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Innate Signaling Networks in Mucosal IgA Class Switching

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Abstract

The past 20 years have seen a growing interest over the control of adaptive immune responses by the innate immune system. In particular, considerable attention has been paid to the mechanisms by which antigen-primed dendritic cells orchestrate the differentiation of T cells. Additional studies have elucidated the pathways followed by T cells to initiate immunoglobulin responses in B cells. In this review, we discuss recent advances on the mechanisms by which intestinal bacteria, epithelial cells, dendritic cells, and macrophages cross talk with intestinal T cells and B cells to induce frontline immunoglobulin A class switching and production.

1. FUNCTIONS OF MUCOSAL IgA

1.1. Intestinal bacteria

The intestinal mucosa becomes exposed to a massive amount of noninvasive bacteria known as commensals shortly after birth (Macpherson, 2006). Commensals establish a mutualistic relationship with the human host as they break down otherwise indigestible food components, generate essential vitamins, limit access to pathogens, stimulate the growth and function of epithelial cells, and facilitate the development of the immune system (Mazmanian and Kasper, 2006). Conversely, the intestinal lumen provides commensals with a stable habitat rich in energy sources derived from the ingested food (Macpherson and Harris, 2004). Remarkably, a single layer of intestinal epithelial cells (IECs) separates the sterile milieu of the intestinal mucosa from trillions of commensals that constantly pose a potential threat of infection and overwhelming inflammatory responses (Sansonetti, 2004). In addition to forming a physical barrier against bacteria and producing multiple immune and nonimmune molecules with antimicrobial activity, IECs instruct the local immune system as to the composition of the commensal microbiota and shape the ensuing innate and adaptive immune responses to generate protection while preserving homeostasis (Sansonetti and Medzhitov, 2009). In general, intestinal homeostasis refers to the complex immune and nonimmune mechanisms that permit the intestinal mucosa to minimize the adverse health effects of commensals even during microenvironmental perturbations. A central component of intestinal homeostasis is immunoglobulin A (IgA).

1.2. Intestinal IgA

The intestinal mucosa has evolved several strategies to control commensals and eventually neutralize pathogens while preventing inflammation-induced bystander damage to the epithelial barrier (Holmgren and Czerkinsky, 2005; Pedron and Sansonetti, 2008). A key strategy to generate immune protection without causing inflammation involves production of massive amounts of IgA, the most abundant antibody isotype in our body (Cerutti and Rescigno, 2008; Macpherson *et al.*, 2008). IgA provides protection against intestinal

microorganisms as a result of its ability to interact with the polymeric Ig receptor (pIgR), an antibody transporter expressed on the basolateral surface of IECs (Mostov, 1994). After binding to pIgR through a joining (J) chain, IgA dimers secreted by intestinal plasma cells translocate across epithelial cells onto the mucosal surface through a process known as transcytosis (Brandtzaeg, 1974; Mestecky *et al.*, 1971; Mostov and Deitcher, 1986; Mostov and Simister, 1985). Transcytosis involves intracellular processing of pIgR into a secretory component (SC) that remains associated with the J chain of the IgA dimer to form a secretory IgA (SIgA) complex (Brandtzaeg and Prydz, 1984; Mestecky *et al.*, 1971, 1999; Phalipon and Corthesy, 2003; Phalipon *et al.*, 2002). SIgA and its dimeric IgA precursor bind to antigen without generating inflammatory products from the complement cascade and without stimulating the release of inflammatory mediators from immune and nonimmune cells (Brandtzaeg *et al.*, 2001; Monteiro and Van De Winkel, 2003).

