INTESTINAL IgA SYNTHESIS: REGULATION OF FRONT-LINE BODY DEFENCES

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Immunoglobulin A is the most abundant immunoglobulin isotype in mucosal secretions. In this review, we summarize recent advances in our understanding of the sites, mechanisms and functions of intestinal IgA synthesis in mice. On the basis of these recent findings, we propose an updated model for the induction and regulation of IgA responses in the gut. In addition, we discuss new insights into the role of IgA in the maintenance of gut homeostasis and into the reciprocal interactions between gut B cells and the bacterial flora.

J CHAIN
A polypeptide produced by immunocytes that is essential for the polymerization of immunoglobulin A and IgM, which is required for binding to the polymeric-immunoglobulin receptor and transport through epithelia.

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In terms of its cellular composition and architecture, its exposure to antigens and its influence on the immune system of the whole body, the mucosal lymphoid tissue of the gastrointestinal tract is one of the most complex tissues of the immune system. Besides other effector functions, this tissue regulates the development of immunoglobulin A immune responses. IgA is the main element of the humoral immune response that has been selected through evolution, together with innate mucosal defences, to provide protection against microbial antigens at mucosal surfaces[1,2].

At least 80% of all plasma cells are located in the intestinal lamina propria, and together, they produce more IgA (in humans, 40–60 mg kg^{-1} day^{-1}) than all other immunoglobulin isotypes combined[3,4]. The IgA is secreted mainly as dimers or larger polymers (pIgA), after incorporation of the J CHAIN and association with a transmembrane epithelial glycoprotein known as the secretory component or polymeric-immunoglobulin receptor (pIgR)[3,5].

The finding that IgA is the most abundant immunoglobulin isotype in mucosal secretions[6,7] led to several important questions regarding the origin of IgA-producing plasma-cell precursors, the sites of antigen-specific induction of IgA production and the migration of the precursors to mucosal sites. These questions have been tackled during the past four decades, giving rise to the concept that Peyer's patches are the main site for the generation of IgA^+ B cells[8]. However, despite the enormous amount of knowledge that has been accumulated over the years, we continue to ask the same basic questions. What is the role of intestinal IgA? Where does IgA class-switching take place? How are the B cells activated, and what interactions regulate IgA class-switching and differentiation? Here, we discuss recent studies in mice that challenge the view that Peyer's patches are the main inductive site for the generation of IgA^+ plasma cells, and we emphasize the importance of cellular interactions outside Peyer's patches, in the gut lamina propria, for the induction of mucosal immune responses. We focus on the cellular and molecular mechanisms of IgA predominance in the intestine, and discuss the role of IgA in the homeostasis of gut-associated lymphoid tissue (GALT).

Precursors and sites for generation of gut IgA

The GALT (FIG. 1), which is the main site of the mucosal immune system, can be divided into two functional compartments, known as inductive and effector sites[9]. The primary inductive sites include the organized lymphoid aggregates that are present in the walls of the small and large intestines. In the small intestine, these inductive sites are represented by Peyer's patches, which consist of many lymphoid follicles located on the antimesenteric side of the bowel. The solitary follicles that are scattered throughout the gut lamina propria —

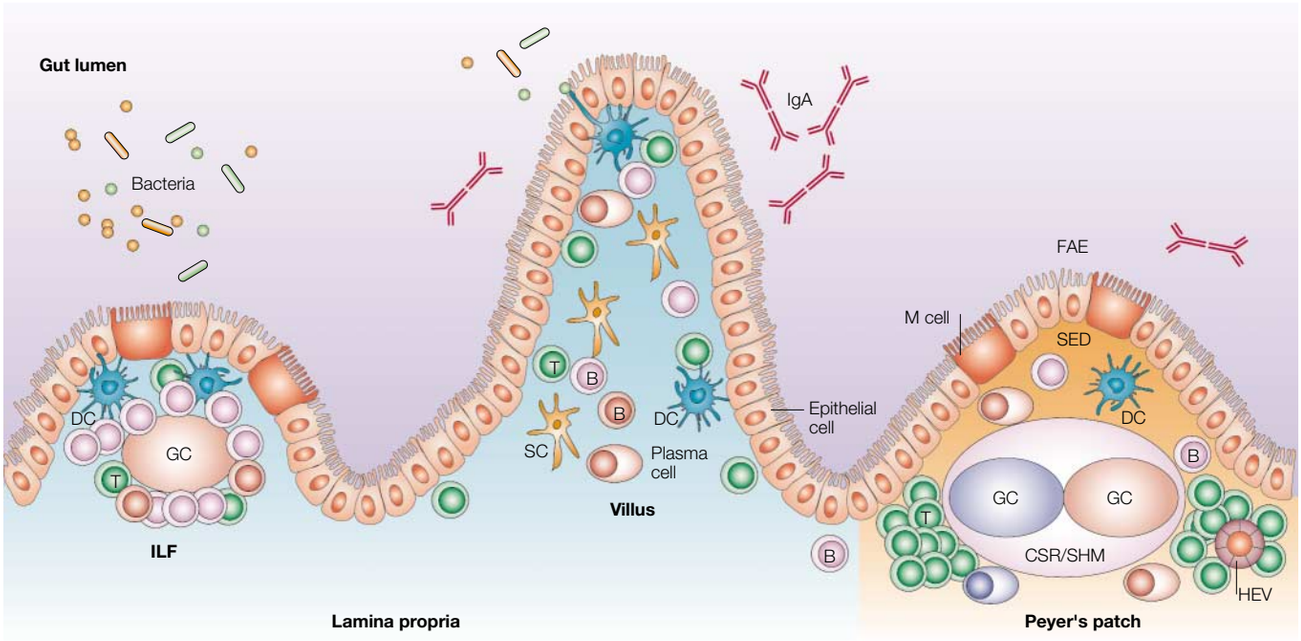


Figure 1 | **Gut-associated lymphoid tissue.** Schematic representation of gut-associated lymphoid tissue (GALT), with organized lymphoid structures — Peyer's patches and isolated lymphoid follicles (ILFs) — and diffuse tissue of the epithelium and the lamina propria. Peyer's patches and ILFs are composed of a specialized follicle-associated epithelium (FAE) containing M cells, a subepithelial dome (SED) rich in dendritic cells (DCs), and B-cell follicle(s) that contain germinal centres (GCs), where follicular B cells efficiently undergo class-switch recombination (CSR) and somatic hypermutation (SHM). Migration of B cells into the mucosa takes place through high endothelial venules (HEVs), located in the interfollicular regions of Peyer's patches, which contain mostly T cells. The diffuse tissues of the lamina propria contain a large number of immunoglobulin A (IgA)$^+$ plasma cells, T and B cells, macrophages, dendritic cells (DCs) and stromal cells (SCs). Lamina-propria DCs take up antigens from the lumen and present them directly to T cells and B cells, which can induce IgA class-switching and differentiation *in situ*. Secreted IgA is transported across the epithelium, where it serves as a first line of defence against pathogens and for the maintenance of gut-flora homeostasis. IgA$^+$ B cells and plasma cells are shown in red, IgG$^+$ cells in blue and IgM$^+$ cells in pink.

