ANATOMICAL BASIS OF TOLERANCE AND IMMUNITY TO INTESTINAL ANTIGENS

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The intestinal immune system has to discriminate between harmful and beneficial antigens. Although strong protective immunity is essential to prevent invasion by pathogens, equivalent responses against dietary proteins or commensal bacteria can lead to chronic disease. These responses are normally prevented by a complex interplay of regulatory mechanisms. This article reviews the unique aspects of the local microenvironment of the intestinal immune system and discuss how these promote the development of regulatory responses that ensure the maintenance of homeostasis in the gut.

COELIAC DISEASE
A chronic inflammatory condition of the upper small intestine in humans that is caused by immunological hypersensitivity to the α-gliadin component of wheat gluten. It is often found in infants after the introduction of solid foods. It causes severe villus atrophy, which can lead to malabsorption and malnutrition if gluten-containing foods are not removed from the diet.

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doi:10.1038/nri1057

The intestinal immune system is the largest and most complex part of the immune system. Not only does it encounter more antigen than any other part of the body, but it must also discriminate clearly between invasive organisms and harmless antigens, such as food proteins and commensal bacteria. Most human pathogens enter the body through a mucosal surface, such as the intestine, and strong immune responses are required to protect this physiologically essential tissue. In addition, it is important to prevent further dissemination of such infections. By contrast, active immunity against non-pathogenic materials would be wasteful, and hypersensitivity responses against dietary antigens or commensal bacteria can lead to inflammatory disorders such as COELIAC DISEASE and CROHN'S DISEASE, respectively. As a result, the usual response to harmless gut antigens is the induction of local and systemic immunological tolerance, known as oral tolerance[1,2]. In addition to its physiological importance, this phenomenon can be exploited for the immunotherapy of autoimmune and inflammatory diseases[3], but it is also an obstacle to the development of recombinant oral vaccines. For these reasons, there is great interest in the processes that determine the immunological consequences of oral administration of antigen.

To some extent, this discrimination between harmful and harmless antigens also occurs in other parts of the immune system, as it partly results from inherent properties of the antigen and associated adjuvants. Nevertheless, it has been proposed that there are also specific features of mucosal tissues that favour the induction of tolerance, the production of immunoglobulin A antibodies and, to a lesser extent, T helper 2 (T_H2)-cell responses[4]. Several features of mucosal tissues might contribute to these effects[4], including a unique ontogeny and anatomical patterning, specialized cells and organs that are involved in the uptake of antigen, distinctive subsets of antigen-presenting cells (APCs) and several unusual populations of B and T cells. In addition, the migration of lymphocytes to the intestine is controlled by a series of unique adhesion molecules and chemokine receptors. In this review, I discuss the anatomical factors which determine the special nature of small intestinal immune responses, and the unique processes and cells involved in the uptake and presentation of antigen to T cells in the gut. In particular, I focus on the local factors that determine the behaviour of APCs and T cells in the gut and discuss recent evidence that challenges the conventional dogma that Peyer's patches are the only site for the initiation of mucosal immunity and tolerance.

I focus on the small intestine, as this tissue has been studied in most detail and it contains the largest proportion of immune cells in the gut. However, the reader

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CROHN'S DISEASE
A form of chronic inflammatory bowel disease that can affect the entire gastrointestinal tract, but is commonest in the colon and terminal ileum. It is characterized by transmural inflammation, strictures and granuloma formation, and is believed to result from an abnormal T-cell-mediated immune response to commensal bacteria.

BRUSH BORDER
The surface layer of the normal small intestine that is comprised of small microvilli coated in a rich glycocalyx of mucus and other glycoproteins. The microvilli contain many of the digestive enzymes and transporter systems that are involved in the metabolism and uptake of dietary materials. The brush border provides a large surface area for absorption.

should be aware that each compartment of the intestine, from the oro-pharynx to the stomach and to the rectum, has its own specializations, which might have individual effects on immune regulation in response to local antigens.

GALT anatomy and intestinal immune responses

The gut-associated lymphoid tissue (GALT) can be divided into effector sites, which consist of lymphocytes scattered throughout the epithelium and lamina propria of the mucosa, and organized tissues, that are responsible for the induction phase of the immune response (FIGS 1,2). These are the Peyer's patches and mesenteric lymph nodes (MLNs), as well as smaller, isolated lymphoid follicles, which have the appearance of microscopic Peyer's patches and are distributed throughout the wall of the small and large intestines[5].

Peyer's patches. The Peyer's patches are macroscopic lymphoid aggregates that are found in the submucosa along the length of the small intestine (FIG. 2). In mice, the pre-natal development of Peyer's patches is distinct from that of peripheral lymphoid tissues, being induced by the production of lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) by recirculating $CD3^-CD4^+$ progenitor cells that respond to the local expression of interleukin-7 (IL-7). The entry of $CD3^-CD4^+$ progenitor cells into the developing Peyer's patches also uniquely requires expression of the chemokine receptor CXCR5. The effects of $LT\alpha_1\beta_2$ involve the selective activation of the p52–RelB heterodimeric subunit of nuclear factor (NF)-κB, mediated by signalling through the lymphotoxin-β receptor (LTβR) that is expressed by vascular-cell adhesion molecule 1 $(VCAM1)^+$ mesenchymal cells. The p55 form of the tumour-necrosis factor receptor (TNFRp55) might also have an accessory role in the development of Peyer's patches[6–11].

Mature Peyer's patches consist of collections of large B-cell follicles and intervening T-cell areas. The lymphoid areas are separated from the intestinal lumen by a single layer of columnar epithelial cells, known as the follicle-associated epithelium (FAE), and a more diffuse area immediately below the epithelium, known as the subepithelial dome (SED) (FIG. 2). The FAE differs from the epithelium that covers the villus mucosa, as it has lower levels of digestive enzymes and a less pronounced BRUSH BORDER, also it is infiltrated by large numbers of B cells, T cells, macrophages and dendritic cells (DCs). The most notable feature of the FAE is the presence of microfold (M) cells, which are specialized enterocytes that lack surface microvilli and the normal thick layer of mucus (FIG. 2). M cells differentiate from enterocytes under the influence of membrane-bound $LT\alpha_1\beta_2$ that is present on local lymphoid cells, mainly B cells[12–14]. They bind invasive pathogens, such as *Salmonella*, *Shigella*, *Yersinia* and reoviruses, and other particulate antigens.

