10. *Int. Immunol.* **22**, 953–962 (2010).
114. Melillo, J. A. *et al.* Dendritic cell (DC)-specific targeting reveals Stat3 as a negative regulator of DC function. *J. Immunol.* **184**, 2638–2645 (2010).
115. Takeda, K. *et al.* Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* **10**, 39–49 (1999).
116. Murai, M. *et al.* Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nature Immunol.* **10**, 1178–1184 (2009).
117. Hadis, U. *et al.* Intestinal tolerance requires gut homing and expansion of FoxP3$^+$ regulatory T cells in the lamina propria. *Immunity* **34**, 237–246 (2011).
This study defines the anatomical and mechanistic pathways that control the induction and secondary expansion of T_{Reg} cells in the intestine.
118. Shaw, M. H., Kamada, N., Kim, Y. G. & Nunez, G. Microbiota-induced IL-1β, but not IL-6, is critical for the development of steady-state T_H17 cells in the intestine. *J. Exp. Med.* **209**, 251–258 (2012).
119. Zigmond, E. *et al.* Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. *Immunity* **40**, 720–733 (2014).
120. Shouval, D. S. *et al.* Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. *Immunity* **40**, 706–719 (2014).
121. Bain, C. C. *et al.* Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nature Immunol.* http://dx.doi.org/10.1038/ni.2967 (2014).
122. Bain, C. C. & Mowat, A. M. Macrophages in intestinal homeostasis and inflammation. *Immunol. Rev.* **260**, 102–117 (2014).
123. Hume, D. A., Perry, V. H. & Gordon, S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: macrophages associated with epithelia. *Anat. Rec.* **210**, 503–512 (1984).
124. Nagashima, R., Maeda, K., Imai, Y. & Takahashi, T. Lamina propria macrophages in the human gastrointestinal mucosa: their distribution, immunohistological phenotype, and function. *J. Histochem. Cytochem.* **44**, 721–731 (1996).
125. Weber, B., Saurer, L. & Mueller, C. Intestinal macrophages: differentiation and involvement in intestinal immunopathologies. *Semin. Immunopathol.* **31**, 171–184 (2009).
126. Ogino, T. *et al.* Increased Th17-inducing activity of CD14$^+$ CD163^{low} myeloid cells in intestinal lamina propria of patients with Crohn's disease. *Gastroenterology* **145**, 1380–1391 (2013).
127. Hume, D. A., Allan, W., Hogan, P. G. & Doe, W. F. Immunohistochemical characterisation of macrophages in human liver and gastrointestinal tract: expression of CD4, HLA-DR, OKM1, and the mature macrophage marker 25F9 in normal and diseased tissue. *J. Leukoc. Biol.* **42**, 474–484 (1987).
128. Mahida, Y. R., Patel, S., Gionchetti, P., Vaux, D. & Jewell, D. P. Macrophage subpopulations in lamina propria of normal and inflamed colon and terminal ileum. *Gut* **30**, 826–834 (1989).
129. Rugtveit, J., Brandtzaeg, P., Halstensen, T. S., Fausa, O. & Scott, H. Increased macrophage subset in inflammatory bowel disease: apparent recruitment from peripheral blood monocytes. *Gut* **35**, 669–674 (1994).
130. Carlsen, H. S., Yamanaka, T., Scott, H., Rugtveit, J. & Brandtzaeg, P. The proportion of CD40$^+$ mucosal macrophages is increased in inflammatory bowel disease whereas CD40 ligand (CD154)$^+$ T cells are relatively decreased, suggesting differential modulation of these costimulatory molecules in human gut lamina propria. *Inflamm. Bowel Dis.* **12**, 1013–1024 (2006).
131. Devosse, T. *et al.* Formyl peptide receptor-like 2 is expressed and functional in plasmacytoid dendritic cells, tissue-specific macrophage subpopulations, and eosinophils. *J. Immunol.* **182**, 4974–4984 (2009).
132. McElrath, M. J. *et al.* Comprehensive assessment of HIV target cells in the distal human gut suggests increasing HIV susceptibility toward the anus. *J. Acquir. Immune Def. Syndr.* **63**, 263–271 (2013).