known as isolated lymphoid follicles (ILFs) — which are architecturally similar to Peyer's patches, have been assumed for a long time to have similar functions, but this has been shown only recently[10–12]. These structures contain a large number of B2 CELLS, which are derived from precursor cells generated in the bone marrow[11,13]. The intestinal lamina propria outside Peyer's patches and ILFs is considered to be the main GALT effector site that is involved in the final differentiation of IgA$^+$ B cells to IgA-producing plasma cells and in the secretion of IgA into the gut lumen[3].

However, these functional distinctions between inductive and effector sites are not absolute, because certain immune responses can be induced in the mucosal epithelium and in the diffuse tissue of the lamina propria, and some effector functions might occur in the Peyer's patches[9]. For experimental reasons, it is important to point out that the cells in these compartments cannot be separated completely, and the term 'lamina propria' or 'lamina-propria cells' is used generally to refer to cells that have been isolated from the lamina propria outside Peyer's patches.

The idea that intestinal IgA$^+$ plasma-cell precursors reside in the Peyer's patches began with the cellular studies of Craig and Cebra in a rabbit system. They showed that adoptively transferred Peyer's patch cells could repopulate the intestine and spleen of irradiated recipients with IgA$^+$ plasma cells much more efficiently than could cells isolated from lymph nodes[14,15]. These original observations were extended further in a syngeneic cell-transfer system in mice, which showed that Peyer's patch cells can effectively repopulate the gut lamina propria with IgA$^+$ plasma cells, indicating that Peyer's patches are an important site of IgA$^+$ B-cell development[16,17]. IgA$^+$ B-cell development in Peyer's patches seems to depend on antigenic stimulation and the induction of germinal centres[18–20]. The specialized germinal-centre microenvironment — which allows strong interactions between B cells, antigens trapped on follicular dendritic cells and local CD4$^+$ T cells — facilitates B-cell proliferation, CLASS-SWITCH RECOMBINATION (CSR) and SOMATIC HYPERMUTATION (SHM). Indeed, in conventionally reared mice, germinal centres are found continuously in Peyer's patches, and these structures contain a higher proportion of actively dividing IgA$^+$ B cells than germinal centres from the spleen or peripheral lymph nodes[18,21]. This led to the proposal that Peyer's patch germinal centres are intrinsically different to other germinal centres, owing to constant antigenic stimulation, as well as to the presence of special regulatory T cells and dendritic cells (DCs) that promote preferential class-switching to IgA (reviewed in REF. 22).

B2 CELLS
IgM^{low}IgD^{hi}Mac1$^-$B220^{hi}CD23$^+$ cells that are produced continuously in adult bone marrow and secrete antibodies with high affinity and fine specificity.

CLASS-SWITCH RECOMBINATION (CSR). Alters the immunoglobulin heavy-chain constant-region (C_H) gene that will be expressed from the Cμ region to one of the other C_H genes. This results in a switch of immunoglobulin isotype from IgM/IgD to IgG, IgA or IgE, without altering antigen specificity.

SOMATIC HYPERMUTATION (SHM). Results in the accumulation of point mutations in the variable-region genes of immunoglobulin heavy and light chains. B cells that express high-affinity immunoglobulins on their surface are selected by limited amounts of the antigens, giving rise to high-affinity antibodies.

B1 CELLS
Self-renewing $IgM^{hi}IgD^{low}Mac1^{+}B220^{lo}CD23^{-}$ cells that are dominant in the peritoneal and pleural cavities. B1 cells recognize self-components, as well as common bacterial antigens, and they secrete antibodies that tend to have low affinity and broad specificity.

NATURAL IgMS
These antibodies normally circulate in the blood of non-immunized mice. They are highly cross-reactive, and bind with low affinity to both microbial and self-antigens. A large proportion of natural IgMs is derived from peritoneal B1 cells.

The potential role of ILFs in the induction of IgA immune responses was indicated only recently[11]. ILFs are induced to develop only after birth in parallel with the bacterial colonization of the gut, and the number, size and cellular composition of ILFs are highly variable and dependent on the bacteria that are present[11,12]. Such high developmental plasticity of the ILFs, together with observations that germ-free mice have few ILFs that do not contain germinal centres or IgA^{+} B cells, indicates that ILFs might have an active role in the induction of local immune responses[11,23].

Another source of B cells that contribute to intestinal IgA^{+} plasma cells is the peritoneal cavity. Although it was suggested that peritoneal B cells do not contribute to gut immune responses in humans[24], in mice, B1 CELLS in the peritoneal cavity were found to generate large amounts of intestinal IgA[25]. Unlike the B1-cell-derived NATURAL IgMS, which are produced even in germ-free mice, the production of intestinal IgA requires the presence of a commensal microflora, which indicates that the production of intestinal IgA is probably induced in response to antigenic stimulation[26]. Moreover, unlike the IgA responses that are generated in the germinal centres of Peyer's patches, which require the help of T cells, IgA production by B1 cells seems to be T-cell independent. This was shown in lethally irradiated T-cell receptor β-chain and γ-chain knockout mice, reconstituted with allotype-marked peritoneal B1 cells together with host-derived bone-marrow cells; most intestinal IgA^{+} plasma cells were derived from peritoneal B1 cells[26].

