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The regulation of IgA class switching

Andrea Cerutti
Department of Pathology and Laboratory Medicine, Weill Medical College of Cornell University, and Weill Graduate School of Medical Sciences of Cornell University, 1300 York Avenue, New York, New York 10021, USA

Andrea Cerutti: acerutti@med.cornell.edu

Abstract

IgA class switching is the process whereby B cells acquire the expression of IgA, the most abundant antibody isotype in mucosal secretions. IgA class switching occurs via both T-cell-dependent and T-cell-independent pathways, and the antibody targets both pathogenic and commensal microorganisms. This Review describes recent advances indicating that innate immune recognition of microbial signatures at the epithelial-cell barrier is central to the selective induction of mucosal IgA class switching. In addition, the mechanisms of IgA class switching at follicular and extrafollicular sites within the mucosal environment are summarized. A better understanding of these mechanisms may help in the development of more effective mucosal vaccines.

IgA has been selected throughout evolution to provide a first line of immune protection at mucosal surfaces — vulnerable frontline sites that are exposed to potentially harmful commensal, airborne, ingested and sexually transmitted agents. Growing evidence indicates that IgA uses a high-affinity binding system to neutralize microbial toxins and pathogens, and a low-affinity binding system to prevent commensal bacteria from breaching the mucosal surface[1]. This latter process is known as immune exclusion and has a fundamental role in the intestine, which is home to a number of commensal bacteria exceeding that of human cells by an estimated order of magnitude[2].

Remarkably, intestinal IgA achieves both immune protection and immune exclusion in a non-inflammatory manner, thereby promoting the establishment of a sustainable host–microbial mutualism[3]. The complex relationship between IgA and the intestinal microbiota is further exemplified by the fact that IgA responses are highly dependent on intestinal colonization by commensal microorganisms. Indeed, the number of IgA-secreting B cells is dramatically reduced in the intestine of germ-free animals and these cells are virtually absent in neonates before their exposure to bacteria[3].

In this Review, I summarize recent advances in our understanding of the function, regulation and geography of IgA class switching. In addition to analysing the signalling pathways underlying IgA class switching, I discuss new evidence indicating that commensal bacteria

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The author declares no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
AID|APRIL|BAFF|BAFFR|BCMA|CD40L|TACI|TGFβ1|TSLP

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regulate intestinal IgA responses by promoting the crosstalk between B cells and multiple components of the mucosal innate immune system, including epithelial cells and dendritic cells (DCs).

Function of IgA class switching

Antibody diversification is essential for the immune system to mount protective humoral responses. B cells diversify their antibody repertoire through three main genetic alterations that occur in two distinct phases of B-cell development. In the antigen-independent phase, B-cell precursors lodged in the bone marrow generate antigen recognition diversity by assembling the exons that encode immunoglobulin heavy (H) and light (L) chain variable regions from individual variable (V), diversity (D) and joining (J) gene segments through V(D)J gene recombination[4]. This process is initiated by a lymphoid-cell- and sequence-specific RAG1 (recombination- activating gene 1)–RAG2 endonuclease complex and is completed by the non-homologous end-joining machinery[4]. Productive assembly of V_HDJ_H and V_LJ_L exons allows the expression of IgH and IgL chains as cell-surface IgM by newly generated B cells[4]. After further differentiation and expression of IgD, B cells emerging from the bone marrow migrate to secondary lymphoid organs, where they initiate the antigen-dependent phase of B-cell development.

In the presence of antigen, mature B cells diversify their antibody repertoire through somatic hypermutation (SHM) and class switching[5,6]. These processes take place in the germinal centres of secondary lymphoid follicles[7] and require the DNA-editing enzyme activation-induced cytidine deaminase (AID)[8]. The process of SHM introduces point mutations at high rates into V_HDJ_H and V_LJ_L exons, thereby providing the structural correlate for selection by antigen of high-affinity immunoglobulin variants[5]. Class switching substitutes the IgH constant region μ (C_μ) and C_δ genes encoding primary IgM and IgD isotypes with C_γ, C_α or C_ε genes through a process known as class-switch recombination (CSR)[9]. This molecular event generates secondary IgG, IgA and IgE isotypes that have the same antigen specificity as IgM and IgD, but different effector functions[10]. Indeed, secondary isotypes can activate multiple innate immune effector cells, including phagocytes, by binding to specific Fc receptors[10]. Together with post-IgA CSR modifications, IgA CSR generates multiple forms of membrane-bound IgA and of secreted IgA (sIgA), each characterized by a distinct location in the body and by distinct functions. Remarkably, some of these forms of IgA substantially differ in humans and mice (TABLE 1). Unlike mouse IgA, which comprises only one class, human IgA comprises two subclasses, IgA1 and IgA2, the latter being more abundant in the intestinal and genitourinary tracts. In addition, circulating IgA is predominantly monomeric in humans, but largely dimeric and oligomeric in mice.

Mucosal IgA

Mucosal secretions contain IgA dimers and oligomers in both mice and humans[1]. These IgA polymers originate from the interaction of IgA monomers with the J chain, a polypeptide synthesized by antibody-secreting cells[11]. In addition to assembling monomeric IgA, the J chain interacts with the polymeric immunoglobulin receptor (pIgR), an antibody-transporting protein expressed on the basolateral surface of mucosal epithelial cells[12]. The pIgR shuttles IgA across epithelial cells through a transcytotic process that culminates in the translocation of sIgA complexes to the mucosal surface[1]. These complexes comprise a secretory component that originates from the endocytic cleavage of pIgR and that confers mucophilic properties to sIgA[13,14]. Remarkably, sIgA neutralizes toxins and pathogens without causing inflammation because of its inability to fix and activate the complement cascade[1]. In addition, sIgA anchors commensal bacteria to the mucus, thereby impeding their entry to the underlying intestinal mucosa[1]. Furthermore, sIgA promotes the establishment of a mutualistic host–microbe relationship by down-modulating the

expression of pro-inflammatory epitopes by commensal bacteria[15]. Moreover, sIgA neutralizes microbial compounds with pro-inflammatory activity, such as lipopolysaccharide (LPS)[16], and facilitates the formation of a biofilm that favours the growth of commensals while attenuating that of pathogens[17]. This property might depend on the ability of sIgA to agglutinate intestinal bacteria through carbohydrates associated with the Fcα region[1]. The secretory component would further increase the contributing capacity of sIgA to biofilm formation by anchoring intestinal bacteria to the mucus layer lining the epithelial-cell surface[14]. Finally, sIgA enhances mucosal immunity by delivering bacterial cargo to M cells (microfold or membrane cells)[18], a specialized epithelial-cell type that is found adjacent to intestinal Peyer's patches[19].

Systemic IgA

Circulating IgA is largely present as a monomer in humans, although circulating IgA polymers are also present[1]. By contrast, circulating IgA is largely polymeric in mice[1]. Systemic IgA binds to various receptors expressed by granulocytes, monocytes, macrophages, DCs, eosinophils, follicular DCs, hepatocytes, hepatic Kupffer cells and renal mesangial cells, including the myeloid-cell-specific type I Fc receptor for IgA (FcαRI; also known as CD89), the Fcα/Fcμ receptor, the asialoglycoprotein receptor and the transferrin receptor[20]. The effector functions of these receptors remain poorly understood, although growing evidence indicates that FcαRI provides a second line of defence against intestinal bacteria that invade the portal venous system[21]. In particular, FcαRI might facilitate the internalization of IgA-opsonized bacteria by hepatic Kupffer cells and other phagocytic cells in a non-inflammatory context[19,22]. Indeed, FcαRI engagement by IgA triggers the recruitment of SHP1 (SRC-homology-2-domain-containing protein tyrosine phosphatase 1), a crucial negative regulator of multiple pro-inflammatory receptors[20]. Finally, it must be noted that, as mice do not express FcαRI, most of the data documenting the *in vivo* function of FcαRI were derived from mice expressing a human *FCAR* transgene (TABLE 1).