133. Rugtveit, J. *et al.* Respiratory burst of intestinal macrophages in inflammatory bowel disease is mainly caused by CD14$^+$L1$^+$ monocyte derived cells. *Gut* **37**, 367–373 (1995).
134. Smythies, L. E. *et al.* Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. *J. Clin. Invest.* **115**, 66–75 (2005).
135. Smythies, L. E. *et al.* Inflammation anergy in human intestinal macrophages is due to Smad-induced IκBα expression and NF-κB inactivation. *J. Biol. Chem.* **285**, 19593–19604 (2010).
136. Smith, P. D. *et al.* Intestinal macrophages and response to microbial encroachment. *Mucosal Immunol.* **4**, 31–42 (2011).
137. Bogunovic, M. *et al.* Origin of the lamina propria dendritic cell network. *Immunity* **31**, 513–525 (2009).
138. Schulz, O. *et al.* Intestinal CD103$^+$, but not CX3CR1$^+$, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J. Exp. Med.* **206**, 3101–3114 (2009).
139. Cerovic, V. *et al.* Intestinal CD103$^-$ dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunol.* **6**, 104–113 (2013).
140. Persson, E. K., Scott, C. L., Mowat, A. M. & Agace, W. W. Dendritic cell subsets in the intestinal lamina propria: Ontogeny and function. *Eur. J. Immunol.* **43**, 3098–3107 (2013).
This review describes the different well-defined subsets of intestinal DCs and how their composition varies along the gastrointestinal tract.
141. Edelson, B. T. *et al.* Peripheral CD103$^+$ dendritic cells form a unified subset developmentally related to CD8α$^+$ conventional dendritic cells. *J. Exp. Med.* **207**, 823–836 (2010).
142. Cerovic, V. *et al.* Lymph-borne CD8α$^+$ dendritic cells are uniquely able to cross-prime CD8$^+$ T cells with antigen acquired from intestinal epithelial cells. *Mucosal Immunol.* http://dx.doi.org/10.1038/mi.2014.40 (2014).
143. Schlitzer, A. *et al.* IRF4 transcription factor-dependent CD11b$^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* **38**, 970–983 (2013).
144. Persson, E. K. *et al.* IRF4 transcription-factor-dependent CD103$^+$CD11b$^+$ dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity* **38**, 958–969 (2013).
145. Poulin, L. F. *et al.* DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. *Blood* **119**, 6052–6062 (2012).
146. Watchmaker, P. B. *et al.* Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. *Nature Immunol.* **15**, 98–108 (2014).
147. Satpathy, A. T. *et al.* Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nature Immunol.* **14**, 937–948 (2013).
148. Welty, N. E. *et al.* Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *J. Exp. Med.* **210**, 2011–2024 (2013).
149. Wendland, M. *et al.* CCR9 is a homing receptor for plasmacytoid dendritic cells to the small intestine. *Proc. Natl Acad. Sci. USA* **104**, 6347–6352 (2007).
150. Baumgart, D. C. *et al.* Aberrant plasmacytoid dendritic cell distribution and function in patients with Crohn's disease and ulcerative colitis. *Clin. Exp. Immunol.* **166**, 46–54 (2011).
151. Kwa, S. *et al.* Plasmacytoid dendritic cells are recruited to the colorectum and contribute to immune activation during pathogenic SIV infection in rhesus macaques. *Blood* **118**, 2763–2773 (2011).
152. Smit, J. J. *et al.* The role of intestinal dendritic cells subsets in the establishment of food allergy. *Clin. Exp. Allergy* **41**, 890–898 (2011).
153. Mizuno, S. *et al.* CCR9$^+$ plasmacytoid dendritic cells in the small intestine suppress development of intestinal inflammation in mice. *Immunol. Lett.* **146**, 64–69 (2012).

154. Karlis, J. *et al.* Characterization of colonic and mesenteric lymph node dendritic cell subpopulations in a murine adoptive transfer model of inflammatory bowel disease. *Inflamm. Bowel Dis.* **10**, 834–847 (2004).