Although the relative contribution and repertoire specificity of B1- and B2-cell-derived IgA are not known, the T-cell-independent production of intestinal IgA by B1 cells might be crucial for preventing systemic invasion by intestinal bacteria. This was indicated by the following observations. First, commensal bacteria bind mostly B1-cell-derived intestinal IgA, and less so B2-cell-derived intestinal IgA[27]. Second, normal mice that have intestinal B1-cell-derived IgA specific for commensal bacteria do not have serum IgA or IgG with the same specificities[26]. By contrast, mice with IgA deficiency ($IgA^{-/-}$ *aly/aly* mice) have serum IgG that is specific for commensal bacteria, and this IgG is produced by B2 cells in a T-cell-dependent manner[26].

IgA^{+} B-cell migration to the lamina propria

From Peyer's patches, IgA^{+} B cells migrate to the draining mesenteric lymph nodes (MLNs), where they proliferate further and differentiate into plasmablasts. Plasmablasts home preferentially to the gut lamina propria through the thoracic duct and blood[28,29]. The tissue specificity of IgA^{+} B-cell homing is the result of complex interactions between receptors that are present on the lymphocytes and their ligands expressed on the vascular endothelium of the target tissues. These interactions trigger an ordered sequence of events, which begins with the transient binding of lymphocytes to the endothelium, followed by transmigration across the vascular wall (reviewed in REF. 30). It is clear that $\alpha_4\beta_7$ integrin expressed by lymphocytes and mucosal vascular addressin cell adhesion molecule 1 (MADCAM1) expressed by blood vessels in the lamina propria form the main receptor–ligand pair that is required for the homing of lymphocytes to the lamina propria[31–33].

Although this interaction is important for mucosal lymphocyte homing, as shown by the reduced size of Peyer's patches and the decreased number of IgA^{+} plasma cells in the lamina propria of β_7-integrin-knockout mice[34], it cannot explain the preferential homing of IgA^{+}, but not IgM^{+} or IgG^{+}, plasma cells to the gut lamina propria. This puzzle led to the search for chemotactic factors that are secreted by the intestine that selectively attract the circulating precursors of IgA^{+} plasma cells. Despite progress in understanding the interactions between chemokines and their receptors, and their importance for migration across the endothelium and in lymphoid tissues[35,36], it was only recently that a chemotactic factor specific for mouse IgA^{+} B cells was identified — thymus-expressed chemokine (TECK; also known as CCL25)[37]. Other than the thymus, CCL25 is produced mainly by the epithelium of the small intestine[38,39]. Importantly, *in vitro* studies have shown that IgA^{+}, but not IgM^{+} or IgG^{+}, plasma cells migrate in response to CCL25, owing to selective expression of the CCL25 receptor (CC-chemokine receptor 9, CCR9) on IgA^{+} plasma-cell precursors[37]. So, in mice, CCL25 is probably one of the chemokines that are responsible for the selective migration of circulating IgA^{+} B cells to the intestinal lamina propria.

Furthermore, the presence of B cells and plasma cells in the lamina propria depends crucially on lamina-propria stromal cells, as shown recently by studies of lymphotoxin-α (LTα)-deficient and LTβ receptor (LTβR)-deficient mice[40,41]. The deficiency of IgA^{+} plasma cells in LTα-deficient mice was thought initially to be due to the absence of Peyer's patches and MLNs in these mice (reviewed in REF. 42). However, when reconstituted with bone marrow from normal mice, LTα-deficient mice developed normal levels of IgA in their intestines, indicating that the presence of organized lymphoid structures (Peyer's patches or MLNs) is not necessary and that the lamina-propria environment might be sufficient for the generation of IgA^{+} B cells[40]. Also, the absence of B cells in the lamina propria of LTα-deficient mice was not due to an intrinsic B-cell defect, because bone-marrow cells or peritoneal-cavity B cells of LTα-deficient mice could migrate to the lamina propria and differentiate into IgA-producing cells when injected into normal mice or recombination-activating gene 2 (*Rag2*)$^{-/-}$ mice, which are deficient in T and B cells[40,43]. This is probably because LTβR on lamina-propria stromal cells was activated by LT-expressing lymphoid or non-lymphoid resident cells in normal or *Rag2*$^{-/-}$ mice, respectively. Indeed, a functional LTβR on lamina-propria stromal cells seems to be crucial for the presence of B cells in the gut lamina propria[40,41]. This conclusion is based on several observations. First, LTβR-deficient mice lack B-cell populations in their lamina propria, which is indicated by the low amount or absence of IgA in their intestinal secretions[41]. This phenotype is similar to that of *aly/aly* mice[44], which have impaired signalling through LTβR because of a point