Human IgA subclasses

The human IgA1 and IgA2 subclasses are encoded by two distinct $C_{\alpha}1$ and $C_{\alpha}2$ genes and possess a seemingly identical receptor-binding profile, but a different distribution in the body[1,10]. Indeed, the circulating IgA pool is comprised mostly of IgA1, whereas the mucosal IgA pool contains both IgA1 and IgA2 (REF. [23]). IgA2 is particularly abundant at sites colonized by a large microbiota, including the distal intestinal tract and the urogenital tract[24–26]. Another difference between IgA1 and IgA2 relates to the fact that IgA1 has a longer hinge region than IgA2 (REF. [27]). This feature renders IgA1 more susceptible to degradation by bacterial proteases that target the hinge region of IgA[23,28]. Furthermore, compared to IgA1 antibodies, IgA2 antibodies seem to have superior Fcα-mediated, mannose-dependent agglutinating properties against enteric microorganisms and exhibit more V_H-mediated reactivity against LPS, a key component of Gram-negative bacteria residing in the distal gut[23,25].

Mechanism of IgA class switching

Mature B cells acquire IgA expression by undergoing CSR of C_{μ} to C_{α} (FIG. 1). CSR involves an exchange of upstream donor C_{μ} and C_{δ} genes with a downstream acceptor C_H gene through a recombinatorial process that is guided by switch (S) regions[10]. S regions are located upstream of each C_H gene, except C_{δ}, and consist of highly repetitive 1–12 kilobase sequences with G-rich non-template strands[9]. Each S region is preceded by a short intronic (I) exon and a promoter that initiates germline C_H gene transcription when the B cell is exposed to activating stimuli[9]. Germline transcription is crucial for CSR as it renders the S region a substrate for AID, an inducible APOBEC (apolipoprotein B mRNA-editing

enzyme, catalytic component 1) family member encoded by *AICDA*[9]. AID is essential for CSR, as *Aicda*-knockout mice or patients with *AICDA* mutations develop hyper-IgM type 2 syndrome (HIGM2) and fail to generate class-switched antibodies, including IgA[29–31].

Germline C_α gene transcription yields a primary I_α–S_α–C_α transcript that is later spliced to form a non-coding germline I_α–C_α transcript[10]. The primary transcript physically associates with the template strand of the DNA to form a stable DNA–RNA hybrid[32]. This structure generates R loops in which the displaced non-template strand exists as a G-rich single-stranded DNA[33]. AID deaminates cytosine residues on both strands of the S-region DNA, thereby generating multiple DNA lesions that are ultimately processed into double-stranded DNA breaks[6,9]. Fusion of double-stranded DNA breaks at S_α and S_μ through the non-homologous end-joining pathway induces looping-out deletion of the intervening DNA, thereby juxtaposing V_HDJ_H to C_α (REFS [6,9]). This process yields a chromosomal V_HDJ_H–C_α sequence, which encodes the IgA protein, and an extrachromosomal switch circle, which encodes a chimeric I_α–C_μ switch circle transcript[34]. Together with AID transcripts and switch circles, switch circle I_α–C_μ transcripts have a short half-life and therefore their detection indicates ongoing CSR[34,35].

T-cell-dependent IgA class switching

Most antigens, including microbial proteins, initiate protective humoral responses in germinal centres, which are specialized follicular environments that foster B-cell proliferation, AID expression and antibody gene diversification through CSR and SHM[7,8]. In general, germinal-centre reactions are highly dependent on cognate interactions between antigen-specific B cells and $CD4^+$ T cells that express CD40 ligand (CD40L; also known as CD154), a tumour-necrosis factor (TNF) family member that engages CD40 on B cells[36]. Antigen exposed on the surface of follicular DCs selects germinal-centre B cells expressing a high-affinity B-cell receptor (BCR), selected B cells thereafter differentiate into long-lived memory B cells and antibody-secreting plasma cells[37]. In the gut, T-cell-dependent antibody responses are strongly biased towards IgA and involve activation of B cells by antigen in the organized lymphoid tissue of Peyer's patches, mesenteric lymph nodes and isolated lymphoid follicles[1,2].

In addition to inducing AID, B-cell-activating signals induce germline C_H gene transcription[9]. This process confers specificity to CSR[38], because the promoter upstream of each C_H gene responds only to a specific set of signal-induced transcription factors[10]. Promoters upstream of C_α genes, or I_α promoters, become activated in response to transforming growth factor-β1 (TGFβ1)[39–41], a cytokine that is secreted by many cell types, including various subsets of $CD4^+$ T cells[42,43]. Together with CD40L, TGFβ1 is essential for the induction of T-cell-dependent IgA class switching[10].

Role of TGFβ1 in germline C_α gene transcription

TGFβ1 is a pleiotropic cytokine that belongs to the TGFβ superfamily[42]. TGFβ1 is derived from the proteolytic cleavage of a pro-region, known as LAP (latency associated peptide), which undergoes dimerization and thereby forms an active molecule of 25 kDa[42]. Active TGFβ1 engages a heterotetrameric TGFβ receptor (TGFβR) complex composed of two type I and two type II transmembrane proteins that have serine/threonine kinase activity[42]. Signals emanating from the TGFβR induce both activating and inhibitory effects in a broad range of target cells. In B cells, low concentrations of TGFβ1 initiate C_α gene transcription (FIG. 2), whereas high concentrations suppress B-cell proliferation and differentiation, including antibody secretion[44]. This dual role is important for the homeostasis of the immune system. Indeed, mice with defective TGFβ1 signalling develop inflammatory and autoimmune disorders[42].

TGFβR signals through mothers against decapentaplegic homologue 2 (SMAD2), SMAD3 and SMAD4 proteins, which form homo- and hetero-oligomeric complexes that bind to SMAD-binding elements (SBEs) on the promoters of target genes[42,45–47]. This signalling pathway is negatively regulated by SMAD7 (REF. [42]). The activation of I_α promoters requires the cooperation of SMAD proteins with runt-related transcription factor 3 (RUNX3; also known as CBFα3)[45,47,48], a TGFβ1-inducible member of the RUNX family of proteins with a DNA-binding runt domain[49]. SMAD proteins and RUNX3 bind to a direct repeat unit on I_α promoters that is known as the TGFβ1 responsive element (TGFβRE)[50]. This conserved *cis*-regulatory DNA region contains two tandemly arrayed RUNX-binding elements (RBEs) that are adjacent to SBEs[45,47,48]. The proximity of SBEs and RBEs provides a structural correlate for the physical interaction of SMAD proteins with RUNX3, which in turn is central to the activation of I_α promoters by TGFβ1 (REFS [45,47,48]). This cytokine is important not only for the initiation of C_α gene transcription *in vitro*, but also for the induction of IgA class switching *in vivo*. Indeed, B-cell-conditional TGFβRII-deficient mice, SMAD2-deficient and SMAD3-deficient mice produce less IgA under steady-state and immunizing conditions in both systemic and mucosal sites, including the Peyer's patches[51–53]. Conversely, B cells from mice lacking SMAD7 show increased IgA CSR in response to TGFβ1 (REF. [54]).

In addition to SBEs and RBEs, both mouse and human I_α promoters contain a cyclic AMP response element (CRE) associated with the TGFβRE[50]. This CRE site binds CRE binding protein (CREB), a TGFβ1-inducible factor that cooperates with SMAD and RUNX3 proteins[46]. Downstream of the CRE site, the mouse I_α promoter contains an Ets site, which binds ELF1 (Ets-like factor 1) and PU.1 (REF. [55]). These Ets family members cooperate with SMAD and CREB proteins to activate I_α (REF. [55]). Finally, near its main transcription initiation site, the mouse I_α promoter has a second CRE site and a site for PAX5 (paired box protein 5; also known as BSAP), which represses C_α gene transcription under basal conditions[56].