155. Yrlid, U. *et al.* Plasmacytoid dendritic cells do not migrate in intestinal or hepatic lymph. *J. Immunol.* **177**, 6115–6121 (2006).
156. Dasgupta, S., Erturk-Hasdemir, D., Ochoa-Reparaz, J., Reinecker, H. C. & Kasper, D. L. Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. *Cell Host Microbe* **15**, 413–423 (2014).
157. Rothkotter, H. J., Kirchhoff, T. & Pabst, R. Lymphoid and non-lymphoid cells in the epithelium and lamina propria of intestinal mucosa of pigs. *Gut* **35**, 1582–1589 (1994).
158. Bischoff, S. C. *et al.* Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. *Histopathology* **28**, 1–13 (1996).
159. Rothenberg, M. E., Mishra, A., Brandt, E. B. & Hogan, S. P. Gastrointestinal eosinophils. *Immunol. Rev.* **179**, 139–155 (2001).
160. Bain, C. C. *et al.* Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6C^{hi} monocyte precursors. *Mucosal Immunol.* **6**, 498–510 (2013).
161. Bischoff, S. C. Physiological and pathophysiological functions of intestinal mast cells. *Semin. Immunopathol.* **31**, 185–205 (2009).
162. Yu, L. C. & Perdue, M. H. Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress. *Immunol. Rev.* **179**, 61–73 (2001).
163. Carlens, J. *et al.* Common γ-chain-dependent signals confer selective survival of eosinophils in the murine small intestine. *J. Immunol.* **183**, 5600–5607 (2009).
164. Straumann, A. *et al.* Cytokine expression in healthy and inflamed mucosa: probing the role of eosinophils in the digestive tract. *Inflamm. Bowel Dis.* **11**, 720–726 (2005).
165. Mishra, A., Hogan, S. P., Lee, J. J., Foster, P. S. & Rothenberg, M. E. Fundamental signals that regulate eosinophil homing to the gastrointestinal tract. *J. Clin. Invest.* **103**, 1719–1727 (1999).
166. Ahrens, R. *et al.* Intestinal macrophage/epithelial cell-derived CCL11/eotaxin-1 mediates eosinophil recruitment and function in pediatric ulcerative colitis. *J. Immunol.* **181**, 7390–7399 (2008).
167. Lampinen, M., Waddell, A., Ahrens, R., Carlson, M. & Hogan, S. P. CD14$^+$CD33$^+$ myeloid cell–CCL11–eosinophil signature in ulcerative colitis. *J. Leukoc. Biol.* **94**, 1061–1070 (2013).
168. Brandt, E. B. *et al.* The α4bβ7-integrin is dynamically expressed on murine eosinophils and involved in eosinophil trafficking to the intestine. *Clin. Exp. Allergy* **36**, 543–553 (2006).
169. Wen, T. *et al.* The pan-B cell marker CD22 is expressed on gastrointestinal eosinophils and negatively regulates tissue eosinophilia. *J. Immunol.* **188**, 1075–1082 (2012).
170. Chu, V. T. *et al.* Eosinophils promote generation and maintenance of immunoglobulin-A-expressing plasma cells and contribute to gut immune homeostasis. *Immunity* **40**, 582–593 (2014).
This study provides novel information on the potential functions of intestinal eosinophils.
171. Dvorak, A. M., Monahan, R. A., Osage, J. E. & Dickersin, G. R. Crohn's disease: transmission electron microscopic studies. II. Immunologic inflammatory response. Alterations of mast cells, basophils, eosinophils, and the microvasculature. *Hum. Pathol.* **11**, 606–619 (1980).
172. Strobel, S., Miller, H. R. & Ferguson, A. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. *J. Clin. Pathol.* **34**, 851–858 (1981).
173. Nishida, Y. *et al.* Different distribution of mast cells and macrophages in colonic mucosa of patients with collagenous colitis and inflammatory bowel disease. *Hepatogastroenterology* **49**, 678–682 (2002).