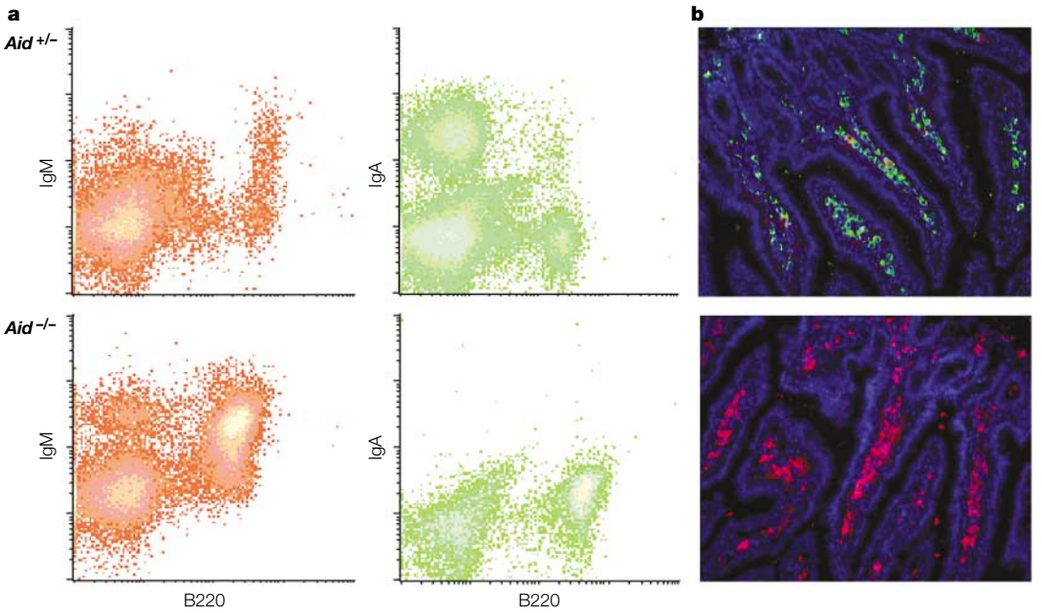


Figure 2 | **Lamina-propria lymphoid cells in normal and AID-deficient mice.** In the presence of activation-induced cytidine deaminase (AID) and in the special microenvironment of the lamina propria, B220$^+$IgM$^+$ B cells undergo class-switch recombination and differentiation to IgA$^+$ plasma cells, as indicated by the presence of B220$^+$IgA$^+$ B cells and B220$^-$IgA$^+$ plasma cells. AID deficiency leads to a massive accumulation of B220$^+$IgM$^+$ B cells and B220$^-$IgM$^+$ plasma cells. **a** | Cells isolated from the lamina propria of *Aid*$^{+/-}$ and *Aid*$^{-/-}$ mice were stained for B220 and IgM or IgA, and analysed by fluorescence-activated cell sorting (FACS). **b** | The photographs show a three-colour histological comparison of wild-type (top) and AID-deficient (bottom) mouse small intestine (IgA, green; IgM, red; nuclei, blue).

mutation in the downstream signalling molecule nuclear factor-κB-inducing kinase (Nik)[45]. Second, when injected into LTβR-deficient mice, bone-marrow cells from normal mice failed to restore the number of B cells and plasma cells in the lamina propria of the recipient mice[40]. Third, when a segment of *Rag2*$^{-/-}$ mouse intestine (with intact LTβR signalling on lamina-propria stromal cells) was transplanted next to the intestine of recipient LTα-deficient mice (in which LT–LTβR interactions were completely absent), IgA$^+$ plasma cells were detected only in the *Rag2*$^{-/-}$ segment, but not in the host intestine[40]. Fourth, the administration of LTβR antagonists after birth led to a marked decrease in the number of lamina-propria B cells and plasma cells[41]. Therefore, signalling through LTβR on lamina-propria stromal cells is absolutely necessary for the presence of IgM$^+$ B cells and IgA$^+$ plasma cells in the small intestine. Although a decrease in the local concentration of adhesion molecules and chemokines might be one explanation for the absence of lamina-propria B cells as a result of impaired LTβR signalling[40,43,46], the molecular mechanisms by which LT–LTβR interactions selectively affect B-cell homing to the gut lamina propria are unresolved.

What factors are required for the migration of B1 cells to the gut lamina propria? Although less so than B2 cells, peritoneal B1 cells do recirculate actively through the bloodstream[47]. Homing of B1 cells to the peritoneal cavity was found to depend mainly on CXCL13 (B-lymphocyte chemoattractant, BLC), a chemokine that is produced by cells in the OMENTUM and by peritoneal macrophages[47]. The migration of B1 cells out of the peritoneal cavity was proposed to depend on Nik, because transfer of peritoneal cells from *aly/aly* mice into the peritoneal cavity of *Rag2*$^{-/-}$ mice failed to generate GALT IgA$^+$ plasma cells[43].

OMENTUM
A bilayered sheet of mesothelial cells connecting the spleen, pancreas, stomach and transverse colon, terminating in an 'apron-like' structure that contains adipocytes.

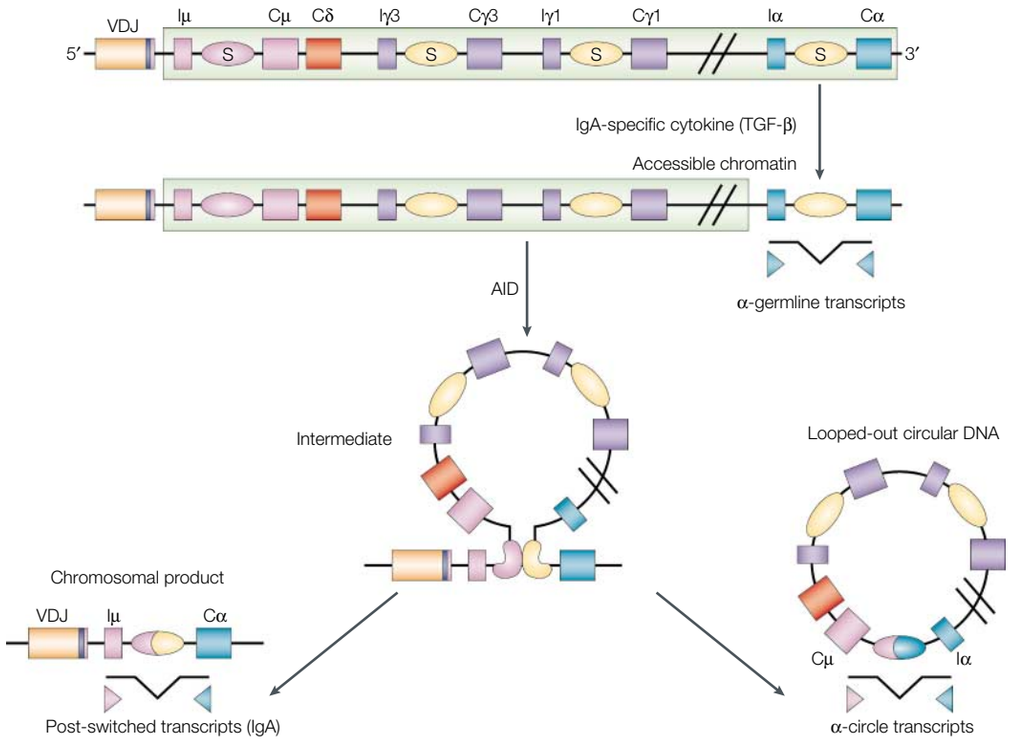
IgA switching in the lamina propria

As mice that lack or have poorly developed Peyer's patches contain a large number of IgA$^+$ plasma cells in their lamina propria, it seems that IgA$^+$ B cells can be generated in lymphoid tissues other than Peyer's patches[48,49]. The notion that Peyer's patches are not the only location for the induction of mucosal IgA responses was strengthened by studies on the progeny of mice treated during gestation with LTβR–immunoglobulin fusion protein, which is known to block the development of Peyer's patches[50,51]. Because these mice, but not tumour-necrosis factor (TNF) and LTα double-knockout mice, which lack Peyer's patches and MLNs, have antigen-specific IgA in their lamina propria, it was proposed that MLNs have an important role in the induction of IgA mucosal immune responses[51]. Although it is clear that this pathway contributes to the generation of IgA$^+$ plasma-cell precursors derived from both B1 and B2 cells[27], it is not absolutely required, as shown by LTα-deficient mice, which lack both Peyer's patches and MLNs but develop normal IgA$^+$ plasma cells in the lamina propria after injection with normal lymphocytes[40].