Role of CD40L in CSR to C_α

CD40L is an essential requirement for T-cell-dependent class switching, including IgA class switching (FIG. 2), and CD40L cooperates with TGFβ1 to induce IgA CSR *in vitro*[57–61]. Furthermore, CD40L- and CD40-deficient mice exhibit impaired systemic and intestinal IgA responses to T-cell-dependent stimuli *in vivo*[62,63]. A significant systemic IgA deficiency can also be observed in humans affected with CD40L or CD40 signalling defects due to HIGM1 and HIGM3 syndromes, respectively[64,65].

CD40L expressed on the surface of antigen-activated $CD4^+$ T cells activates B cells by engaging the CD40 receptor[36]. By recruiting TNF-receptor-associated factor (TRAF) adaptor proteins[36], CD40 forms a signalling platform that activates the IκB kinase (IKK) enzymatic complex[66]. This IKK complex mediates phosphorylation of inhibitor of NF-κB (IκB) proteins, which retain nuclear factor-κB (NF-κB) in a cytoplasmic inactive form[66]. Phosphorylation of IκBα by the IKK complex is followed by ubiquitylation and proteasome-dependent degradation of IκBα, which allows NF-κB to translocate into the nucleus[66]. Here, NF-κB binds to *cis*-regulatory κB sites, thereby determining the activation of promoters located upstream of key B-cell genes, including I_γ and I_ε (REF. [10]).

In addition to triggering IgG and IgE class switching[10], NF-κB has an important role in IgA class switching, as this process is impaired in B cells from NF-κB-deficient mice[67]. Yet, I_α promoters contain only one κB site located downstream of the Ets site[55]. This κB site neither induces nor enhances the activation of I_α promoters[55], suggesting that NF-κB regulates IgA CSR at a level other than germline C_α gene transcription. Most likely, NF-κB mediates the

induction of AID expression[68], an essential requirement for IgA CSR, in addition to germline C_α gene transcription[9].

Remarkably, CD40L can induce IgA class switching in combination with cytokines other than TGFβ1, including interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-10 and VIP (vasoactive intestinal peptide)[58,60,61,67,69,70]. These cytokines may enhance the production of endogenous TGFβ1 by B cells exposed to CD40L, thereby triggering IgA CSR through an autocrine TGFβ1-dependent loop[61]. In addition, they may augment the proliferation and plasma-cell differentiation of the B cells that have switched to IgA in response to CD40L and autocrine TGFβ1 (REF. [10]).

T-cell-independent IgA class switching

T-cell-dependent antibody responses take at least 5 to 7 days to develop, which is too much of a delay to neutralize pathogens that replicate quickly, commensal bacteria and dietary antigens. To compensate for this limitation, specialized B-cell subsets can rapidly produce IgM as well as class-switched IgG and IgA in a $CD4^+$ T-cell-and CD40L-independent manner[71]. In mice, intestinal T-cell-independent IgA responses rely on a peritoneal B-1-cell subset, which has ontogenic, phenotypic and genotypic features that are distinct from conventional (or B-2) B cells[72–74]. Indeed, B-1 cells express unmutated IgA antibodies (that is, they have not been subjected to SHM) that recognize multiple specificities with low affinity[1,2]. These antibodies mediate immune exclusion of commensals and provide limited protection against some pathogens, including rotaviruses and *Salmonella typhimurium*[75–77]. Systemic T-cell-independent IgA responses also exist and these appear to require B cells in the marginal zone of the spleen[1,78]. Similar to B-1 cells, mouse marginal-zone B cells express polyreactive IgA (and IgM) antibodies that may provide a second line of defence against commensal bacteria that breach the epithelial-cell barrier[1,71].

T-cell-independent IgA responses are also present in humans, as patients with severe $CD4^+$ T-cell deficiency due to HIV infection, as well as patients lacking CD40 retain intestinal IgA class switching[26]. Although humans seem to lack B-1 cells, they have additional B-cell subsets that might be involved in T-cell-independent IgA responses, including IgM^+ memory B cells[79]. These B cells can be detected in the circulation and in the marginal zone of the spleen, express mutated V(D)J genes, and undergo CD40-independent IgM and IgG production in response to bacterial polysaccharides, a canonical T-cell-independent antigen[79,80]. An additional human B-cell subset that is possibly involved in T-cell-independent IgA responses is the transitional B-cell subset, which expresses polyreactive antibodies encoded by unmutated V(D)J genes[81–83].

T-cell-independent antigens initiate IgA class switching by linking B cells with multiple innate immune pathways. Whereas some T-cell-independent antigens, such as LPS, activate B cells through Toll-like receptors (TLRs)[84], others, such as polysaccharides, activate B cells through their BCR[85]. T-cell-independent antigens can also provide additional B-cell-stimulating signals through DCs. Positioned as sentinels throughout the body, DCs sample T-cell-independent antigens from the environment and thereafter convey them to a non-degradative endocytic pathway[86]. Subsequent recycling of the endocytosed antigen to the plasma membrane is followed by its presentation to B cells[86–90]. During this process, DCs release soluble class-switch-inducing factors related to CD40L, including B-cell activating factor (BAFF; also known as BLyS) and a proliferation-inducing ligand (APRIL)[91–93] (see later).

Role of microbial TLR ligands in CSR to C_α

The TLR4 ligand LPS, along with TGFβ1, can also initiate germline C_α gene transcription and CSR from C_μ to C_α in mouse B cells[84,94] (FIG. 3); however, the mechanism by which TLRs trigger IgA CSR in B cells remains unclear. TLRs activate NF-κB by recruiting various adaptor proteins, including myeloid differentiation primary-response protein 88 (MyD88), to their cytoplasmic tail[95]. MyD88 forms a signalling complex with multiple downstream elements, including IL-1-receptor-associated kinases (IRAKs) and TRAF6, thereby causing IKK activation and subsequent nuclear translocation of NF-κB[96]. Surprisingly, NF-κB is not required for the activation of I_α promoters, which is highly dependent on other transcription factors[45–48,55,97]. Instead, NF-κB may be required by TLRs to induce the expression of AID[26,34,98,99].

Although sufficient to initiate IgA CSR[41], TGFβ1 and LPS require additional signals, such as BCR engagement by dextran-conjugated IgD-specific antibodies, to induce significant IgA expression and secretion[58,78]. These additional signals may be needed to optimize the expansion and differentiation of the B cells that have undergone IgA CSR in response to TGFβ1 and LPS. However, they may also be necessary to introduce crucial epigenetic changes rendering the S_α region more accessible to the CSR machinery. Indeed, mouse B cells undergo increased histone 3 acetylation at the S_α region on exposure to a range of stimuli including TGFβ1, LPS and a BCR ligand[100]. In addition, IgA CSR and production are enhanced by histone H3 methyltransferase Suv39h1 both *in vitro* and *in vivo*[101]. Suv39h1 might sequester an S_α-specific repressor by inducing relocation of proteins associated with heterochromatin, such as the polycomb group proteins HPC2 and HP1β (REF. [101]). Alternatively, Suv39h1 might induce transcriptional inhibition of an S_α-specific repressor. A candidate for such a repressor is LSF (late SV40/CP2 factor), a protein that binds S_α (and S_μ) segments and inhibits IgA CSR[102].