Mesenteric lymph nodes. The MLNs are the largest lymph nodes in the body. Their development is distinct from that of both Peyer's patches and peripheral lymph

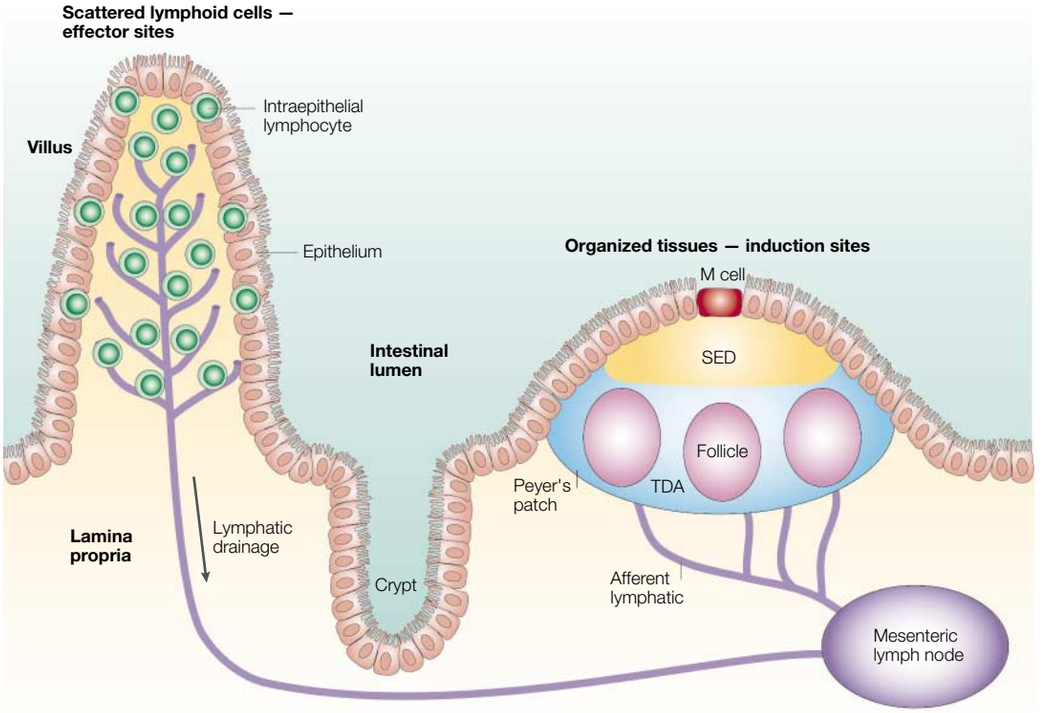

Figure 1 | **Schematic representation of the lymphoid elements of the intestinal immune system**. The organized tissues of the Peyer's patches and mesenteric lymph nodes (MLNs) are involved in the induction of immunity and tolerance, whereas the effector sites are scattered throughout the lamina propria and epithelium of the mucosa. Both the Peyer's patches and villus lamina propria are drained by afferent lymphatics that go to the MLNs. SED, subepithelial dome; TDA, thymus-dependent area.

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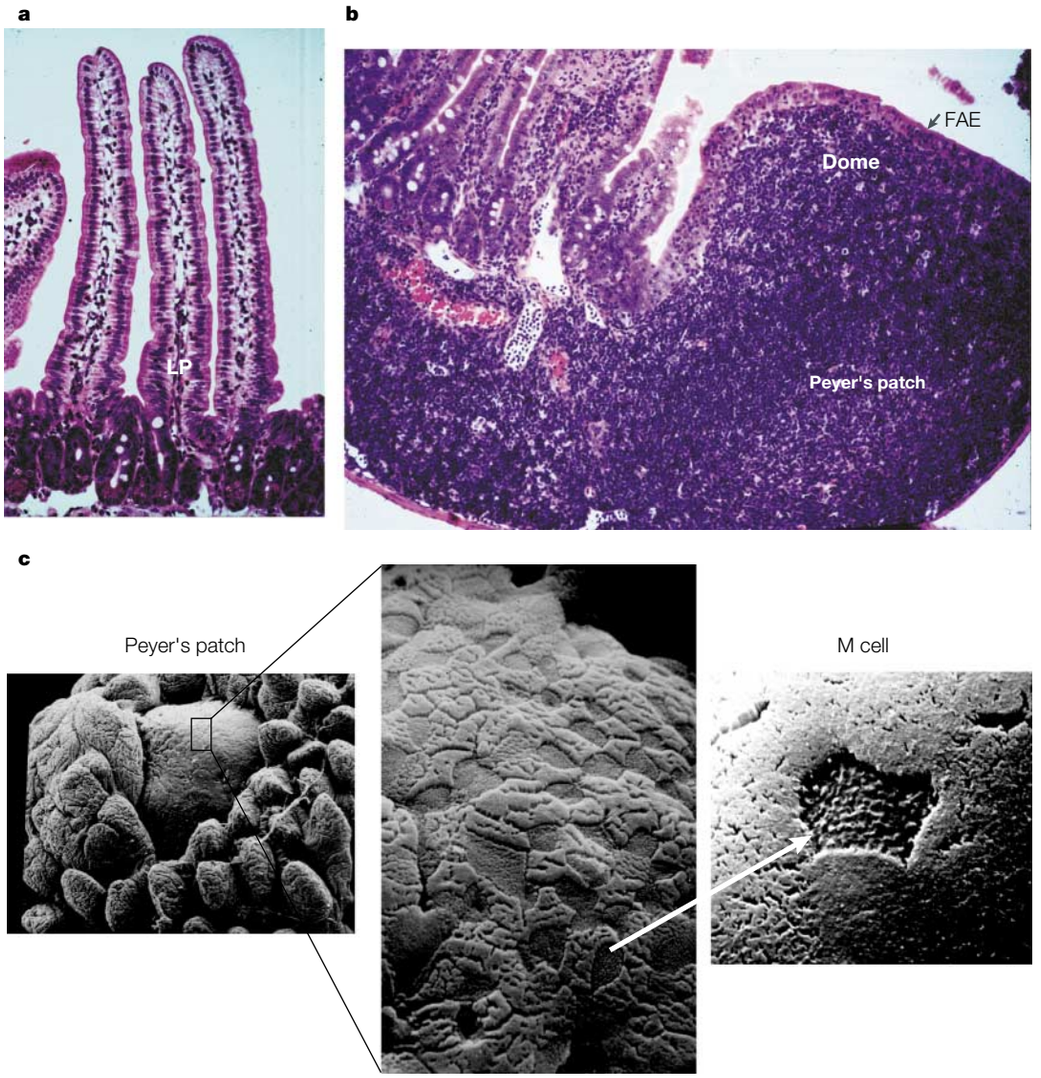