1.3. Function of intestinal IgA

Besides neutralizing toxins and pathogens, mucosal SIgA excludes commensal bacteria from the sterile milieu of the lamina propria through as yet poorly understood mechanisms that may include entrapment of microorganisms in the mucus layer topping the surface of IECs (Macpherson *et al.*, 2008; Phalipon and Corthesy, 2003). This process is known as immune exclusion and involves the interaction of the SC portion of SIgA with mucin proteins that constitute the building blocks of mucus (Phalipon *et al.*, 2002). In addition, SIgA binds to microbial proteins involved in epithelial attachment and thereby avoids the penetration of commensals across the epithelium (Macpherson *et al.*, 2001). SIgA further contributes to mucosal immunity by neutralizing intracellular proinflammatory components such as lipopolysaccharide (LPS) (Fernandez *et al.*, 2003). In addition, SIgA favors the growth of commensal bacteria in biofilms that prevent the outgrowth of pathogens through a mechanism that involves competition for biological niches and sources of energy (Bollinger *et al.*, 2006). Furthermore, SIgA promotes the establishment of a symbiotic relationship between host and commensals by down-modulating the expression of proinflammatory bacterial epitopes (Peterson *et al.*, 2007). Moreover, SIgA favors the maintenance of appropriate bacterial communities within specific intestinal segments (Suzuki *et al.*, 2004) and facilitates the sampling of antigen by binding to receptors expressed on microfold (M) cells, an epithelial cell type specialized in antigen capture (Kadaoui and Corthesy, 2007; Mantis *et al.*, 2002; Neutra, 1999). If bacteria elude SIgA and penetrate the subepithelial area, IgA dimers released by plasma cells provide a second line of defense by transporting bacteria back into the lumen through the pIgR (Brandtzaeg *et al.*, 2001). Alternatively, IgA dimers clear bacteria by interacting with FcαRI, a high-affinity IgA receptor that is expressed on dendritic cells (DCs) and neutrophils and is also known as CD89 (Pasquier *et al.*, 2005; Phalipon and Corthesy, 2003). Remarkably, intestinal macrophages express little or no FcαRI and therefore do not release inflammatory cytokines upon exposure to IgA oligomers (Brandtzaeg *et al.*, 2001). As discussed earlier, IgA is also unable to fix and activate the classical complement cascade, which further explains the noninflammatory nature of this mucosal antibody isotype (Mestecky *et al.*, 1999). Finally, recent data point to a possible role of dimeric IgA in the establishment of a symbiotic relationship between host and commensal bacteria in intestinal lymphoid structures denominated Peyer's patches (Obata *et al.*, 2010). This symbiosis would be instrumental to maximize the efficiency of commensal-specific IgA responses by the intestinal immune system.

2. MECHANISMS OF MUCOSAL IgA PRODUCTION

2.1. Antibody diversification

Antibodies diversify through three Ig gene-modifying processes that occur in distinct phases of B cell development. Bone marrow B cell progenitors generate antigen recognition

diversity by assembling the antigen-binding variable regions of Ig heavy (H) and light (L) chain genes from individual variable (V), diversity (D), and joining (J) gene segments through V(D)J recombination (Schlissel, 2003). This antigen-independent process is initiated by a recombinase activating gene complex comprising RAG-1 and RAG-2 endonucleases and is completed by the nonhomologous end-joining machinery (Bassing *et al.*, 2002). Immature B lymphocytes that have completed functional V(D)J recombination initially express only IgM, but then differentiate into transitional and mature B cells that express both IgM and IgD through alternative splicing of a long mRNA. These mature B cells colonize peripheral lymphoid organs, where the antigen-dependent phase of B cell ontogeny takes place. In the presence of antigen, mature B cells undergo a second wave of Ig gene remodeling through class switch recombination (CSR) and somatic hypermutation (SHM), two processes that require the DNA-editing enzyme activation-induced cytidine deaminase (AID) and mediate isotype switching and affinity maturation, respectively (Chaudhuri and Alt, 2004; Chaudhuri *et al.*, 2003; Muramatsu *et al.*, 2000; Stavnezer *et al.*, 2008). SHM introduces point mutations to the variable regions of antibodies, thereby providing the structural correlate for selection by antigen of high-affinity immunoglobulin variants (Odegard and Schatz, 2006). CSR alters the effector function of antibodies without changing their antigen-binding specificity by replacing C_{μ} and C_{δ} exons encoding IgM and IgD with C_{γ} , C_{α} , or C_{ϵ} exons encoding IgG, IgA, or IgE (Chaudhuri and Alt, 2004). Such secondary isotypes mediate novel effector functions by engaging specific Fc receptors on innate immune cells (Stavnezer, 1996).

2.2. IgA class switching

Mature B cells acquire IgA expression by undergoing CSR from C_{μ} to C_{α} . This event involves the exchange of an upstream donor C_{μ} gene with a downstream acceptor C_{α} gene through a DNA recombination process guided by switch (S) regions (Cerutti, 2008b; Manis *et al.*, 2002). S regions are intronic guanine- and cytosine-rich DNA sequences located 5' of each C_H gene and preceded by a promoter that initiates germline C_H gene transcription when the B cell is exposed to appropriate activating stimuli (Stavnezer *et al.*, 2008). Activation of the I_{α} promoter yields a primary I_{α} - S_{α} - C_{α} transcript that is later spliced to form a noncoding germline I_{α} - C_{α} transcript (Cerutti, 2008b; Stavnezer *et al.*, 2008). The primary I_{α} - S_{α} - C_{α} transcript associates with the template strand of the DNA to form a stable DNA-RNA hybrid that is recognized by a DNA-editing protein complex comprising AID (Cerutti, 2008b; Chaudhuri and Alt, 2004). In general, germline transcription is essential for CSR as it renders the S region substrate for AID (Chaudhuri and Alt, 2004). This enzyme deaminates cytosine residues on both strands of S region DNA (Chaudhuri *et al.*, 2003), thereby generating multiple DNA lesions that are ultimately processed into double-stranded DNA breaks (Stavnezer *et al.*, 2008). Fusion of double-stranded DNA breaks at S_{α} and S_{μ} through the nonhomologous end-joining pathway induces looping-out deletion of the intervening DNA, thereby juxtaposing V_HDJ_H to C_{α} (Cerutti, 2008b). This event yields a chromosomal V_HDJ_H - C_{α} sequence, which encodes the IgA protein, and an extrachromosomal S_{α} - S_{μ} switch circle, which encodes a chimeric I_{α} - C_{μ} switch circle transcript (Cerutti, 2008b; Kinoshita *et al.*, 2001).