Direct evidence for class-switching to IgA-producing B cells outside Peyer's patches and MLNs was obtained by studies of activation-induced cytidine deaminase

Box 1 | **Events and markers in class-switch recombination**

One genetic alteration that amplifies the diversity of the immune response is class-switch recombination (CSR). This complex process takes place in activated B cells, and it changes the immunoglobulin heavy-chain constant-region (C_H) gene that will be expressed from the Cμ region to one of the other C_H genes. The result is a 'switch' of the immunoglobulin isotype from IgM/IgD to IgG, IgA or IgE, with similar antigenic specificity but with different biological properties. CSR takes place between two regions comprising repetitive sequences of palindrome-rich motifs, known as S regions, and it results in a looped-out deletion of the intervening DNA segments. CSR is preceded by the expression of germline transcripts initiated from intronic promoters (I), which are located 5′ to the S regions and are regulated specifically by various cytokines. The close association between isotype specificity of germline transcription and the recombination targeting of S regions by stimulation with a certain cytokine has led to the accessibility model — that germline transcription opens the chromatin structure of a specific S region and renders it accessible to the putative recombinase. Activation-induced cytidine deaminase (AID), expression of which is induced specifically in activated B cells, seems to be the only B-cell-specific marker that is essential for CSR[100], and it is involved most probably in recognition and cleavage of the target DNA by the 'switch recombinase'. CSR is accompanied by looping-out deletion of the DNA fragment containing Cμ and other C_H genes from the chromosome, followed by repair and ligation of the broken DNA ends by the ubiquitously expressed non-homologous end-joining (NHEJ) repair system. The resultant circular DNA contains an I promoter that is still responsive to cytokines and that directs the production of I–Cμ transcripts known as circle transcripts. These circle transcripts are dependent on AID, and they disappear more quickly than germline transcripts, AID or circular DNAs after removal of class-switch stimulation, making them the best available marker for active CSR. D, diversity; J, joining; TGF-β, transforming growth factor-β; V, variable.



(AID)-deficient mice[52]. AID, a potential RNA-editing enzyme, is expressed specifically by germinal-centre B cells[53], and AID deficiency in humans and mice causes a complete block of CSR and SHM[52,54]. Interestingly, AID-deficient mice accumulate a large number of IgM^+ B cells and IgM^+ plasma cells in their lamina propria[55] (FIG. 2). In addition, the lamina propria of normal mice contains IgM^+ B cells and a small number of IgA^+ B cells, as well as IgA^+ plasma cells[55,56] (FIG. 2). These two lamina-propria B-cell populations are found either in ILFs or scattered among IgA^+ plasma cells in the villi. The accumulation of IgM^+ B cells and IgM^+ plasma cells in the lamina propria of AID-deficient mice, together with the presence of IgM^+ B cells and IgA^+ B cells in the lamina propria of normal mice, indicates that IgA^+ B cells might be generated in the ILFs, or the lamina propria outside the ILFs, from IgM^+ B cells.

If this is the case, then ongoing CSR should be detectable at these sites. However, until recently, the detection of active, ongoing CSR was not easy, as no molecular marker was known that appears during CSR and disappears rapidly after the class-switching event. This problem has been overcome recently by the finding that the I promoter, located on the circular

M CELLS
(Microfold cells). Specialized epithelial cells that deliver antigens by transepithelial vesicular transport from the gut lumen directly to intraepithelial lymphocytes and to subepithelial lymphoid tissues.

DNA that is released during CSR, is still active and directs the production of I–Cμ transcripts, known as 'circle transcripts'[57] (BOX 1). Kinetic analyses in stimulated spleen B cells or in a B-lymphoma cell line[58] showed that circle transcripts appear shortly before or simultaneously with surface immunoglobulin expression and disappear more rapidly than other markers of CSR, such as the expression of AID, germline transcripts[59] or circular DNA[60–62], within one day of the class-switching event[55,57]. Importantly, IgA$^+$ B cells isolated from the lamina propria express not only α-germline transcripts and AID, but also α-circle transcripts. This molecular profile provides direct evidence that lamina-propria IgA$^+$ B cells are generated *in situ* from IgM$^+$ B cells. Lamina-propria IgM$^+$ B cells seem to be committed to class-switching to IgA, because they express α-germline transcripts and AID. Indeed, when stimulated *in vitro*, lamina-propria IgM$^+$ B cells generate IgA$^+$ B cells, which, under the influence of factors that are produced by lamina-propria stromal cells, differentiate to IgA$^+$ plasma cells. Moreover, both *in vitro* culture experiments and *in vivo* transfer studies of IgA-depleted cells into *Rag2*$^{-/-}$ mice have shown that lamina-propria IgM$^+$ B cells generate IgA$^+$ B cells and plasma cells more quickly and more efficiently than do their Peyer's patch counterparts[55].

Antigen recognition and activation in the gut

How are lamina-propria B cells activated for *in situ* IgA class-switching, and how do the immune cells at this site 'sense' the intestinal microflora?