Figure 2 | **Histological appearance of Peyer's patches and intestinal mucosa**. **a** | Normal small intestine showing the characteristic architecture of finger-like villi that are covered by a single layer of columnar epithelial cells, which encloses the central lamina propria (LP). **b** | Peyer's patches are aggregates of secondary lymphoid tissue present in the submucosa of the small intestine. They are separated from the lumen by the follicle-associated epithelium (FAE), which is comprised of columnar epithelial cells and also contains microfold (M) cells, dendritic cells (DCs), T cells, B cells and macrophages. The area immediately beneath the FAE ('dome') is rich in DCs. **c** | Scanning-electron micrographs of Peyer's patches and FAE. At low magnification (left), the dome shape of the Peyer's patch protrudes between villi into the lumen of the intestine. At higher magnification (centre and right), M cells can be seen as epithelial cells with surface microfolds rather than the microvilli that are seen on the surrounding conventional enterocytes. Antigen is taken up preferentially through M cells (right). Images reproduced from REF. 4, with permission from Blackwell Publishing Ltd.

nodes, as it is relatively unaffected by the absence of most of the factors that are involved in the ontogeny of these other organs, including TNF, TNFR, $LT\alpha_1\beta_2$ and LTβR. Instead, these factors might have complementary roles in MLN development, whereas LIGHT, a new ligand for LTβR, might also be required[7,15–17]. Accumulation of lymphocytes in the MLNs also requires both L-selectin and $\alpha_4\beta_7$ integrin adhesion molecules, which normally direct lymphocytes to enter peripheral and mucosal tissues, respectively[18]. As a result of these unique anatomical features, the MLNs might be a crossroads between the peripheral and mucosal recirculation pathways.

Induction of intestinal immune responses. It has been assumed for many years that M cells provide the main, if not the only, way in which complex antigens can gain access to the intestinal immune system. M cells probably do not process antigens themselves — they do not express MHC class II molecules — and instead, they are believed to pass on intact antigen to professional APCs, either in the epithelium or in the underlying dome region. From there, the APCs move to the T-cell areas and/or B-cell follicles, where they can interact with naive lymphocytes (FIG. 3). DCs are probably the APC involved in this process, and several DC subsets have been

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described recently in Peyer's patches (see later). In Peyer's patches, B cells undergo immunoglobulin class switching from expression of IgM to IgA under the influence of several local factors, including transforming growth factor-β (TGF-β), IL-10 and cellular signals that are delivered by DCs and T cells[19].

The lymphocytes that are primed in the Peyer's patches exit through the draining lymphatics to the MLNs, where they reside for an undefined period of further differentiation, before they migrate into the bloodstream through the thoracic duct and finally accumulate in the mucosa (FIG. 3). The exit of lymphocytes into the mucosa occurs because lymphocytes that are primed by antigen in the GALT lose expression of L-selectin and selectively upregulate expression of $\alpha_4\beta_7$ integrin. This directs the emigration of lymphocytes from the bloodstream by interacting with the ligand for $\alpha_4\beta_7$ integrin, mucosal addressin cell-adhesion molecule 1 (MADCAM1), which is expressed at high levels by the vasculature of mucosal surfaces[20,21]. In parallel, expression of the chemokine receptor CCR9 is induced by gut-derived T cells, allowing them to respond to the chemokine CCL25, also known as TECK (thymus-expressed chemokine), which is expressed selectively by small-bowel epithelial cells[10,22,23]. This pattern of adhesion-molecule and chemokine-receptor expression is distinct from that of T cells that are primed in peripheral lymphoid organs, which acquire the $\alpha_4\beta_1$ integrin VLA4 (very late antigen 4) and the chemokine receptor CCR4 and so cannot migrate to mucosal surfaces[23]. This is the molecular explanation as to why mucosal vaccination is required to protect against mucosal infections, whereas parenteral vaccines are generally ineffective against such infections.

Box 1 | **Regulatory T cells and intestinal immunity**

T_H3 cells
A population of $CD4^+$ T cells that produce transforming growth factor-β (TGF-β) and can be isolated by repeated restimulation of mesenteric lymph nodes or spleen lymphocytes from mice that have been fed low doses of antigen for tolerance induction. Similar cells have been identified directly *in vivo*[96].

T_R1 cells
A population of $CD4^+$ T cells that produce interleukin-10 (IL-10). They can be generated *in vitro* in the presence of antigen, IL-10, IL-15 and/or type I interferon. These cells have not been isolated during oral tolerance *in vivo*, but have been shown to produce bystander suppression of experimental colitis in mice that have been fed antigen[75].

$CD4^+CD25^+$ regulatory T cells
Intrathymically derived regulatory T cells with a potent ability to prevent autoreactivity *in vivo*. Although few reports have described the induction of these cells by specific antigen in the periphery, one study has identified ovalbumin-specific $CD4^+CD25^+$ T cells with regulatory activity after feeding tolerogenic doses of antigen to mice[97].

$CD8^+$ suppressor T cells
The first identified population of regulatory T cells thought to be involved in oral tolerance[98]. However, their functions and characteristics have not been clearly defined.

γδ T cells
Several studies in knockout mice indicate that these cells have an important role in some models of oral tolerance. In fact, tolerance can be transferred to normal mice by injection of γδ T cells isolated from fed mice[99].