2.3. Binding modes of intestinal IgA

Mucosal IgA antibodies form high- and low-affinity binding systems that originate from different B cell types and likely serve distinct functions. High-affinity IgA originates from B cells lodged in intestinal lymphoid follicles (Martinoli *et al.*, 2007). Follicular B cells undergo IgA CSR and SHM in the specialized microenvironment of the germinal center through a T cell-dependent (TD) pathway that involves engagement of CD40 on B cells by CD40 ligand (CD40L) on CD4⁺ T cells (Cerutti and Rescigno, 2008; Suzuki and Fagarasan, 2009). High-affinity IgA neutralizes microbial toxins and pathogens in addition to

recognizing commensal bacteria (Macpherson *et al.*, 2008). Low-affinity IgA derives from B-1 cells lodged in the peritoneal cavity and intestinal lamina propria as well as conventional B-2 cells lodged in isolated lymphoid follicles, at least in mice (Macpherson and Uhr, 2004; Macpherson *et al.*, 2000; Tsuji *et al.*, 2008). These B cells undergo IgA CSR and perhaps some limited degree of SHM upon receiving T cell-independent (TI) activating signals from Toll-like receptors (TLRs), a family of innate antigen receptors that recognize highly conserved molecular signatures associated with microbes (Medzhitov, 2001; Takeda *et al.*, 2003). In addition to generating B cell-intrinsic activation signals, TLRs trigger the release of B cell-stimulating cytokines from DCs, macrophages, and follicular dendritic cells (FDCs), including B cell-activating factor of the tumor necrosis factor (TNF) family (BAFF, also known as BLyS) and a proliferation-inducing ligand (APRIL), two IgA-inducing molecules structurally related to CD40L (Cerutti, 2008b; Cerutti and Rescigno, 2008; Dillon *et al.*, 2006; Schneider, 2005; Suzuki and Fagarasan, 2009). Together with high-affinity IgA antibodies, low-affinity IgA antibodies minimize the interaction of commensals with the surface of epithelial cells, and eventually detect and kill bacteria and other microorganisms that manage to penetrate the epithelial barrier (Macpherson *et al.*, 2008).

3. MUCOSAL IgA Pathways Involving T Cells

3.1. TD IgA production in intestinal follicles

Most antigens initiate mucosal IgA responses through a TD reaction that takes place in the inductive site of mucosal lymphoid follicles (Fig. 2.1), including Peyer's patches and mesenteric lymph nodes (Macpherson *et al.*, 2008; Suzuki and Fagarasan, 2009). These organized structures comprise a germinal center that fosters B cell clonal expansion, AID expression, CSR, SHM, and antigen-mediated selection of high-affinity B cell mutants through cognate interaction between B cells expressing CD40 and CD4⁺ T helper (Th) cells expressing CD40L (Fagarasan *et al.*, 2010; MacLennan, 1994; Muramatsu *et al.*, 2007; Vinuesa *et al.*, 2009). Compared to systemic lymphoid follicles, intestinal lymphoid follicles have a less stringent requirement for signals from the B cell receptor (BCR) complex, which is a multimolecular antigen-binding structure comprising transmembrane Ig (Allen *et al.*, 2007; Casola *et al.*, 2004). In general, BCR captures antigen to convey it into intracellular compartments that generate immunogenic peptides in the context of major histocompatibility class-II (MHC-II, the mouse equivalent of human HLA-II) molecules (Batista and Harwood, 2009; Batista *et al.*, 2001). In addition to generating powerful B cell-activating signals, BCR allows B cells to present immunogenic peptides to CD4⁺ T cells. The resulting activation, expansion, and differentiation of CD4⁺ T cells lead to the initiation of TD B cell responses specific for a given antigen (McHeyzer-Williams and Ahmed, 1999). Yet, B cells from Peyer's patches remain competent for TD production of antigen-specific IgA even in the absence of BCR, which implies that noncognate antigenic signals play a key role in IgA-mediated mucosal immunity (Casola *et al.*, 2004). Consistent with this possibility, lack of myeloid differentiation primary response gene 88 (MyD88), an adaptor protein that transduces signals from TLRs, causes an impairment of IgA production in intestinal Peyer's patches (Casola *et al.*, 2004; Suzuki *et al.*, 2010; Tezuka *et al.*, 2007). The central role of TLRs in intestinal IgA responses can be also inferred from recent studies showing that some commensals colonize Peyer's patches and induce commensal-specific IgA responses under homeostatic conditions (Obata *et al.*, 2010).