174. Lloyd, G., Green, F. H., Fox, H., Mani, V. & Turnberg, L. A. Mast cells and immunoglobulin E in inflammatory bowel disease. *Gut* **16**, 861–865 (1975).
175. Miller, H. R. & Pemberton, A. D. Tissue-specific expression of mast cell granule serine proteinases and their role in inflammation in the lung and gut. *Immunology* **105**, 375–390 (2002).
176. Miller, H. R. *et al.* Granule proteinases define mast cell heterogeneity in the serosa and the gastrointestinal mucosa of the mouse. *Immunology* **65**, 559–566 (1988).
177. Kunkel, E. J. *et al.* 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 (2000).
178. Pabst, O. *et al.* Chemokine receptor CCR9 contributes to the localization of plasma cells to the small intestine. *J. Exp. Med.* **199**, 411–416 (2004).
179. Svensson, M. *et al.* CCL25 mediates the localization of recently activated CD8αβ$^+$ lymphocytes to the small-intestinal mucosa. *J. Clin. Invest.* **110**, 1113–1121 (2002).
180. Feng, N. *et al.* Redundant role of chemokines CCL25/TECK and CCL28/MEC in IgA$^+$ plasmablast recruitment to the intestinal lamina propria after rotavirus infection. *J. Immunol.* **176**, 5749–5759 (2006).
181. Hieshima, K. *et al.* CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. *J. Immunol.* **173**, 3668–3675 (2004).
182. Hu, S., Yang, K., Yang, J., Li, M. & Xiong, N. Critical roles of chemokine receptor CCR10 in regulating memory IgA responses in intestines. *Proc. Natl Acad. Sci. USA* **108**, E1035–E1044 (2011).
183. Kim, S. V. *et al.* GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. *Science* **340**, 1456–1459 (2013).
This paper describes a novel colon-specific chemoattractant.
184. Agace, W. W. & Persson, E. K. How vitamin A metabolizing dendritic cells are generated in the gut mucosa. *Trends Immunol.* **33**, 42–48 (2012).
185. Spencer, S. P. *et al.* Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. *Science* **343**, 432–437 (2014).
186. Jaensson-Gyllenback, E. *et al.* Bile retinoids imprint intestinal CD103$^+$ dendritic cells with the ability to generate gut-tropic T cells. *Mucosal Immunol.* **4**, 438–447 (2011).
187. Wong, J. M., de Souza, R., Kendall, C. W., Emam, A. & Jenkins, D. J. Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* **40**, 235–243 (2006).
188. Meresse, B., Malamut, G. & Cerf-Bensussan, N. Celiac disease: an immunological jigsaw. *Immunity* **36**, 907–919 (2012).
189. Knights, D., Lassen, K. G. & Xavier, R. J. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. *Gut* **62**, 1505–1510 (2013).
This paper reports that correlating the genetic basis of Crohn's disease and immune responses to microbes may help to explain its anatomical localization.
190. Kaser, A., Zeissig, S. & Blumberg, R. S. Inflammatory bowel disease. *Annu. Rev. Immunol.* **28**, 573–621 (2010).
191. Deuring, J. J. *et al.* Genomic ATG16L1 risk allele-restricted Paneth cell ER stress in quiescent Crohn's disease. *Gut* **63**, 1081–1091 (2013).
192. Jostins, L. *et al.* Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* **491**, 119–124 (2012).
193. Raverdeau, M. & Mills, K. H. Modulation of T cell and innate immune responses by retinoic acid. *J. Immunol.* **192**, 2953–2958 (2014).
194. Wehkamp, J. *et al.* Reduced Paneth cell α-defensins in ileal Crohn's disease. *Proc. Natl Acad. Sci. USA* **102**, 18129–18134 (2005).
195. Wehkamp, J., Schmid, M. & Stange, E. F. Defensins and other antimicrobial peptides in inflammatory bowel disease. *Curr. Opin. Gastroenterol.* **23**, 370–378 (2007).
196. Becker, C. *et al.* Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J. Clin. Invest.* **112**, 693–706 (2003).
197. Maloy, K. J. & Powrie, F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* **474**, 298–306 (2011).
198. Chassaing, B. & Darfeuille-Michaud, A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* **140**, 1720–1728 (2011).