The lymphocytes that enter the mucosa redistribute into distinct compartments. B-cell blasts mature into IgA-producing plasma cells and remain in the lamina propria. $CD4^+$ T cells also remain in the lamina propria, but are distributed more evenly throughout the villus–crypt unit. $CD8^+$ T cells migrate preferentially to the epithelium, although ~40% of T cells in the lamina propria are also $CD8^+$. The functions of mucosal T cells are still largely uncertain, but cells with a 'memory' phenotype predominate in both the epithelium and the lamina propria, indicating that they have been exposed to antigen. $CD4^+$ T cells in the lamina propria are of particular importance to local immune regulation. They are generally unresponsive to T-cell receptor (TCR)-mediated proliferative signals, but in humans, they can be induced to proliferate when CD2 is used as an accessory molecule. They produce large amounts of cytokines, particularly interferon-γ (IFN-γ), but also IL-4 and IL-10 (REFS 24–26). Lamina-propria $CD8^+$ T cells can also have potent cytotoxic T-lymphocyte (CTL) activity[27]. Some of these antigen-experienced lamina-propria T cells might be true effector cells, and might help local B cells to produce IgA. Alternatively, they might be 'effector memory' cells[28], as indicated by the recent findings that antigen-specific memory $CD4^+$ and $CD8^+$ T cells accumulate preferentially in non-lymphoid tissues, in particular the intestinal mucosa[29,30]. Finally, lamina-propria $CD4^+$ T cells might be regulatory T cells and therefore responsible for maintaining local tolerance to environmental antigens[31]. Many of the properties of the lamina-propria $CD4^+$ T cells are similar to those of 'anergic' regulatory T cells in other systems, including oral-tolerance protocols[32,33]. The unresponsiveness of lamina-propria T cells to commensal bacteria can be reversed by the depletion of IL-10 or TGF-β[31], and a predominance of regulatory T cells in normal lamina propria would be consistent with the location and functions of this poorly understood population.

Fates of orally administered antigen

The immunological consequences of oral administration of antigen ultimately depend on where and how antigen is taken up and presented to T cells. FIGURE 3 outlines the conventional pathways by which it is conventionally assumed that this might occur after uptake of antigen into Peyer's patches through M cells. Although a considerable amount of work supports this scheme, it fails to address several important issues, and alternative routes might be just as important. These are: the transfer of intestinal antigen and/or APCs from the Peyer's patches or mucosal lamina propria through the draining lymph to the MLNs, followed by local presentation to naive T cells; blood-borne dissemination of antigen to peripheral lymphoid tissues; transfer of antigen to the liver through the portal vein; and local presentation of antigen to T cells by enterocytes or professional APCs in the lamina propria.

Antigen transfer to the mesenteric lymph nodes? Priming of T cells in the Peyer's patches and selective homing to mucosal sites would lead to efficient local immune

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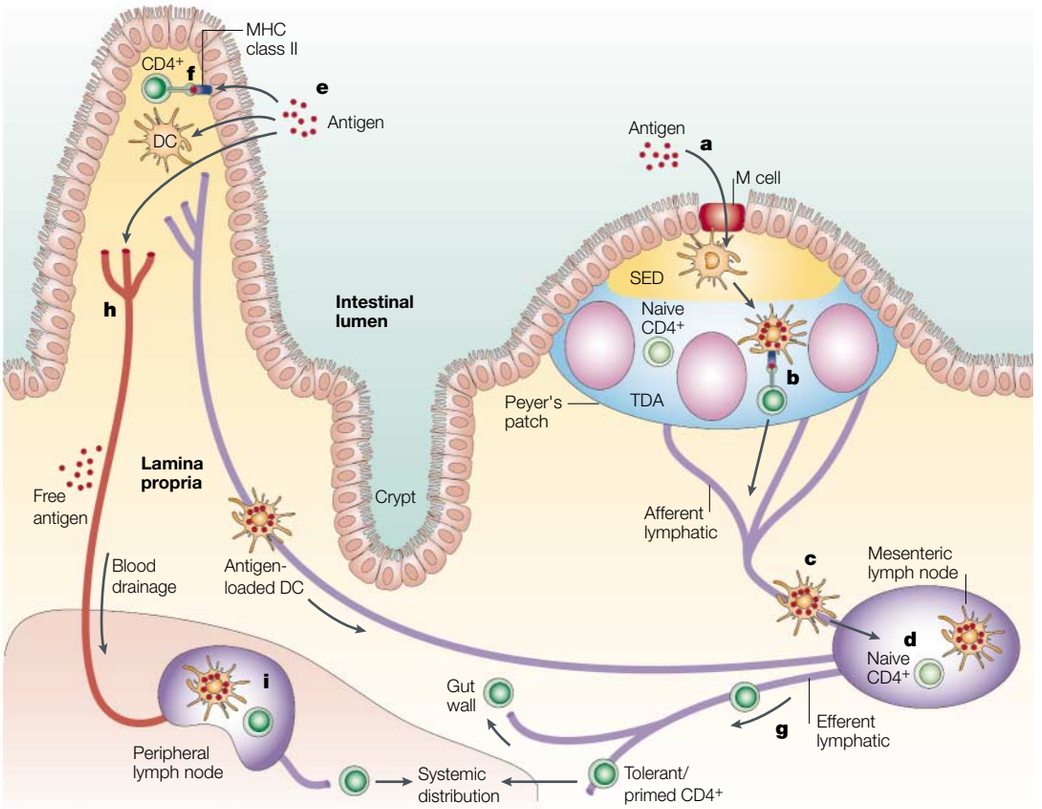

Figure 3 | **Antigen uptake and recognition by CD4+ T cells in the intestine**. Antigen might enter through the microfold (M) cells in the follicle-associated epithelium (FAE) (**a**), and after transfer to local dendritic cells (DCs), might then be presented directly to T cells in the Peyer's patch (**b**). Alternatively, antigen or antigen-loaded DCs from the Peyer's patch might gain access to draining lymph (**c**), with subsequent T-cell recognition in the mesenteric lymph nodes (MLNs) (**d**). A similar process of antigen or antigen-presenting cell (APC) dissemination to MLNs might occur if antigen enters through the epithelium covering the villus lamina propria (**e**), but in this case, there is the further possibility that MHC class II+ enterocytes might act as local APCs (**f**). In all cases, the antigen-responsive CD4+ T cells acquire expression of the $\alpha_4\beta_7$ integrin and the chemokine receptor CCR9, leave the MLN in the efferent lymph (**g**) and after entering the bloodstream through the thoracic duct, exit into the mucosa through vessels in the lamina propria. T cells which have recognized antigen first in the MLN might also disseminate from the bloodstream throughout the peripheral immune system. Antigen might also gain direct access to the bloodstream from the gut (**h**) and interact with T cells in peripheral lymphoid tissues (**i**).

responses or tolerance, but it is more difficult to understand how it could explain the fact that intestinal antigen can also induce systemic priming or tolerance. This paradox could be answered if T-cell priming occurs in the MLNs, either due to the antigen itself reaching the MLNs in the draining lymph (see later), or as a result of APCs that have acquired unprocessed antigen from M cells and then migrated to the MLNs. T cells that are primed in the MLNs would then differentiate and migrate to the mucosa to induce local immune responses. In addition, because the MLNs can act as a crossover point between the peripheral and systemic immune systems, this pathway might also explain the induction of systemic immunity or tolerance in response to intestinal antigens.