Interestingly, B-1 and marginal-zone B cells undergo IgA class switching more effectively than B-2 cells in response to T-cell-independent stimuli. This circumstance possibly reflects the unique antigen recognition profile of B-1 and marginal-zone B cells, which express both germline gene-encoded (that is, TLRs) and somatically recombined (that is, BCRs) antigen receptors[103]. When exposed to BAFF, LPS and TGFβ1, B-1 and marginal-zone B cells switch to IgA expression more readily than B-2 cells[78]. Such distinctive responsiveness may reflect the stimuli available in the microenvironments in which these B-cell subsets usually operate. Similar to B-1 and marginal-zone B cells, human IgM memory and transitional B cells exhibit robust antibody responses to microbial TLR and BCR ligands[83,104]. Yet, the contribution of these human B-cell subsets to T-cell-independent IgA class switching is presently not known.

Role of BAFF and APRIL in CSR to C_α

In addition to engaging TLRs on B cells, microbial products stimulate the release of BAFF and APRIL by DCs (FIG. 3). These two molecules are soluble B-cell-stimulating factors that are structurally and functionally related to CD40L[26,91,99]. In the presence of other cytokines, BAFF and APRIL induce germline C_α gene expression, AID expression and IgA class switching in a CD40-independent manner[26,91,99,105]. This effect depends on expression of the transmembrane activator and calcium-modulating cyclophilin-ligand interactor (TACI) receptor by B cells, as B cells lacking TACI do not express AID or undergo CSR in response to BAFF or APRIL[105]. In agreement with these data, TACI-deficient mice exhibit decreased steady-state serum IgA levels and make less IgA in response to T-cell-independent (but not T-cell-dependent) antigens[106]. The key role of TACI in T-cell-independent IgA responses is further indicated by its elevated expression by B-1 cells[107]. Similar to mice, humans develop selective IgA deficiency when *TNFRSF13b*[108],

the gene encoding TACI, is mutated (BOX 1). *TNFRSF13b* mutations also cause common variable immunodeficiency, which is associated with pan-hypogammaglobulinaemia[108,109]. This more pervasive phenotype may reflect the ability of TACI to enhance CD40-dependent antibody secretion in addition to promoting CD40-independent class switching[110,111].

Box 1

Lessons learned from IgA deficiencies

Selective IgA deficiency and common variable immunodeficiency (CVID) are the most common forms of primary immunodeficiency[137]. Selective IgA deficiency causes an isolated IgA defect, whereas CVID impairs IgM, IgG and IgA responses[108,109,137]. Although often asymptomatic, individuals with selective IgA deficiency and CVID can suffer from respiratory and gastrointestinal infections, respond poorly to T-cell-independent immunogens and develop B-cell lymphoproliferative disorders and autoimmunity[137]. In addition, some individuals with CVID develop chronic intestinal disorders, including inflammatory bowel disease, coeliac disease and nodular lymphoid hyperplasia (NLH), a follicular B-cell hyperplasia of the proximal intestine[2,137]. These nodules are similar to isolated lymphoid follicles and can trigger bleeding and intussusception, a telescoping prolapse of the intestine into an immediate adjacent segment. Hyperstimulation of isolated lymphoid follicles by an altered microflora may have a role in NLH, as mice lacking activation-induced cytidine deaminase (AID), an enzyme essential for IgA class switching[8], develop NLH-like lesions, as well as systemic and mucosal B-cell hyperactivation due to an abnormal expansion of commensal anaerobic bacteria in proximal intestinal segments[31,126]. B-cell hyperactivation is also present in mice lacking transmembrane activator and calcium-modulating cyclophilin-ligand interactor (TACI), a class-switch-inducing receptor involved in T-cell-independent IgA responses[105,106]. Similar to individuals with selective IgA deficiency or CVID, TACI-deficient mice develop autoimmune and lymphoproliferative disorders and produce less IgM, IgA and IgG in response to T-cell-independent antigens[106]. Remarkably, TACI is defective in a subset of individuals with selective IgA deficiency and CVID[108,109]. Overall, the clinical manifestations of IgA deficiencies reflect recent evidence indicating that IgA class switching is important not only to protect against pathogens[77,117], but also to preserve a mutualistic host–microorganism relationship via modulation of the gene-expression profile of commensal bacteria[15]. By preserving the homeostasis of the gut microflora, IgA may prevent the overstimulation of mucosal and systemic B cells and the subsequent expansion of autoreactive and neoplastic clones.

BAFF and APRIL also bind to BAFF receptor (BAFFR; also known as BR3), B-cell maturation antigen (BCMA) and heparan-sulphate proteoglycans (HSPGs)[92,93]. Engagement of BAFFR by BAFF delivers survival signals and, to some extent, CSR-inducing signals to peripheral B cells[105,112]. By contrast, engagement of BCMA by BAFF or APRIL delivers survival signals to plasma cells[113], but has no effect on CSR. Similarly, engagement of HSPGs by APRIL conveys survival and differentiation, but not CSR signals to plasma cells[110]. Of note, HSPGs form highly efficient TACI and BCMA signalling platforms by generating APRIL oligomers through the binding of their glycos-aminoglycan side chains to a basic QKQKKQ amino acid sequence that is proximal to the amino terminus of APRIL[93].

The mechanism by which TACI triggers IgA CSR remains unclear. Similar to CD40, TACI is thought to induce TRAF-dependent activation of the IKK complex, followed by nuclear translocation of NF-κB[114]. This pathway may be crucial to induce AID expression[68], but is unlikely to have a key role in germline C_α gene transcription, which usually requires signals

from TGFβ1 (REF. [55]). Yet, this cytokine is not absolutely required by BAFF and APRIL to induce IgA CSR, at least in murine B cells[105], raising the possibility that TACI activates I_α promoters in a TGFβR-independent manner. Although BAFF and APRIL are sufficient to induce IgA secretion in mouse B cells[105], human B cells require additional stimuli, including BCR or TLR engagement and IL-10 (REFS [26,91,99]), which enhances IgA class switching and production by functioning in synergy with paracrine or autocrine TGFβ1 (REFS [57,61,115]). In summary, the experimental evidence presently available clearly points to the existence of an important innate pathway to IgA class switching that is highly dependent on the engagement of TACI on B cells by BAFF and APRIL. Yet, more studies are needed to elucidate the co-stimuli and signalling events required by TACI to initiate IgA CSR and production.

Geography of IgA class switching

By ensuring the establishment of complex commensal and symbiotic relationships, co-evolution of mammals and bacteria over the past 200 million years has contributed to the development of multiple follicular and extrafollicular layers of protection in the intestinal mucosa (FIG. 4). These layers encompass both T-cell-dependent and T-cell-independent pathways for IgA class switching and production.

T-cell-dependent IgA CSR in Peyer's patches

Prior studies have confirmed that the intestinal IgA inductive sites are the Peyer's patches, isolated lymphoid follicles and mesenteric lymph nodes[1,2,116]. The key role of these organized lymphoid structures in the induction of intestinal IgA was demonstrated in mice that have abnormal follicles as a result of a genetic manipulation or experimental disruption of the signalling pathways required for lymphoid organogenesis[1,2,117]. In general, Peyer's patches are thought to generate high-affinity IgA antibodies to toxins and pathogens through a canonical T-cell-dependent pathway that is orchestrated by DCs. Positioned in the subepithelial dome of Peyer's patches (FIG. 5), DCs capture antigen from the intestinal lumen either directly by extending transepithelial projections or indirectly via M cells[19]. After migrating into the perifollicular area of the Peyer's patches, DCs present the captured antigen to $CD4^+$ T cells, thereby inducing effector T cells that release IgA-inducing cytokines, including TGFβ1, IL-4, IL-6 and IL-10 (REFS [117–119]). This response is enhanced by thymic stromal lymphopoietin (TSLP), a DC-conditioning IL-7-like cytokine derived from epithelial cells that promotes the formation of non-inflammatory T cells with IgA-inducing functions[119,120]. Ultimately, these non-inflammatory effector T cells trigger CSR from C_μ to C_α through a CD40-dependent pathway involving cognate T-cell–B-cell interactions in the germinal centre of mucosa-associated lymphoid follicles[2].