199. Nguyen, H. T. *et al.* Crohn's disease-associated adherent invasive *Escherichia coli* modulate levels of microRNAs in intestinal epithelial cells to reduce autophagy. *Gastroenterology* **146**, 508–519 (2014).
200. Wallace, K. L., Zheng, L. B., Kanazawa, Y. & Shih, D. Q. Immunopathology of inflammatory bowel disease. *World J. Gastroenterol.* **20**, 6–21 (2014).
201. Parkes, M. The genetics universe of Crohn's disease and ulcerative colitis. *Dig. Dis.* **30** (Suppl. 1), 78–81 (2012).
This paper compares the genetic basis of the human IBDs with discrete anatomical preferences.
202. Neurath, M. F., Finotto, S. & Glimcher, L. H. The role of Th1/Th2 polarization in mucosal immunity. *Nature Med.* **8**, 567–573 (2002).
203. Biancheri, P. *et al.* Absence of a role for interleukin-13 in inflammatory bowel disease. *Eur. J. Immunol.* **44**, 370–385 (2014).
204. Strober, W., Fuss, I. J. & Blumberg, R. S. The immunology of mucosal models of inflammation. *Annu. Rev. Immunol.* **20**, 495–549 (2002).
205. Neurath, M. F. Animal models of inflammatory bowel diseases: illuminating the pathogenesis of colitis, ileitis and cancer. *Dig. Dis.* **30** (Suppl. 1), 91–94 (2012).
206. Rubtsov, Y. P. *et al.* Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* **28**, 546–558 (2008).
207. Saleh, M. & Elson, C. O. Experimental inflammatory bowel disease: insights into the host-microbiota dialog. *Immunity* **34**, 293–302 (2011).
208. Fuss, I. J. *et al.* Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J. Clin. Invest.* **113**, 1490–1497 (2004).
209. Heller, F., Fuss, I. J., Nieuwenhuis, E. E., Blumberg, R. S. & Strober, W. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* **17**, 629–638 (2002).
210. Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K. & Muller, W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75**, 263–279 (1993).
211. Glocker, E. O. *et al.* Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N. Engl. J. Med.* **361**, 2033–2045 (2009).
212. Kontoyiannis, D., Pasparakis, M., Pizarro, T. T., Cominelli, F. & Kollias, G. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* **10**, 387–398 (1999).
This paper reports one of the few experimental models of IBD that affects the small intestine.
213. Matsumoto, S. *et al.* Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. *Gut* **43**, 71–78 (1998).
214. Marini, M. *et al.* TNF-α neutralization ameliorates the severity of murine Crohn's-like ileitis by abrogation of intestinal epithelial cell apoptosis. *Proc. Natl Acad. Sci. USA* **100**, 8366–8371 (2003).
215. McNamee, E. N. *et al.* Novel model of T_H2-polarized chronic ileitis: the SAMP1 mouse. *Inflamm. Bowel Dis.* **16**, 743–752 (2010).
216. Han, D. *et al.* Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. *Immunity* **38**, 1211–1222 (2013).
217. Travis, M. A. *et al.* Loss of integrin $\alpha_v\beta_8$ on dendritic cells causes autoimmunity and colitis in mice. *Nature* **449**, 361–365 (2007).
218. Paidassi, H. *et al.* Preferential expression of integrin αvβ8 promotes generation of regulatory T cells by mouse CD103$^+$ dendritic cells. *Gastroenterology* **141**, 1813–1820 (2011).
219. Palma, G. D. *et al.* Influence of milk-feeding type and genetic risk of developing coeliac disease on intestinal microbiota of infants: the PROFICEL study. *PLoS ONE* **7**, e30791 (2012).
220. Sellitto, M. *et al.* Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. *PLoS ONE* **7**, e33387 (2012).
221. Ou, G. *et al.* Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease. *Am. J. Gastroenterol.* **104**, 3058–3067 (2009).
222. Sjoberg, V. *et al.* Intestinal T-cell responses in celiac disease — impact of celiac disease associated bacteria. *PLoS ONE* **8**, e53414 (2013).