Enterocytes in antigen presentation. In recent years, there has been interest in the idea that villus enterocytes can present intestinal antigens to CD4+ T cells. Although the villus epithelium has always been considered to be an impermeable barrier to macromolecules, proteins such as ovalbumin (OVA), can be taken up and processed by enterocytes *in situ*[34]. Intestinal epithelial-cell lines can also process apically absorbed antigen and present it on their basal surface to CD4+ T cells *in vitro*[35]. As enterocytes are MHC class-II-positive in most species, but normally do not express the co-stimulatory molecules that are required for full T-cell activation[36], they are good candidates for tolerogenic APCs *in vivo*. Presentation of antigen by enterocytes to adjacent CD4+ T cells might help to explain local tolerance. However, naive CD4+ T cells are rare in the lamina propria. In addition, lamina-propria T cells do not migrate out of the gut[37] and, therefore, it seems unlikely that this pathway could contribute to systemic tolerance. It remains possible that presentation of antigen to lamina-propria CD4+ T cells by MHC class-II-expressing enterocytes could be involved in maintaining the survival and activity of previously primed regulatory or effector T cells, thereby maintaining local tolerance to environmental antigens or sustaining chronic inflammatory conditions, such as INFLAMMATORY BOWEL DISEASE.

INFLAMMATORY BOWEL DISEASE
A chronic condition of the intestine that is characterized by severe inflammation and mucosal destruction. The commonest forms in humans are ulcerative colitis and Crohn's disease. Animal models indicate that they result from the dysregulation of the local immune response to normally harmless commensal bacteria.

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Table 1 | **The main subsets of DCs in intestinal and other lymphoid tissues**

Lymphoid tissue	Subset percentage		
	$CD11c^+CD11b^+CD8\alpha^-$	$CD11c^+CD11b^-CD8\alpha^+$	$CD11c^+CD11b^-CD8\alpha^-$
Peyer's patches	30–40	30–35	30–35
Lamina propria	50–60	15–20	15–20
MLNs	30–40	30–35	30–40
Spleen	60–75	20–30	<10

DC, dendritic cell; MLNs, mesenteric lymph nodes. Data adapted from REFS 83, 116 and from unpublished observations of A.M.M.

Systemic dissemination of antigen. One finding that might explain the systemic consequences of oral tolerance is that immunologically relevant amounts of fed proteins can spread throughout the mucosal and peripheral immune systems. This material is recognized by antigen-specific T cells[38–42] and might induce functional tolerance[2]. One particularly interesting, but as yet unconfirmed, possibility is that some of this antigen might be incorporated into MHC class-II-positive EXOSOMES that are derived from intestinal epithelial cells[43]. These are lipid vesicles of ~40 nm in diameter, and have been referred to as 'tolerosomes'. They have been detected in the bloodstream of antigen-fed rats and can induce systemic tolerance when transferred into naive recipients. Until the nature and immunological properties of these agents have been substantiated, their role in mucosal immunity must remain contentious. Nevertheless, exosomes are now believed to be important in communicating immunological information from cell to cell in several other situations[44], and the presence of tolerosomes would mean that antigen uptake by enterocytes could have more widespread consequences.

Are Peyer's patches essential?

The conventional dogma, which proposes that the unique features of intestinal immune responses reflect an absolute dependence on Peyer's patches as inductive sites, has been challenged by recent findings. It is particularly controversial whether M cells and Peyer's patches are essential for mucosal immune responses and tolerance to soluble antigens. As M cells are impossible to isolate and there are few, if any, M cell-specific markers, their functions are difficult to study directly. In an attempt to address these issues, several groups have used mice that are deficient in M cells and/or Peyer's patches *in vivo*, but these studies have produced conflicting results.

It has been reported that there is a complete defect in the induction of tolerance after feeding a single high dose of OVA or multiple feeds of TNP–OVA to mice with a selective absence of Peyer's patches created by treating female mice late in gestation with a soluble LTβR–immunoglobulin fusion protein[45]. However, the same workers reported that normal oral tolerance was induced in these Peyer's-patch 'null' mice when the hapten TNBS itself was used as the antigen. Others have also reported that entirely normal oral tolerance to either low or high doses of OVA in these mice[46,47]. In these last studies, oral-tolerance induction was also normal in other mouse models with relatively selective defects in Peyer's-patch development, including TNF-deficient, LTβ-deficient and $LT\alpha^{+/-} \times LT\beta^{+/-}$ heterozygous mice, as well as in mice depleted of TNF and LTα by treatment with specific neutralizing antibodies *in vivo*. Oral tolerance to protein antigens also seems to be normal in B-cell-deficient mice, which have only rudimentary Peyer's patches and lack virtually all M cells[14,48]. Although these experiments can be criticized on the basis that the knockout strategies used can have profound effects on other components of the immune system, including lymph-node development, germinal-centre formation and splenic architecture, an older study reported that surgical removal of Peyer's patches from rats had no effect on oral tolerance[49]. It seems, therefore, that M cells and Peyer's patches might not be necessary for the uptake and processing of antigens in the induction of oral tolerance.

The situation for the induction of active immunity in Peyer's patches is equally contentious. Some decrease in the levels of total IgA in faeces and serum has been reported in mice that have been treated with LTβR–immunoglobulin fusion protein *in utero* and in LTβ-deficient or $LT\alpha^{+/-} \times LT\beta^{+/-}$ heterozygous mice[46,50]. In addition, local and systemic immune responses to intestinally administered soluble OVA or OVA in microspheres are reduced when Peyer's patches are eliminated by treatment with an antibody specific for the IL-7R *in utero*[51]. However, local and systemic specific-antibody responses or $CD4^+$ T-cell responses that are induced by feeding antigen together with the mucosal adjuvant cholera toxin (CT) are relatively intact in mice that have been treated with LTβR–immunoglobulin fusion protein *in utero*[52]. Furthermore, it has been shown that the apparent defects in total IgA production in LT-depleted mice might be related to abnormalities in the mucosa, rather than in the Peyer's patches[50]. As the effects of treatment with antibody specific for the IL-7R might also not be specific to Peyer's patches, I suggest that although Peyer's patches might assist the initiation of active immune responses through the gut, they might not be essential for these to occur.