In addition to triggering the inductive phase of a T-cell-dependent IgA response, mucosal DCs initiate the effector phase of the mucosal humoral response by releasing retinoic acid, a compound derived from vitamin A that upregulates the expression of gut-homing receptors, including $\alpha_4\beta_7$-integrin and CC-chemokine receptor 9 (CCR9), by IgA class-switched B cells[121]. This upregulation enables IgA^+ B cells to migrate from Peyer's patches to the gut lamina propria via the draining mesenteric lymph nodes, the thoracic duct and blood[121]. During this migration, IgA^+ B cells develop into IgA-secreting plasma cells under the influence of cytokines released by lymphoid, stromal and epithelial cells and by DCs. The reason why class switching is biased towards IgA in the Peyer's patches remains unclear, although the release of IL-6, IL-10, TGFβ1, retinoic acid and nitric oxide by gut DCs seems to have an important role[119,121–123].

Peyer's patches can also induce IgA class switching through an alternative T-cell-dependent pathway that does not require BCR signals from canonical cognate T-cell–B-cell

interactions, but that rather relies on TLR signals from commensal bacteria[124]. This observation would explain why a fraction of B-2 cells from Peyer's patches expresses a restricted IgA repertoire that does not show the sequential acquisition of somatic hypermutations that would be expected in a canonical germinal-centre reaction[125]. By using both canonical and alternative T-cell-dependent pathways, Peyer's patches may generate IgA antibodies to control both commensal and pathogenic bacteria[1,117,126].

T-cell-independent IgA CSR in the lamina propria

In mice, T-cell-independent IgA production requires B-1 cells and preferentially targets commensal bacteria[3]. Indeed, experiments in which radiation chimaeras have been generated with allotypic markers to distinguish the IgA derived from B-1 and B-2 cells indicate that up to 50% of the intestinal IgA originates from B-1 cells[73,127], although this point remains controversial[128]. Most of this IgA is produced in a T-cell-independent manner, because MHC-class-II-deficient mice (lacking cognate T-cell–B-cell interactions) or mice deficient for the T-cell receptor β- and γ-chains (lacking T cells) retain B-1-cell-mediated IgA responses to commensal bacteria[74,76]. Recent data indicate that also the human intestine supports T-cell-independent IgA production[26].

But where do B cells undergo T-cell-independent IgA class switching? One probable place is the intestinal lamina propria, as IgA^+ B cells from this site contain hallmarks of ongoing IgA CSR, including *AICDA* gene transcripts, AID protein and I_α–C_μ switch circle transcripts[2,26,129]. In humans, these indicators are still present in the absence of $CD4^+$ T cells, CD40 signalling and/or germinal centres[26], suggesting that lamina-propria B cells can undergo IgA CSR *in situ* in a T-cell-independent manner. Consistent with this possibility, lamina-propria B cells retain IgA CSR in an AID mouse reporter strain that lacks germinal centres as a result of a deletion of the gene for the transcription factor OCA-B (Oct co-activator from B cells; also known as OBF1)[129]. Although still debated[130], the presence of IgA CSR in the lamina propria is in agreement with compelling evidence showing that CSR is not restricted to lymphoid follicles, but also occurs in extrafollicular lymphoid areas[131], including subepithelial areas[99]. In both mice and humans, lamina-propria-derived IgA CSR-inducing signals may target multiple IgM^+ B-cell subsets that originate from various sites, including the peritoneum, mucosal follicles and bone marrow[26,31,73,129,132]. A full characterization of these IgM^+ B-cell subsets is made more difficult by the fact that they may rapidly modify their phenotype on entering the microenvironment of the lamina propria.

Role of DCs in CSR to C_α

How do lamina-propria B cells undergo T-cell-independent IgA CSR in response to T-cell-independent antigens? In humans, DCs acquire B-cell-licensing functions, including IgA-inducing activity, following stimulation by microbial TLR ligands (FIG. 5), such as LPS[91,115]. In mice, intestinal DCs initiate T-cell-independent IgA responses by activating B-1 cells after loading commensal bacteria[74,76]. Consistent with these findings, DCs in the intestinal lamina propria continuously sample antigens from the intestinal lumen through transepithelial projections[133,134]. This sampling activity probably results in the induction of IgA CSR-inducing factors, such as BAFF and APRIL[26,91,99], and in the presentation of T-cell-independent antigens to B cells[86–90]. Of note, bacterial TLR ligands have recently been shown to generate a mucosal DC subset that expresses TNF and inducible nitric oxide synthase (iNOS) and has potent IgA-inducing functions[123]. Nitric oxide can enhance T-cell-independent IgA class switching by stimulating BAFF and APRIL production by lamina-propria DCs and enhance T-cell-dependent IgA class switching by upregulating TGFβR expression by B cells from the Peyer's patches[123]. In this way, TNF^+iNOS^+ DCs would contribute to the striking IgA bias observed in the gut.

Role of epithelial cells in CSR to C_α

DCs are unlikely to be the only inducers of T-cell-independent antibody responses in the gut[135]. Positioned at the interface between the antigen-rich intestinal lumen and the B-cell-rich lamina propria, epithelial cells produce numerous mediators with IgA-inducing function, including IL-10 and TGFβ1. Similar to respiratory epithelial cells[99,136], human colonocytes also express BAFF and APRIL (FIG. 5) and further upregulate this expression on sensing bacteria via TLRs through a mechanism that involves DC activation by TSLP[26,99]. Together with IL-10, APRIL triggers IgA2 CSR in B cells[26], suggesting that epithelial cells are central to the induction of IgA2 at mucosal sites colonized by a large microbiota, such as the colon[24,25]. At these sites, IgA2 may be more beneficial than IgA1, perhaps because IgA2 is more resistant than IgA1 to enzymatic digestion by bacterial proteases[25,28].

The presence of local IgA2 CSR in the human colonic lamina propria is consistent with several observations. First, the colonic lamina propria contains higher levels of IgA2 than IgA1, whereas colonic lymphoid follicles contain more IgA1 than IgA2 (REF. [26]), suggesting that both inductive and migratory events contribute to IgA2 production in the colonic lamina propria. Second, the colonic lamina propria retains IgA2, as well as hallmarks of ongoing CSR, in the absence of functional $CD4^+$ T cells, CD40 signals and germinal centres[26], which indicates that IgA2 responses proceed locally in a T-cell-independent manner. Third, the colonic lamina propria contains traces of both direct IgM-to-IgA2 and sequential IgA1-to-IgA2 CSR events, which are both highly dependent on APRIL[26]. Although direct IgA2 CSR can take place in unmutated or mutated lamina-propria IgM^+ B cells originating from systemic and mucosal sites, sequential IgA2 CSR would occur in mutated lamina-propria $IgA1^+$ B cells arriving from mucosal follicles. In this way, epithelial cells would maximize the diversity of the IgA2 repertoire released onto the mucosal surface. Finally, APRIL may enable epithelial cells to optimize mucosal IgA2 secretion by enhancing plasma-cell survival and differentiation[110,113].

Altogether, the available evidence indicates that human intestinal epithelial cells use APRIL to trigger T-cell-independent production of protease-resistant IgA2 antibodies, which may be more suited than IgA1 antibodies to cope with the dense microbial community of the large intestine. Consistent with its ability to induce IgA1 in addition to IgA2 class switching[26], APRIL is also expressed by epithelial cells at mucosal sites with predominant IgA1 production, such as the respiratory tract[23,26,136]. This implies that APRIL influences the IgA1–IgA2 balance in the context of other unknown factors that are probably specific to each mucosal site.