223. Bilimoria, K. Y. *et al.* Small bowel cancer in the United States: changes in epidemiology, treatment, and survival over the last 20 years. *Ann. Surg.* **249**, 63–71 (2009).
224. American Cancer Society. Cancer Facts & Figures 2013. *American Cancer Society* [online] http://www.cancer.org/research/cancerfactsfigures/cancerfactsfigures/cancer-facts-figures-2013 (2013).
225. O'Keefe, S. J. Nutrition and colonic health: the critical role of the microbiota. *Curr. Opin. Gastroenterol.* **24**, 51–58 (2008).

226. Bongers, G. *et al.* Interplay of host microbiota, genetic perturbations, and inflammation promotes local development of intestinal neoplasms in mice. *J. Exp. Med.* **211**, 457–472 (2014).
227. Huber, S. *et al.* IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature* **491**, 259–263 (2012).
228. Martin, J. C. *et al.* Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid. *Mucosal Immunol.* **7**, 101–113 (2014).
229. Jang, M. H. *et al.* Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proc. Natl Acad. Sci. USA* **101**, 6110–6115 (2004).
230. Rescigno, M. *et al.* Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunol.* **2**, 361–367 (2001).
231. Niess, J. H. *et al.* CX_3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307**, 254–258 (2005).
232. Chieppa, M., Rescigno, M., Huang, A. Y. & Germain, R. N. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J. Exp. Med.* **203**, 2841–2852 (2006).
233. Arques, J. L. *et al. Salmonella* induces flagellin- and MyD88-dependent migration of bacteria-capturing dendritic cells into the gut lumen. *Gastroenterology* **137**, 579–587, 587 e1-2 (2009).
234. Farache, J. *et al.* Luminal bacteria recruit $CD103^+$ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity* **38**, 581–595 (2013).
235. McDole, J. R. *et al.* Goblet cells deliver luminal antigen to $CD103^+$ dendritic cells in the small intestine. *Nature* **483**, 345–349 (2012).
This is a state-of-the-art study using *in vivo* imaging which suggests how antigen may gain access via goblet cells to DCs in the small intestine but not in the colon.
236. Yoshida, M. *et al.* Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. *Immunity* **20**, 769–783 (2004).
237. Mazzini, E., Massimiliano, L., Penna, G. & Rescigno, M. Oral tolerance can be established via gap junction transfer of fed antigens from $CX3CR1^+$ macrophages to $CD103^+$ dendritic cells. *Immunity* **40**, 248–261 (2014).
238. Arijs, I. *et al.* Mucosal gene expression of cell adhesion molecules, chemokines, and chemokine receptors in patients with inflammatory bowel disease before and after infliximab treatment. *Am. J. Gastroenterol.* **106**, 748–761 (2011).
239. Papadakis, K. A. *et al.* CCR9-positive lymphocytes and thymus-expressed chemokine distinguish small bowel from colonic Crohn's disease. *Gastroenterology* **121**, 246–254 (2001).
240. Johansson-Lindbom, B. *et al.* 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 (2003).
241. Lindner, C. *et al.* Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. *J. Exp. Med.* **209**, 365–377 (2012).
242. Stenstad, H., Svensson, M., Cucak, H., Kotarsky, K. & Agace, W. W. Differential homing mechanisms regulate regionalized effector $CD8\alpha\beta^+$ T cell accumulation within the small intestine. *Proc. Natl Acad. Sci. USA* **104**, 10122–10127 (2007).
This detailed study defines how CCL25 controls T cell homing to the small intestine but not to the colon, as well as describing differences within the small intestine itself.
243. Pan, J. *et al.* A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. *J. Immunol.* **165**, 2943–2949 (2000).
244. Kunkel, E. J. *et al.* CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA Ab-secreting cells. *J. Clin. Invest.* **111**, 1001–1010 (2003).
245. Cook, D. N. *et al.* CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity* **12**, 495–503 (2000).
246. Iwasaki, A. & Kelsall, B. L. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3α, MIP-3β, and secondary lymphoid organ chemokine. *J. Exp. Med.* **191**, 1381–1394 (2000).