The model of Peyer's patches as the main, or only, site in the intestine that can, through M CELLS, bind, translocate and present bacterial antigens to B cells (reviewed in REF. 63) has been challenged recently by two

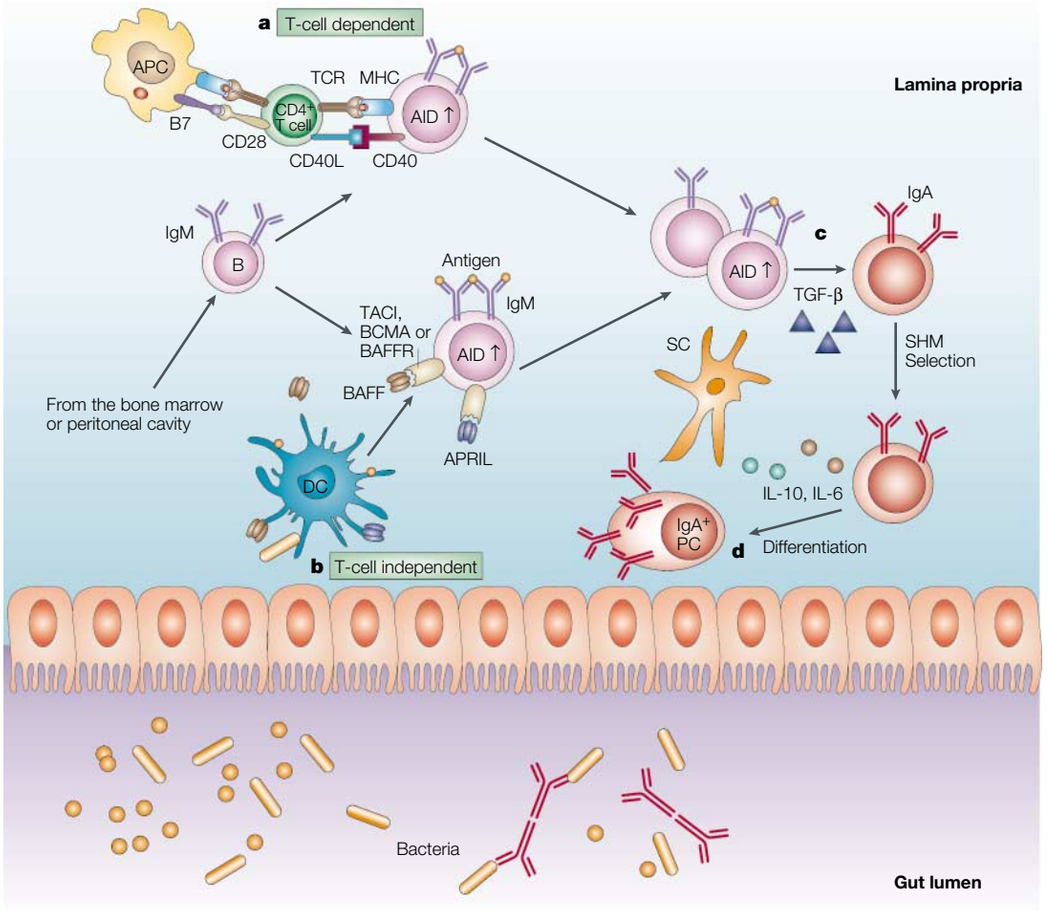


Figure 3 | **Possible pathways for the induction of IgA responses in the gut lamina propria.** From the bone marrow or peritoneal cavity, immunoglobulin M (IgM)$^+$ B cells home to the gut lamina propria, where they are activated by antigens and CD40 ligand (CD40L) expressing T cells (**a**) or, alternatively, by antigens presented by lamina-propria dendritic cells (DCs) or by polyclonal stimuli (**b**). In the presence of cytokines secreted by T cells (**a**), or soluble and membrane-bound BAFF and/or APRIL produced by antigen-presenting cells (APCs) (**b**), activated B cells upregulate expression of activation-induced cytidine deaminase (AID), which is an absolute requirement for class-switch recombination and somatic hypermutation (SHM). The factors that are secreted by lamina-propria stromal cells (SCs), such as interleukin-6 (IL-6), IL-10 and transforming growth factor-β (TGF-β), favour not only preferential class-switching to IgA (**c**), but also the differentiation of antigen-selected cells to IgA$^+$ plasma cells (PCs) (**d**). APRIL, a proliferation-inducing ligand; BAFF, B-cell-activating factor of the tumour-necrosis factor family; BCMA, B-cell maturation antigen; TACI, transmembrane activator and CAML interactor; TCR, T-cell receptor.

COMMON VARIABLE IMMUNODEFICIENCY SYNDROME (CVID). The most common symptomatic primary antibody deficiency, characterized by decreased levels of serum immunoglobulin. Most patients suffer from recurrent infections, predominantly of the respiratory and gastrointestinal tracts. The incidence of malignancies, such as gastric carcinoma or lymphoma, is increased in patients with CVID.

INTUSSUSCEPTION The telescoping or prolapse of one portion of the intestine into an immediately adjacent segment.

findings. First, the presence of M cells is not restricted to the follicular-associated epithelium (FAE) of the Peyer's patches, because the epithelium that covers the ILFs also contains M cells[10,11,64]. Second, DCs located in the gut epithelium[65] or the lamina propria can sample intestinal antigens directly[66].

The role of DCs in surveillance of gut bacteria seems to require that the integrity of the epithelium remains intact. To achieve this, lamina-propria DCs express proteins such as occludin, claudin 1 and zona occludens 1, which are required to open and close the tight junctions between epithelial cells[67], and they project dendrites into the lumen and sample the gut antigens[66]. It has been proposed that lamina-propria DCs that interact with pathogenic bacteria might migrate out of the lamina propria, whereas those that encounter commensals might remain *in situ*, where they can probably present antigens and activate T cells and B cells located in the lamina propria[66]. However, this has not been proven yet.

Lamina-propria T cells have an activated phenotype, as indicated by the high percentage of cells expressing the interleukin-2 receptor (IL-2R) and MHC class II molecules, and by the increased production of cytokines (such as IL-2, IL-4 and IL-5) that are involved in the generation of IgA responses[68,69]. However, even in the absence of T cells, it is possible that local B cells are activated by antigen presentation by lamina-propria DCs or by polyclonal stimulation by microbes captured by DCs (FIG. 3). Activated B cells might then class-switch and differentiate to IgA^+ plasma cells under the influence of factors secreted by lamina-propria stromal cells. In support of this proposal, interactions between lipopolysaccharide (LPS)-stimulated B cells and lamina-propria stromal cells greatly enhanced *in vitro* class-switching to IgA and differentiation to IgA^+ plasma cells, independent of T cells or the CD40 signalling pathway[55]. IgA class-switching, which was induced to an equal extent in lamina-propria, Peyer's patch and spleen B cells, was induced mainly by transforming growth factor-β (TGF-β)[70–73] secreted by lamina-propria stromal cells, whereas stromal-cell-derived IL-6 (REFS 69,74) and IL-10 (REF. 75) might be involved in the final differentiation to plasma cells. Moreover, activated lamina-propria DCs might be sufficient to induce the class-switching of B cells to IgA through a pathway that involves the engagement of BAFF (B-cell-activating factor of the tumour-necrosis factor family) receptors[76]. After stimulation with LPS, interferon-α (IFN-α) and IFN-γ, human DCs were found to upregulate expression of the TNF-family molecules BAFF and a proliferation-inducing ligand (APRIL), which engage the receptors transmembrane activator and CAML interactor (TACI), B-cell maturation antigen (BCMA) and BAFF receptor (BAFFR) on B cells[76]. In a TGF-β-sufficient environment, by as-yet-unknown mechanisms, these interactions can induce class-switching to IgA, by enhancing the accessibility of the IgA locus and by the upregulation of expression of AID[76].