Concluding remarks

Despite recent advances, the functions and mechanisms of mucosal IgA class switching remain unclear. Commensal communities are strikingly different at distinct mucosal sites and may influence the subclass and binding properties of IgA class-switched antibodies in a site-specific manner. However, little is known about the role of individual bacterial species in the induction of IgA class switching and about the mechanisms by which class-switched IgA antibodies modify the composition and mutualistic relationship between the commensal microbiota and the host. We also need more information on the phenotype, IgA class-switch-inductive properties, and activation requirements of individual DC and B-cell subsets at distinct mucosal sites. Furthermore, more data are needed on the follicular and extrafollicular pathways that mediate mucosal IgA class switching and on the role of these pathways in the preservation of mucosal homeostasis and in the generation of immune protection against mucosal pathogens (BOX 2). Addressing these questions will not only

help to develop more efficient mucosal vaccines, but also to prevent and treat mucosal inflammatory disorders.

Box 2

Mucosal vaccines

Mucosal immune responses provide a first line of defence against infectious agents that use mucosal surfaces as a portal site of entry. Growing evidence indicates that the generation of mucosal immunity requires topical immunization[19,138]. Indeed, intramuscular or subcutaneous vaccines induce poor mucosal immune protection compared to oral, nasal, vaginal or rectal vaccines. Unfortunately, only a few mucosal vaccines are currently available and these include oral vaccines against poliovirus, *Salmonella typhimurium*, *Vibrio cholerae* and rotavirus, as well as an inhalable vaccine against influenza virus[19,138]. The development of mucosal vaccines has lagged behind that of other vaccines owing to a limited knowledge of mucosal immunity and to the technical challenges associated with the measurement of correlates of mucosal protection, including IgA antibodies in mucosal secretions. Additional challenges relate to the fact that mucosal vaccines are diluted by mucosal secretions, captured by mucus gels, attacked by enzymes and excluded by epithelial-cell barriers. Furthermore, in the absence of adequate adjuvants, mucosal antigens tend to induce tolerance rather than immunity[19,138]. Hence, an effective mucosal vaccine against infectious agents should avoid physical elimination and enzymatic digestion, target mucosal inductive sites and stimulate the innate immune system to generate effective adaptive immune responses[19,138]. Stimulated by the threat posed by HIV, current research efforts aim at identifying new mucosal delivery systems and adjuvants. Whether mucosally transmitted or injected, HIV preferentially replicates in mucosal districts, where it establishes viral reservoirs. Therefore, consensus is growing for the notion that effective HIV vaccines should elicit both cellular and humoral immune responses in mucosal regions[19,138]. Secreted IgA, with or without broad neutralizing activity, may prevent HIV from contacting mucosal surfaces, adhering to epithelial cells or crossing the epithelial-cell barrier[139]. Indeed, there is some evidence from highly exposed, uninfected individuals that IgA in mucosal secretions increases resistance to sexually transmitted HIV infection[140].

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Glossary

Non-homologous end-joining	The process that joins broken DNA ends independently of extended homology. Components of this pathway include the proteins Ku70, Ku80, ARTEMIS, X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs)
Membrane-bound IgA	IgA protein expressed on the surface of B cells that have undergone IgA class switching. Membrane IgA works as a transmembrane antigen receptor in that it delivers activating signals to effector memory B cells on secondary exposure to antigen

Secreted IgA	(sIgA). IgA protein secreted by early plasmacytoid B cells (or plasmablasts) and terminally differentiated plasma cells. sIgA exists in monomeric, dimeric or oligomeric forms that target local or distant antigens
Biofilm	A population of aggregated microorganisms, typically concentrated at an interface (usually solid–liquid), that is held together by a protective self-produced extracellular polymeric substance matrix
M cells	(Microfold or membrane cells). Specialized epithelial cells that deliver antigens by transepithelial vesicular transport from the gut lumen to intraepithelial dendritic cells and lymphocytes
Peyer's patches	Large lymphoid follicles positioned in the antimesenteric wall of the small intestine and containing a large germinal centre. Each follicle is capped by a dome area and is flanked by a T-cell-rich perifollicular area
Follicular DCs	Specialized non-haematopoietic stromal cells that reside in the follicles and germinal centres. These cells have long dendrites, but are not related to dendritic cells, and carry intact antigen on their surface
Kupffer cells	Large, stellate- or pyramidal-shaped, specialized macrophages that line the sinusoidal vessels of the liver. They regulate local immune responses, and remove microbial particles, endotoxin and other noxious substances that penetrate the portal venous system
Asialoglycoprotein receptor	A C-type lectin receptor that mediates endocytosis of desialylated glycoproteins and clearance of circulating IgA2 by hepatocytes
Transferrin receptor	Also known as CD71, this receptor regulates the cellular import of iron by binding the iron-carrier protein transferrin. In addition, it mediates clearance of circulating IgA1 by renal mesangial cells
Portal venous system	The venous system responsible for directing blood from parts of the gastrointestinal tract to the liver
APOBEC	(Apolipoprotein B RNA-editing, catalytic component). A cytidine deaminase enzyme family including APOBEC1, an RNA editor involved in lipid metabolism, APOBEC3G and APOBEC3F, two DNA editors with antiretroviral activity, and activation-induced cytidine deaminase, a DNA editor mediating immunoglobulin gene diversification
Hyper-IgM syndrome	(HIGM). Congenital immunodeficiency with defective immunoglobulin heavy chain class switching and increased IgM production. The underlying molecular defect involves CD40 ligand (HIGM1), activation-induced cytidine deaminase (HIGM2), CD40 (HIGM3), uracil DNA glycosylase (HIGM4) or other unknown B-cell proteins (HIGM5)

Isolated lymphoid follicles	Small lymphoid aggregates located in the antimesenteric wall of the small intestine and containing B cells, dendritic cells, stromal cells and some T cells. They may contain germinal centers
VIP	(Vasoactive intestinal peptide). A peptide hormone released by mucosal postsynaptic parasympathetic nerve fibres and intrinsic neurons of the intestinal lamina propria. In addition to stimulating water and electrolyte secretion and intestinal wall motility, VIP activates lymphocytes via a constitutive VIPR1 receptor and an inducible VIPR2 receptor

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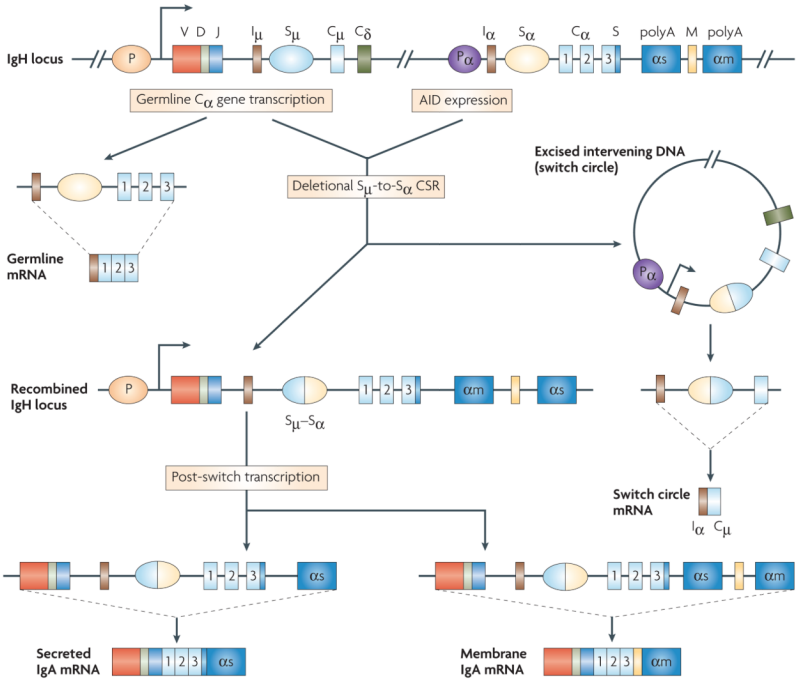