247. Wang, C., Kang, S. G., Lee, J., Sun, Z. & Kim, C. H. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. *Mucosal Immunol.* **2**, 173–183 (2009).
248. Kitamura, K., Farber, J. M. & Kelsall, B. L. CCR6 marks regulatory T cells as a colon-tropic, IL-10-producing phenotype. *J. Immunol.* **185**, 3295–3304 (2010).
249. Salazar-Gonzalez, R. M. *et al.* CCR6-mediated dendritic cell activation of pathogen-specific T cells in Peyer's patches. *Immunity* **24**, 623–632 (2006).
250. McDonald, K. G. *et al.* CC chemokine receptor 6 expression by B lymphocytes is essential for the development of isolated lymphoid follicles. *Am. J. Pathol.* **170**, 1229–1240 (2007).
251. Varona, R. *et al.* CCR6-deficient mice have impaired leukocyte homeostasis and altered contact hypersensitivity and delayed-type hypersensitivity responses. *J. Clin. Invest.* **107**, R37–R45 (2001).
252. Zhao, X. *et al.* CCL9 is secreted by the follicle-associated epithelium and recruits dome region Peyer's patch $CD11b^+$ dendritic cells. *J. Immunol.* **171**, 2797–2803 (2003).
253. Cha, H. R. *et al.* Downregulation of Th17 cells in the small intestine by disruption of gut flora in the absence of retinoic acid. *J. Immunol.* **184**, 6799–6806 (2010).
254. Chang, S. Y. *et al.* Lack of retinoic acid leads to increased langerin-expressing dendritic cells in gut-associated lymphoid tissues. *Gastroenterology* **138**, 1468–1478, 1478 e1-6 (2010).
255. Coombes, J. L. *et al.* A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-β and retinoic acid-dependent mechanism. *J. Exp. Med.* **204**, 1757–1764 (2007).
256. Iwata, M. *et al.* Retinoic acid imprints gut-homing specificity on T cells. *Immunity* **21**, 527–538 (2004).
257. Klebanoff, C. A. *et al.* Retinoic acid controls the homeostasis of pre-cDC-derived splenic and intestinal dendritic cells. *J. Exp. Med.* **210**, 1961–1976 (2013).
258. Mora, J. R. *et al.* Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* **314**, 1157–1160 (2006).
259. Mora, J. R., Iwata, M. & von Andrian, U. H. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nature Rev. Immunol.* **8**, 685–698 (2008).
260. Mucida, D. *et al.* Reciprocal T_H17 and regulatory T cell differentiation mediated by retinoic acid. *Science* **317**, 256–260 (2007).
261. Sun, C. M. *et al.* Small intestine lamina propria dendritic cells promote *de novo* generation of Foxp3 T reg cells via retinoic acid. *J. Exp. Med.* **204**, 1775–1785 (2007).
262. Suzuki, K. *et al.* The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut. *Immunity* **33**, 71–83 (2010).
263. Takahashi, H. *et al.* TGF-β and retinoic acid induce the microRNA miR-10a, which targets Bcl-6 and constrains the plasticity of helper T cells. *Nature Immunol.* **13**, 587–595 (2012).
264. Hammerschmidt, S. I. *et al.* Stromal mesenteric lymph node cells are essential for the generation of gut-homing T cells *in vivo*. *J. Exp. Med.* **205**, 2483–2490 (2008).
265. Molenaar, R. *et al.* Expression of retinaldehyde dehydrogenase enzymes in mucosal dendritic cells and gut-draining lymph node stromal cells is controlled by dietary vitamin A. *J. Immunol.* **186**, 1934–1942 (2011).
266. Kiss, E. A. & Diefenbach, A. Role of the aryl hydrocarbon receptor in controlling maintenance and functional programs of $ROR\gamma t^+$ innate lymphoid cells and intraepithelial lymphocytes. *Front. Immunol.* **3**, 124 (2012).