The MLNs as the crucial checkpoint

In contrast to the rather contradictory evidence on Peyer's patches, there seems little doubt that MLNs have a crucial role in the induction of mucosal immunity and tolerance. All studies using adoptively transferred TCR-transgenic T cells agree that antigen recognition occurs in the MLNs within a few hours of feeding protein antigen[38–40,42,53–58]. In additon, it is impossible to induce oral tolerance in LTα-deficient or (LTα-deficient × TNF-deficient) mice,

EXOSOMES
Small lipid-bilayer vesicles that are released from dendritic cells and other cells. They are comprised of either cell membranes or the membranes of intracellular vesicles. They might contain antigen–MHC complexes and interact with antigen-specific lymphocytes directly, or they might be taken up by further antigen-presenting cells.

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which lack MLNs; conversely MLNs are present in all the models of Peyer's-patch null mice in which tolerance is usually maintained[46,47]. Total and specific IgA-antibody responses are also absent in mice that lack MLNs, despite responses to parenterally administered antigens being preserved in these mice[50,52].

This role for MLNs in the priming or tolerizing of antigen-specific T cells is not surprising, given the known importance of draining lymph nodes in initiating responses to most antigens that come from the tissues. The most contentious issue is how and where the antigen-specific T cells in the MLNs first encounter antigen. The evidence from Peyer's-patch null mice indicates that these T cells might not necessarily migrate to the MLNs after initial activation in the Peyer's patches. It seems more probable that presentation to naive T cells occurs in the MLNs themselves, due to antigen that is brought there by APCs that traffic to the MLNs after being loaded with antigen in the mucosa or Peyer's patches[59]. Recent work indicates that some of the DCs in the intestinal lymph might also contain apoptotic enterocytes, which provides a way in which antigen that has associated with epithelial cells could gain access to naive T cells in the MLNs[60].

It is also possible that free antigen in the afferent lymph that drains from the gut could have a role in priming T cells in the MLNs, especially if high doses of soluble antigen are given. Conversely, lower doses of proteins and other forms of antigen, such as particles or invasive organisms, might be more likely to be taken up only by specialized APCs in the local environment of the gut wall.

A central role for mucosal dendritic cells?

Dendritic cells in the Peyer's patches. One of the most important features of immune regulation in the intestine is the presence of distinctive subsets of DCs in most parts of the GALT (TABLE 1). DCs are abundant in the Peyer's patches and have unusual phenotypic and functional characteristics[61,62]. Several DC subsets have recently been detailed in mouse Peyer's patches by Kelsall and colleagues [63–65]. In addition to the conventional subsets of $CD8\alpha^{-}CD11b^{+}$ ('myeloid') and $CD8\alpha^{+}CD11b^{-}$ ('lymphoid') DCs that are found throughout the immune system in mice, the Peyer's patches also contain large numbers of $CD8\alpha^{-}CD11b^{-}$ DCs. The lineage and roles of this unusual subset are unknown, but both $CD8\alpha^{-}CD11b^{-}$ and $CD8\alpha^{-}CD11b^{+}$ DCs are found outside the organized lymphoid areas, especially in the dome region, which is immediately beneath the FAE. Their presence is dependent on the production of macrophage inflammatory protein 3α (MIP3α), also known as CCL20, by local epithelial cells[64–66]. Each of the DC subsets in Peyer's patches have distinct properties, the most notable being the production of IL-10 by the predominant $CD8\alpha^{-}CD11b^{+}$ DC subset. Interestingly, Peyer's-patch DCs also differ from their peripheral counterparts in their response to activation after ligation of the co-stimulatory molecule RANK (receptor activator of NF-κB), which results in the production of IL-10 by Peyer's-patch DCs, rather than IL-12, which is produced by splenic DCs under the same conditions[67]. In parallel, Peyer's-patch DCs polarize antigen-specific T cells to produce T_H2 cytokines and IL-10 (REF. 65), and treatment of mice with RANK-ligand preferentially enhances oral tolerance[67]. This subset is similar to the relatively immature, IL-10-producing DCs that are found in the normal respiratory tract[68,69] and would be consistent with a population that has a specialized role in the induction of tolerance.

Dendritic cells in the lamina propria. As discussed, Peyer's patches may not be essential for the induction of immune responses in the small intestine and there might be antigen uptake across the epithelium overlying the villus mucosa and into the lamina propria. Although this antigen might not necessarily be presented directly to T cells by enterocytes, the underlying lamina propria contains many DCs, which are ideally situated to pick up any material that is transported between or through the epithelial cells. This route has been largely ignored and lamina-propria DCs are poorly characterized. Nevertheless, our recent work has indicated that these cells might account for a large proportion of the uptake of fed protein antigens in mice (F. Chirdo, O. Millington and A.M.M., unpublished observations). In addition, the lamina propria seems to contain several unusual subsets of DCs, including some that are similar to the IL-10-inducing DCs that have been described in Peyer's patches as well as a population that is equivalent to the subset of potentially tolerogenic PLASMACYTOID DENDRITIC CELLS that have been described recently in mice[70] (TABLE 1). An analogous population of DCs, which has a relatively immature phenotype, has also been described in the normal human colon[71]. If confirmed, these findings would extend earlier observations showing that an unidentified population of APCs can be isolated from the lamina propria of mice that are fed protein antigen and can induce tolerance when transferred to naive recipients[72]. Therefore, lamina-propria DCs might be crucial for the induction of tolerance to intestinal antigens, such as foods.