So, lamina-propria B cells that are activated either in a BAFF-independent manner by polyclonal stimulation or after antigen presentation by BAFF-expressing DCs might class-switch preferentially to IgA under the influence of cytokines that are secreted by lamina-propria stromal cells, then undergo terminal differentiation to IgA^+ plasma cells (FIG. 3). Taken together, these observations indicate that the lamina propria (inside or outside ILFs) might be a site where T-cell-independent IgA responses are generated[77].

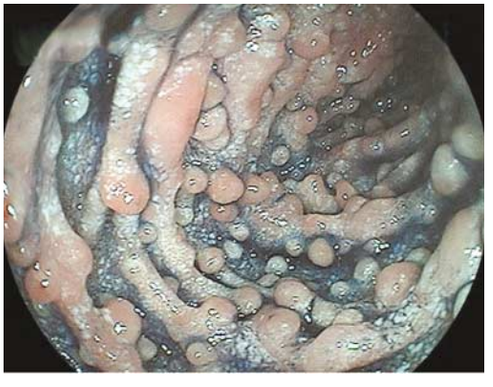
Biological relevance of IgA for gut homeostasis

What has led to the evolution of such a sophisticated system to generate large amounts of IgA in the intestine? In other words, what is the physiological importance of IgA secretion in the gut?

Selective IgA deficiency is the most common humoral immunodeficiency in humans, occurring at a frequency of about 1 in 500–2,000 (REF. 78). Furthermore, some patients with COMMON VARIABLE IMMUNODEFICIENCY SYNDROME (CVID), who have low levels of IgA and IgG, as well as a marked reduction in SHM, suffer from frequent gastrointestinal infections and develop a lymphoproliferative disorder of the small intestine known as

Box 2 | Nodular lymphoid hyperplasia

Nodular lymphoid hyperplasia is a rare lymphoproliferative disorder that is associated with common variable immunodeficiency syndrome (CVID), intestinal lymphoma and Gardner syndrome. But, it can be found also in the small intestine of adults without immunodeficiency. The hyperplastic lymphoid follicles are found most often in the small intestine as numerous small protrusions of 3–6 mm in diameter, and they are morphologically identical to the isolated lymphoid follicles (ILFs) that are present normally in the gastrointestinal tract. The symptoms are caused usually by underlying conditions, such as gastrointestinal infections or malabsorption, and only rarely by large follicles that can cause INTUSSUSCEPTION or bleeding. Although nodular follicular hyperplasia in itself does not require therapy, it is important to differentiate it from other polyposis syndromes with which it is often confused, to define the aetiology and eventually to treat the associated diseases (reviewed in REF. 81). The figure shows an endoscopic image of a duodenal segment with hyperplasia of ILFs in an adult patient (courtesy of K. Suzuki, Department of Gastroenterology, Kyoto University Hospital, Japan).



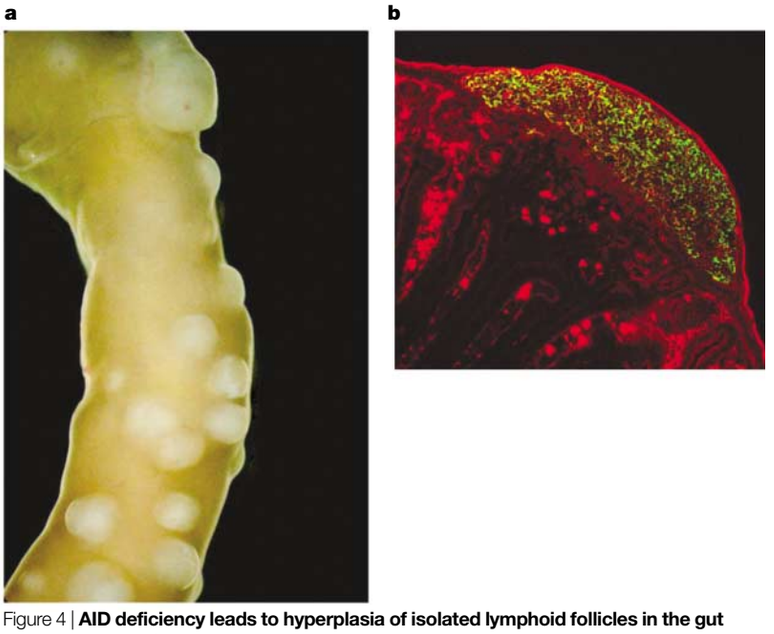


Figure 4 | **AID deficiency leads to hyperplasia of isolated lymphoid follicles in the gut lamina propria. a** | A duodenal segment of the small intestine from an AID-deficient mouse showing many protruding follicles. **b** | These follicles consist of IgM$^+$ B cells on a follicular dendritic cell (FDC) network (IgM, red; FDC, green).

nodular follicular hyperplasia[79–81] (BOX 2). The mechanisms for the development of follicular hyperplasia in humans remain unknown, although it has been proposed to occur as a result of local immune responses to gut antigens[79–81].