267. Veldhoen, M. & Brucklacher-Waldert, V. Dietary influences on intestinal immunity. *Nature Rev. Immunol.* **12**, 696–708 (2012).
This review discusses the effects of AHR ligands and other dietary components on the function of intestinal immune cells.
268. Lee, J. S. *et al.* AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nature Immunol.* **13**, 144–151 (2012).
269. Qiu, J. *et al.* The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity* **36**, 92–104 (2012).
270. Li, Y. *et al.* Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell* **147**, 629–640 (2011).
271. MacDonald, T. T. & Carter, P. B. Requirement for a bacterial flora before mice generate cells capable of mediating the DTH reaction to sheep red blood cells. *J. Immunol.* **122**, 2624–2629 (1979).
272. Talham, G. L., Jiang, H. Q., Bos, N. A. & Cebra, J. J. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect. Immun.* **67**, 1992–2000 (1999).
273. Ivanov, I. I. *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485–498 (2009).
274. Goto, Y. *et al.* Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity* **40**, 594–607 (2014).
275. Zelante, T. *et al.* Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* **39**, 372–385 (2013).
276. Atarashi, K. *et al.* T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* **500**, 232–236 (2013).
This study describes the presence of bacteria in the human colon that drive the selective generation of T_{Reg} cells.
277. Atarashi, K. *et al.* Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* **331**, 337–341 (2011).
278. Round, J. L. & Mazmanian, S. K. Inducible $Foxp3^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl Acad. Sci. USA* **107**, 12204–12209 (2010).
279. Furusawa, Y. *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450 (2013).
280. Arpaia, N. *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**, 451–455 (2013).
281. Smith, P. M. *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic T_{reg} cell homeostasis. *Science* **341**, 569–573 (2013).
282. Singh, N. *et al.* Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* **40**, 128–139 (2014).
283. Cadwell, K. *et al.* Virus-plus-susceptibility gene interaction determines Crohn's disease gene *Atg16L1* phenotypes in intestine. *Cell* **141**, 1135–1145 (2010).
284. Virgin, H. W. The virome in mammalian physiology and disease. *Cell* **157**, 142–150 (2014).
285. Ahmed, S. *et al.* Mucosa-associated bacterial diversity in relation to human terminal ileum and colonic biopsy samples. *Appl. Environ. Microbiol.* **73**, 7435–7442 (2007).
286. Hu, S. *et al.* Regional differences in colonic mucosa-associated microbiota determine the physiological expression of host heat shock proteins. *Am. J. Physiol. Gastrointest. Liver Physiol.* **299**, G1266–G1275 (2010).
This study describes some of the immunological consequences of anatomical differences in the composition of the intestinal microbiota.
287. Belkaid, Y. & Hand, T. W. Role of the microbiota in immunity and inflammation. *Cell* **157**, 121–141 (2014).
288. Unthank, J. L. & Bohlen, H. G. Lymphatic pathways and role of valves in lymph propulsion from small intestine. *Am. J. Physiol.* **254**, G389–G398 (1988).
289. Miller, M. J., McDole, J. R. & Newberry, R. D. Microanatomy of the intestinal lymphatic system. *Ann. NY Acad. Sci.* **1207** (Suppl. 1), E21–E28 (2010).
290. Kawashima, Y., Sugimura, M., Hwang, Y.-C. & Kudo, N. The lymph system in mice. *Japanese J. Veterinary Res.* **12**, 69–78 (1964).

Acknowledgements
The authors would like to thank A. Sasor and E. Toth at Skåne University Hospital, Sweden, for the intestinal section and endoscopy images, respectively. W.W.A. is supported by grants from the Swedish Medical Research Council, the Swedish National Health Service and a Sapere Aude senior researcher grant from the Danish Research Council. A.M.M is supported by grants from the Medical Research Council, UK, the Wellcome Trust and Tenovus Scotland.

Competing interests statement
The authors declare no competing interests.

FURTHER INFORMATION
Cancer Stats, Cancer Research UK:
http://www.cancerresearchuk.org/cancer-info/cancerstats

ALL LINKS ARE ACTIVE IN THE ONLINE PDF