DCs in the villus mucosa might also be involved in the uptake of other antigens, such as intestinal pathogens, and might have a central role in the subsequent induction of active immunity. Recent *in vitro* studies have shown that DCs can migrate into epithelial monolayers in the presence of pathogenic bacteria and can extend cellular processes into the lumen to internalize the organism, before retreating again beneath the epithelium. A similar phenomenon has been observed *in vivo* when isolated loops of intestine were challenged with live bacteria[73]. These findings indicate that lamina-propria DCs might respond to luminal pathogens by migrating into an intact epithelium to sample the environment, before returning to the lamina propria and initiating protective immunity. As discussed, it is probable that antigen-loaded DCs from the lamina propria interact with naive T cells mainly in the MLNs, rather than in the mucosa itself. There is rapid and constitutive trafficking of DCs from the lamina propria to the MLNs and this process can be increased by the presence of

PLASMACYTOID DENDRITIC CELLS
A subset of dendritic cells (DCs) with a microscopic appearance similar to plasmablasts. In humans, these DCs are the main producers of type I interferon (IFN) in response to virus infections. Recent studies have identified a similar subset of type I IFN-producing DCs in mice, which are characterized by expression of B220 and Ly6C/G, and which might be tolergenic in nature.

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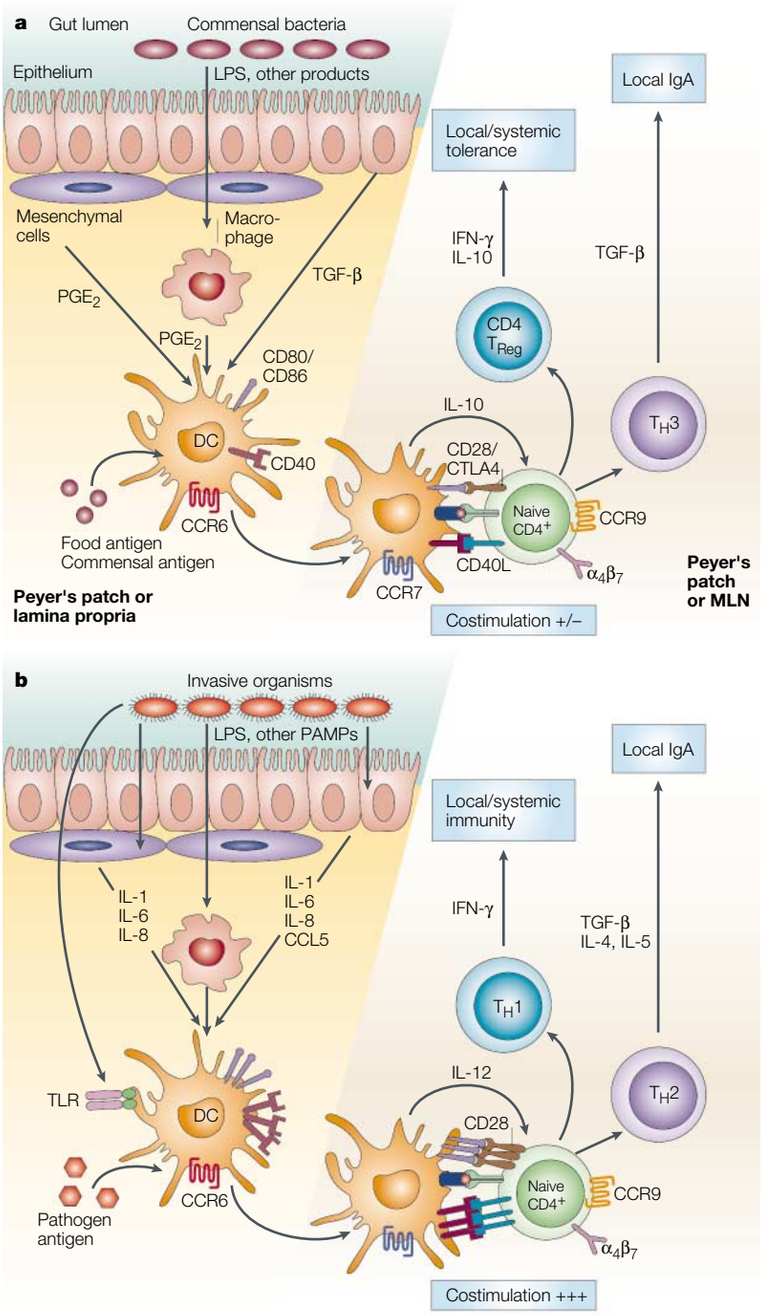

Figure 4 | **Model of the role of the intestinal microenvironment in polarizing immune functions**. **a** | Food proteins and products of commensal bacteria are taken up by dendritic cells (DCs) and in the absence of inflammation, prostaglandin E_2 (PGE_2) (produced constitutively by mesenchymal cells and macrophages), and transforming growth factor-β (TGF-β) and perhaps interleukin-10 (IL-10), which are produced by epithelial cells, result in the partial maturation of DCs in the Peyer's patch or lamina propria. The antigen is then presented to naive $CD4^+$ T cells in the mesenteric lymph node (MLN) or Peyer's patch. These T cells differentiate into regulatory T cells, which produce IL-10 and interferon-γ (IFN-γ), and/or T helper (T_H) 3 cells, which produce TGF-β. The immunological consequences are local IgA production, systemic tolerance and local immune homeostasis. **b** | When pathogens are encountered, local inflammation is induced by the effects of pathogen products mediated through Toll-like receptors (TLRs) that are expressed by mesenchymal cells, macrophages and epithelial cells. As a result, DCs in the Peyer's patch or lamina propria mature completely after taking up antigen and produce IL-12. After migrating to the MLN, these DCs prime gut-homing T_H1 cells, which produce IFN-γ and cause further inflammation. CCR, CC-chemokine receptor; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern.

inflammatory stimuli, such as lipopolysaccharide (LPS)[59]. Together, these findings indicate that lamina-propria DCs might be much more important for the surveillance of the intestinal milieu and the shaping of the intestinal immune responses than previously thought. The properties of these cells under different conditions now need to be explored.

Dendritic cells in the mesenteric lymph nodes. Unusual phenotypic subsets of DCs have also been described in the MLNs[74]. Although it has not been formally proven that these DCs are derived from the intestinal mucosa, recent work has indicated that DCs from the MLNs of antigen-fed mice produce IL-10 or TGF-β and preferentially stimulate antigen-specific $CD4^+$ T cells to produce IL-10 and/or TGF-β[48,69]. This cytokine pattern is similar to that of the T_R1 or T_H3 'regulatory' T cells that have been identified in the GALT of mice fed tolerogenic doses of proteins, which have been implicated in several models of oral tolerance[1,75] (BOX 1). TGF-β-producing T cells probably have an important role in immunoglobulin-class switching to IgA production also. A further unique property of DCs in the MLNs seems to be an ability to induce the expression of $\alpha_4\beta_7$ integrin by naive T cells[76].