Although the importance of IgA-mediated protection at mucosal surfaces is not questioned[82], the role of intestinal IgA in regulation of the gut microflora and how dysregulation of the gut bacteria might affect the GALT system leading to pathological manifestations are controversial issues. This is due partly to the heterogeneity of the clinical manifestations in IgA-deficient patients and the existence of compensatory mechanisms — such as overproduction of IgM or IgG — that make it difficult to assess the biological role of IgA. This is also a problem in animal models that have been generated to investigate the physiological role of IgA — such as IgA-, J-chain- or pIgR-deficient mice. Under normal conditions, these mice seem to be healthy, although they have a greater number of activated B cells in Peyer's patches (IgA-deficient mice)[83], impaired intestinal anti-toxin protection (J-chain-deficient mice)[84] or elevated levels of serum IgG that reacts with intestinal bacteria (pIgR-deficient mice)[85] compared with wild-type mice.

An animal model that seems to recapitulate the pathology of CVID in humans is AID deficiency in mice. AID-deficient mice develop many protruding follicular structures along the small intestine, which indicate hypertrophia of ILFs[12] (FIG. 4). This resembles the nodular follicular hyperplasia that is seen in humans with CVID (BOX 2). Furthermore, AID-deficient mice have a large increase in the number of non-pathogenic, but anaerobic, bacteria in all segments of the small intestine[12]. As appropriate antibiotic treatment of the anaerobic bacteria abolished not only the ILF hyperplasia, but also the induction of germinal-centre formation in all lymphoid tissues, continuous antigenic stimulation by an excessive population of intestinal anaerobic bacteria is probably responsible for both local and systemic B-cell activation, as well as ILF hyperplasia and germinal-centre formation, in AID-deficient mice[12,52]. So, it seems that the IgA that is secreted into the gut lumen functions not only to protect against viral and bacterial pathogens, but also for homeostasis of the gut flora, which is essential to prevent over-stimulation of the non-mucosal immune system.

Hypermutation and antigenic selection

Besides CSR, another AID-dependent process that occurs mostly in germinal centres and induces further diversification of the immunoglobulin repertoire of activated B cells is SHM[86–90]. Multiple rounds of mutation of variable (V), diversity (D) and joining (J) exons, followed by the selection of B cells with enhanced binding to antigens, result in so-called 'affinity maturation' of the humoral response. SHM is likely to be more frequent at mucosal sites[87], where constant antigenic pressure from a diverse bacterial flora[91] requires a vast repertoire of antibody specificities.

Indeed, IgA$^+$ and IgM$^+$ plasma cells isolated from human small intestine[92–94] or mouse small intestine[95] (S.F. and T.H., unpublished observations) are heavily mutated in their heavy-chain variable-region (V_H) genes, and the frequency of mutation is more than two-fold greater than that of splenic IgA$^+$ or IgM$^+$ plasma cells. SHM of intestinal B cells and plasma cells might be important for homeostasis of the mucosal immune system, because IgA-deficient mice, which have mutated IgMs in their intestinal secretions, do not have the ILF hyperplasia that is observed in AID-deficient mice (D. Metzger, personal communication).

That antigenic pressure leads to selection of gut B cells was indicated by repertoire studies of non-mutated B cells isolated from the ILFs of AID-deficient mice[12]. Not only was the repertoire of ILF B cells more diverse than that of B cells from Peyer's patches or spleen, but also individual ILFs contained different dominant V_H gene and V_H–D–J_H combinatorial diversity, indicating that selection and clonal expansion of B cells probably take place *in situ*, depending on the prevailing antigenic diversity of local bacteria[12].

A model for the development of IgA responses

Taken together, the available evidence indicates that the simplest model of IgA$^+$ plasma-cell generation in the gut lamina propria would be: recruitment of IgM$^+$ B cells; followed by activation and proliferation of IgM$^+$ B cells, in either a T-cell-dependent or -independent manner, through interactions with lamina-propria DCs and lamina-propria stromal cells; followed by class-switching to IgA, then SHM and selection of IgA$^+$ B cells by antigens captured by DCs; and finally, differentiation to IgA$^+$ plasma cells (FIG. 3).

This model would help to explain an apparently unexpected finding — that µMT$^{-/-}$ MICE, which have

µMT$^{-/-}$ MICE
These mice carry a stop codon in the first membrane exon of the µ-chain constant region. They lack IgM$^+$ B cells, and B-cell development is arrested at the pre-B-cell stage.

a developmental block at the pro-B-cell stage[96], do have intestinal IgA[95]. This IgA seems to be induced in response to the intestinal flora, or after experimental intestinal bacterial infection, even in the absence of surface IgM and T cells. The IgA repertoire in these mice was found to be diverse, with point mutations in the complementarity-determining regions, which strongly indicates the involvement of antigenic selection[95]. One additional assumption required to explain the above observation is that the BCR-null B cells of $\mu MT^{-/-}$ mice might be recruited to the lamina propria and rescued from apoptosis in a T-cell-independent manner[76,97]. If so, they might proliferate and class-switch to IgA in response to soluble factors and cellular interactions in the lamina propria. Once surface IgA is expressed, antigenic stimulation might induce SHM, followed by positive selection and further differentiation to IgA^+ plasma cells.

Concluding remarks

Although recent findings have indicated new complexities of the regulation and biological importance of IgA^+ B-cell differentiation in the gut, more studies are required to understand the role of non-follicularly organized lymphoid elements in the gut lamina propria in the induction of mucosal immune responses. Further analyses of the interactions between B cells, T cells, DCs, macrophages and stromal cells after antigen presentation in the gut lamina propria are required to solve outstanding questions regarding the molecular mechanisms that are responsible for the induction of immunity or tolerance in the intestinal mucosa.

Furthermore, a more comprehensive understanding of the relationships between commensal bacteria[98,99] and the innate and adaptive immune systems should offer new approaches for the therapy of gut inflammatory pathology and for the design of oral vaccinations.

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Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
$\alpha_4\beta_7$ | AID | APRIL | BAFF | BAFFR | BCMA | CCL25 | CCR9 | CD40 | claudin 1 | CXCL13 | IFN-α | IFN-γ | IL-2 | IL-2R | IL-4 | IL-5 | IL-6 | IL-10 | LTα | LTβR | MADCAM1 | Nik | occludin | pIgR | *Rag2* | TACI | TGF-β | TNF | zona occludens 1

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
CVID | Gardner syndrome

FURTHER INFORMATION

Tasuku Honjo's lab: http://www.kyoto-u.ac.jp/kokuryu/kyotouniv/cur03.htm

Access to this interactive links box is free online.