Figure 1. Recombinatorial and transcriptional events underlying IgA class switching

The immunoglobulin heavy chain (IgH) locus of mature B cells contains a rearranged variable (V) diversity (D) joining (J) exon encoding the antigen-binding domain of an immunoglobulin. Following rearrangement of the light chain, B cells produce intact IgM and IgD through a transcriptional process driven by a promoter (P) upstream of the VDJ exon. Production of downstream IgG, IgA or IgE with identical antigen specificity occurs through class-switch recombination (CSR). Appropriate stimuli induce germline transcription of the constant heavy chain α ($C_α$) gene from the promoter ($P_α$) of the intronic α ($I_α$) exon through the switch α ($S_α$) region between $I_α$ and $C_α$ exons. In addition to yielding a sterile $I_α$–$C_α$ mRNA, germline transcription renders the $C_α$ gene substrate for activation-induced cytidine deaminase (AID), an essential component of the CSR machinery. By generating and repairing DNA breaks at $S_μ$ and $S_α$, the CSR machinery rearranges the IgH locus, thereby yielding a deletional recombination product known as the switch circle. This episomal DNA transcribes a chimeric $I_α$–$C_μ$ mRNA under the influence of signals that activate $P_α$. Post-switch transcription of the IgH locus generates mRNAs for both secreted IgA and membrane IgA. $C_α$ 1–3, exons that encode the $C_α$ chain of IgA; S, 3′ portion of $C_α3$ encoding the tailpiece of secreted IgA; M, exon encoding the transmembrane and cytoplasmic portions of membrane-bound IgA; αs, polyadenylation site for secreted IgA mRNA; αm, polyadenylation site for membrane-bound IgA mRNA.

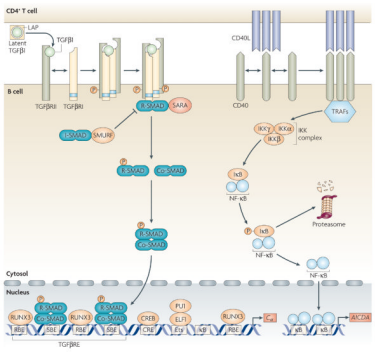

Figure 2. Signalling events leading to T-cell-dependent IgA class switching

$CD4^+$ T cells release the active transforming growth factor-β1 (TGFβ1) after processing of a latency-associated peptide (LAP). TGFβ1 forms a heteromeric TGFβ receptor (TGFβR) complex on B cells comprising TGFβRII and TGFβRI subunits. TGFβR undergoes degradation on binding by I-SMAD (inhibitory SMAD (mothers against decapentaplegic homologue)) proteins, such as SMAD7, which recruits ubiquitin ligases of the SMURF (SMAD ubiquitylation regulatory factor) family to TGFβRI. Alternatively, the TGFβR remains on the B-cell surface to activate SMAD proteins. In the presence of TGFβ1, TGFβRII kinases phosphorylate TGFβRI, leading to the activation of TGFβRI kinases. These kinases induce the phosphorylation of receptor-regulated SMAD (R-SMAD) proteins, including SMAD2 and SMAD3, thereby releasing them from the plasma membrane-anchoring protein SARA (SMAD anchor for receptor activation). After forming homo-oligomeric complexes, as well as hetero- oligomeric complexes with SMAD4 — a co-mediator SMAD (Co-SMAD) protein — R-SMAD proteins translocate to the nucleus, where they bind to SMAD-binding elements (SBEs) on target gene promoters, including constant heavy chain α (C_α) gene promoters. These SMAD complexes further associate with constitutive and TGFβR-induced co-factors, including runt-related transcription factor 3 (RUNX3), which binds to RUNX-binding elements (RBEs), cyclic AMP response element binding protein (CREB), which binds to a cyclic AMP response element (CRE), and Ets-like factor 1 (ELF1), which binds to an Ets-binding site. In addition to TGFβ, $CD4^+$ T cells express CD40 ligand (CD40L), which elicits oligomerization of CD40 on B cells, recruitment of tumour-necrosis-factor-receptor-associated factors (TRAFs) to CD40, activation of the IκB kinase (IKK) complex, phosphorylation of IκB (inhibitor of nuclear factor-κB (NF-κB)), and IκB degradation. IκB-free NF-κB translocates to the nucleus to induce the activation-induced cytidine deaminase (*AICDA*) gene promoter. Although NF-κB binds to an NF-κB-binding (κB) site on the C_α promoter, it has a marginal role in the transcription of the C_α gene.

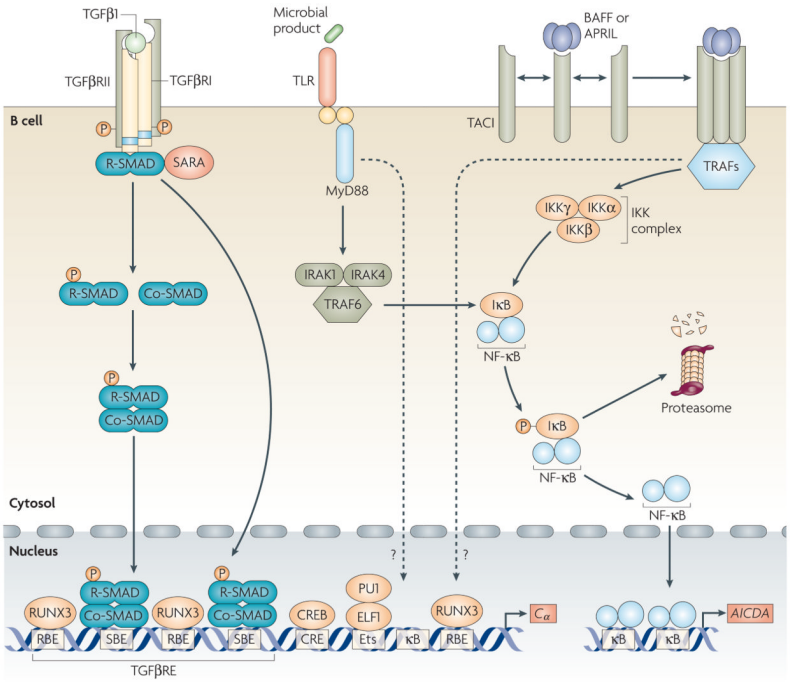

Figure 3. Signalling events leading to T-cell-independent IgA class switching

Dendritic cells (DCs) activate transforming growth factor-β1 (TGFβ1) by inducing the processing of a latency-associated peptide (LAP). TGFβ1 activates the constant heavy chain α (C_α) gene promoter (as shown in Figure 2). DCs also present bacterial products to B cells, thereby activating Toll-like receptors (TLRs). By recruiting myeloid differentiation primary-response protein 88 (MyD88), interleukin-1-receptor-associated kinase 1 (IRAK1) and IRAK4, TLRs induce activation of the IκB (inhibitor of nuclear factor-κB (NF-κB)) kinase (IKK) complex, phosphorylation and degradation of IκB. IκB-free NF-κB translocates to the nucleus to induce the promoter of the activation-induced cytidine deaminase (*AICDA*) gene. DCs further activate B cells by engaging transmembrane activator and calcium-modulating cyclophilin-ligand interactor (TACI) through B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL). TACI activates NF-κB after recruiting tumour-necrosis-factor-receptor-associated factors (TRAFs) to its cytoplasmic domain, thereby triggering *AICDA* gene expression. It is unknown whether TLRs and TACI also activate the C_α promoter. Co-SMAD, co-mediator SMAD; CRE, cyclic AMP response element; CREB, CRE-binding protein; ELF1, Ets-like factor 1; κB, NF-κB-binding site; RUNX3, runt-related transcription factor 3; RBE, RUNX-binding element; SARA, SMAD anchor for receptor activation; SBE, SMAD-binding element; SMAD, mothers against decapentaplegic homologue; TGFβR, TGFβ1 receptor.