A role for the liver? Before considering why intestinal DCs and their functions are so unusual, it is important to mention the possible role of the liver in determining the immunological effects of intestinal antigen, in particular oral tolerance. The liver receives a large amount of intestinally derived antigen through the portal veins, and intestinal pathogens, such as *Salmonella*, can become established readily in the liver. The liver also contains unusual populations of 'regulatory' T cells and DCs[77–79], and injection of antigen directly into the portal vein produces a state of systemic tolerance not unlike that found after feeding antigen[80]. There is also some direct evidence that the liver might be required for the induction of oral tolerance itself[81]. Although an opposite result was obtained in an earlier study[82], the possibility that the immunological microenvironment of the liver has an accessory role in intestinal immunity cannot be disregarded at present.

Features that influence local immune regulation

The intestinal immune system is characterized by a distinct profile of adhesion molecules, cytokines, chemokines and cells. In addition, it has a predisposition to the induction of tolerance and a bias towards productive responses that are dominated by the production of IgA antibodies and, to a lesser extent, T_H2-cell responses. It seems reasonable to assume that these features result, in part, from the unusual nature of the lymphoid tissues that are associated with the intestine. This idea is supported by the fact that MLNs and Peyer's patches have been reported to have a cytokine profile that is dominated by IL-4 and IL-10, as well as having a generally immunosuppressive environment that can affect newly arrived lymphocytes[5,83–85]. Whether these are the local factors that determine the specialized nature of the

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DANGER SIGNALS
Cell-wall components and other products of pathogens that alert the innate immune system to the presence of potentially harmful invaders, usually by interacting with Toll-like receptors and other pattern-recognition receptors expressed by tissue cells and dendritic cells, for example.

intestinal immune response remains to be proven. However, the ability of protein antigens to induce tolerance in the respiratory tract is determined by special properties of the draining cervical lymph nodes, which are not shared by other lymphoid tissues[86], indicating that mucosal lymphoid organs might indeed be unique.

Several features of mucosal tissues might contribute to this process (FIG. 4), including the unique range of factors that control their ontogeny and anatomical patterning, as described earlier. Environmental factors that are derived from the intestinal lumen, such as bacterial products and dietary materials, might then interact with these genetically determined factors to produce a unique immunological niche.

I propose that the mucosal DCs are the cells that integrate these genetic and environmental factors to shape T-cell responses to local antigen in ways such that homeostasis is maintained. In turn, the behaviour of intestinal DCs is controlled by an intimate interplay with other local cell types, including epithelial cells and mesenchymal cells. Intestinal epithelial cells probably provide the first level of regulatory control, both through their role in taking up antigen and through their ability to respond to luminal materials by producing mediators that can influence DCs. Enterocytes produce pro-inflammatory chemokines and cytokines in response to pathogens and their products, and recent work shows that the signals that are responsible for this activation can be modified or inhibited by interactions between epithelial cells and commensal bacteria[87,88]. So, the epithelial barrier might send crucial signals to the underlying immune cells about the nature of the luminal environment.

The features of DCs in the lamina propria, Peyer's patches and MLNs indicate that, under physiological conditions, most antigens are presented by mucosal DCs in a manner that favours the generation of 'regulatory' or IgA-promoting T cells, which have an inherent ability to migrate to the mucosa. It is controversial why intestinal DCs have these unusual characteristics. One possibility is that the DC precursors that migrate to the intestine are a functionally distinct lineage, which are attracted into the mucosa under the control of unique chemokines and adhesion molecules. Given the plasticity of DCs in other tissues, this seems unlikely and it is more reasonable to think that precursor DCs are modified after their arrival in the tissue. TGF-β and IL-10 are candidates for this local modulatory activity, with TGF-β being produced constitutively by many cells in the intestine, including epithelial cells and mesenchymal cells[89]. In addition, recent studies have indicated that mucosal stromal cells constitutively produce cyclo-oxygenase 2 (COX2)-dependent prostaglandin E_2 (PGE_2) under the influence of the physiological levels of LPS that is absorbed from intestinal flora[90,91]. DCs themselves might also express COX2 and produce PGE_2 in response to LPS[92]. As PGE_2 is known to polarize DC differentiation towards an IL-10-producing, inhibitory phenotype[92,93], this would help to explain the prevalence of such DCs in the normal gut.

The idea that the immunological environment of the gut seems to make great efforts to ensure that tolerance is the default response to antigen is often challenged on the basis that this would be a dangerous strategy for host survival in the face of continuous exposure to pathogens. Therefore, it is important to emphasize that this is not a 'hard-wired' functional phenotype and that the local immune system can clearly generate protective immune responses when necessary. Similar to resting DCs that are present in the respiratory tract[68], DCs in Peyer's patches and MLNs can produce IL-12 when activated by the appropriate stimuli[63,65,69]. In addition, activation of local DCs by IL-1 can prevent oral tolerance that is induced by feeding proteins to mice, even when tolerance is favoured by the recruitment of increased numbers of normally tolerogenic DCs, by the use of the cytokine FLT3 (Fms-like tyrosine kinase 3) ligand[94]. So, DANGER SIGNALS that are released by local pathogens can overcome the default status of the intestinal immune system, by enhancing the migration of DCs from the mucosa[95] and allowing the activation of DCs that is necessary to promote productive immunity when required.

Concluding remarks

The result of the interactions between intestinal contents, unique anatomical features, and immune and non-immune cells is an environment that favours the induction of IgA antibodies and regulatory-T-cell-dependent tolerance[31]. This ensures that a homeostatic balance is maintained between the intestinal immune system and its antigen load, retaining the ability to recognize both dangerous and harmless antigens as foreign, and preserving the integrity of the intestinal mucosa. The inappropriate immune responses to foods and commensal bacteria that are responsible for coeliac disease and Crohn's disease are due to dysregulation of these crucial processes. This highlights the fine line that the intestinal immune system must tread to allow pathogen-derived danger signals to overcome the normally downregulatory environment when protective immunity is necessary.

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Acknowledgements

I would like to thank L. Cousins for critical review of the manuscript.

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