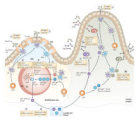

Figure 4. Map of IgA class switching in the gut

Dendritic cells (DCs) in the subepithelial dome (SED) of the Peyer's patches capture antigen by interacting with microfold (M) cells or by extending transepithelial projections into the lumen. During this process, DCs are induced to express tumour-necrosis factor (TNF) and inducible nitric oxide synthase (iNOS) (and are therefore referred to as tiDCs), which present antigen to perifollicular $CD4^+$ T cells, thereby inducing them to differentiate into effector T cells releasing IgA-inducing cytokines. T cells also interact with antigen-specific IgM^+IgD^+ naive B cells. Together with follicular dendritic cells (FDCs), this interaction fosters a germinal centre (GC) reaction that includes somatic hypermutation (SHM) and IgA class-switch recombination (CSR). The resulting IgA^+ effector B cells home to the gut lamina propria, where they differentiate into plasma cells that secrete high-affinity IgA. Human $IgA1^+$ effector B cells can also undergo sequential IgA2 CSR on receiving T-cell-independent signals from bacteria-activated epithelial cells, DCs and tiDCs. Similar signals trigger direct IgA CSR in various B-cell subsets, including unmutated IgM^+IgD^+ B-1 cells from the peritoneum and mutated IgM^+IgD^- effector B cells from Peyer's patches. These local CSR events generate plasma cells secreting low- or high-affinity IgA. FAE, follicle-associated epithelium; FM, follicular mantle; HEV, high endothelial venule; sIgA, secreted IgA.

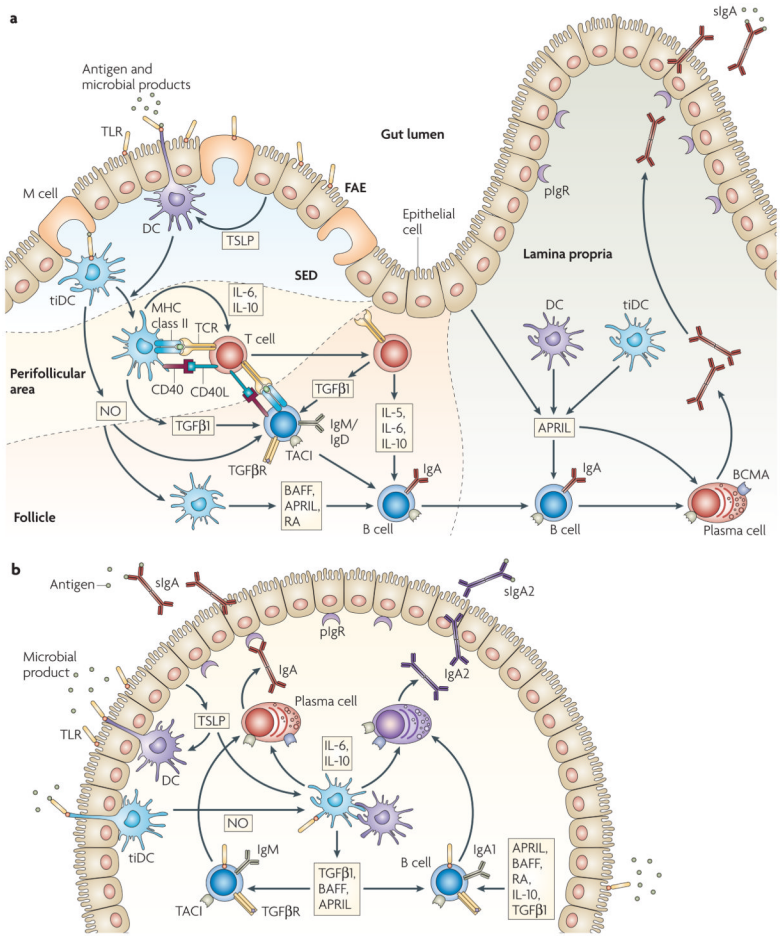

Figure 5. Cellular interactions causing IgA class switching in the gut

a | While capturing antigen, intestinal dendritic cells (DCs) are exposed to microbial Toll-like receptor (TLR) ligands and epithelial-cell-derived cytokines, including thymic stromal lymphopoietin (TSLP). These signals promote the generation of tiDCs, which are DCs that express tumour-necrosis factor and inducible nitric oxide synthase; these cells present antigen to $CD4^+$ T cells in the perifollicular area of Peyer's patches. In addition, tiDCs transfer antigen to follicular IgM^+IgD^+ naive B cells and induce them to upregulate the expression of TGFβ1 receptor (TGFβR) through nitric oxide (NO). During cognate interactions with $CD4^+$ T cells, B cells undergo IgA class-switch recombination (CSR) in response to CD40 ligand (CD40L) and transforming growth factor-β1 (TGFβ1) from activated T cells. IgA expression requires interleukin-5 (IL-5), IL-6 and IL-10 from activated T cells, as well as B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) from tiDCs. After being imprinted by retinoic acid (RA) from DCs, IgA^+ effector B cells migrate to the lamina propria, where they differentiate into IgA-secreting plasma cells. This differentiation is enhanced by APRIL secreted by epithelial cells, DCs and tiDCs. **b** | After sensing microorganisms via TLRs, epithelial cells from intestinal villi release APRIL, thereby triggering direct IgA CSR in lamina-propria IgM^+ B cells and sequential IgA2 CSR in lamina-propria $IgA1^+$ B cells in a T-cell-independent manner. This pathway may also involve APRIL, BAFF and TGFβ1 from DCs and tiDCs exposed to microbial TLR ligands, NO and TSLP. Epithelial cells, DCs and tiDCs promote plasma-cell differentiation via APRIL, BAFF, IL-6 and IL-10. BCMA, B-cell maturation antigen; FAE, follicle-associated epithelium; M cell, microfold cell; pIgR, polymeric immunoglobulin

receptor; SED, subepithelial dome; sIgA, secreted IgA; TACI, transmembrane activator and calcium-modulating cyclophilin-ligand interactor.

Table 1

Differences in IgA class switching and production between mice and humans

Parameter	Mice	Humans	References
Organization of the C_α locus	One C_α locus	Two C_α loci	6,8,9
Modes of IgA CSR	No sequential IgA1-to-IgA2 CSR	Sequential IgA1-to-IgA2 CSR	6,8,9,99
B cells undergoing IgA CSR	B-1-and B-2-cell subsets	No canonical B-1-cell subset	1,103,127
Requirements for IgA CSR	LPS induces IgA CSR via TLR4 in B cells	B cells lack TLR4 and are unresponsive to LPS	9,84
Requirements for IgA secretion	IL-5 increases IgA secretion	IL-5 does not influence IgA secretion	9
Types of IgA produced	One IgA class only	Two subclasses, IgA1 and IgA2	1,9,21–23
Form of systemic IgA antibodies	Mostly IgA oligomers	Mostly IgA1 monomers	1,21
Form of mucosal IgA antibodies	IgA oligomers	Mostly IgA1 and IgA2 oligomers	1,21
Effector functions of IgA antibodies	No FcαRI expression	FcαRI expressed by innate immune cells	18

CSR, class-switch recombination; FcαRI, type I Fc receptor for IgA; IL-5, interleukin-5; LPS, lipopolysaccharide; TLR4, Toll-like receptor 4.