3.2. Intestinal IgA-inducing follicular T cells

The mechanism underlying skewed IgA production in Peyer's patches and other intestinal lymphoid follicles remains poorly understood, but growing evidence indicates that these organized structures favor the generation of CD4⁺ T cells with IgA-inducing effector functions (Xu-Amano *et al.*, 1993). Indeed, intestinal DCs prime naïve CD4⁺ T cells to

differentiate into Th2 cells, T regulatory (Treg) cells, and T follicular helper (Tfh) cell subsets that express CD40L and release IL-4, IL-5, IL-6, IL-10, IL-21, and/or transforming growth factor- β (TGF- β) (Cong *et al.*, 2009; Rimoldi *et al.*, 2005a,b; Tsuji *et al.*, 2009; Xu-Amano *et al.*, 1993; Yamamoto *et al.*, 1996). In addition to triggering IgA CSR in naïve B cells, such cytokines can induce differentiation of IgA-expressing germinal center B cells into IgA-secreting plasma cells (Cerutti, 2008b; Cerutti and Rescigno, 2008). As discussed later, intestinal DCs promote Th2, Treg, and Tfh cell differentiation upon receiving “conditioning” signals from the local mucosal environment, including IECs (Cong *et al.*, 2009; Rimoldi *et al.*, 2005a; Tsuji *et al.*, 2009). While the presence of Th2 and Tfh cells in Peyer’s patches is consistent with the key role of these T cell subsets in systemic B cell responses (Linterman *et al.*, 2010), intriguing new data suggest that Peyer’s patches may also include Treg cells (Cong *et al.*, 2009; Tsuji *et al.*, 2009). These tolerogenic T cells would promote mucosal homeostasis in two ways: by dampening inflammatory Th1 and Th17 cell responses and by initiating noninflammatory IgA responses (Fagarasan *et al.*, 2010; Weaver and Hatton, 2009). Both functions seem to involve Treg production of TGF- β , a T cell-suppressing cytokine essential for B cell production of IgA, at least in mice (Cazac and Roes, 2000; Cong *et al.*, 2009; Tsuji *et al.*, 2009; Weaver and Hatton, 2009). Although sufficient to initiate germline C α gene transcription, TGF- β requires an additional signal from CD40L to upregulate AID expression and complete IgA CSR (Cerutti, 2008b; Cerutti and Rescigno, 2008). In agreement with this notion, CD40L and TGF- β 1 are both expressed by intestinal Treg and Tfh cells and are both essential for the induction of IgA in Peyer’s patches (Cazac and Roes, 2000; Cong *et al.*, 2009; Tsuji *et al.*, 2009). Interestingly, intestinal Treg cells can differentiate into Tfh cells, which release IL-21 in addition to TGF- β (Tsuji *et al.*, 2009). Together with IL-5 and IL-6, which enhance plasma cell differentiation, IL-21 and TGF- β promote the formation of high-affinity IgA-secreting plasma blasts that migrate to the effector site of the lamina propria, where J chain-linked dimeric IgA molecules are released (Avery *et al.*, 2010; Brandtzaeg *et al.*, 2001; Cerutti, 2008b; Cerutti and Rescigno, 2008; Dullaers *et al.*, 2009). In the lamina propria, a Th17 cell subset developmentally linked to Treg cells may facilitate the generation and retention of IgA-secreting plasma cells and perhaps even the release of SIgA across IECs through a mechanism involving IL-17 (Jaffar *et al.*, 2009; Murai *et al.*, 2010; Uematsu *et al.*, 2009). Thus, distinct mucosal T cell subsets may operate at different intestinal sites to regulate the production and release of IgA (Fagarasan *et al.*, 2010).

3.3. Intestinal IgA-inducing FDCs

In addition to T cells, Peyer’s patches contain a meshwork of FDCs that extensively interact with B cells (El Shikh *et al.*, 2010; Gonzalez *et al.*, 2009). FDCs are ontogenetically different from conventional DCs in that they originate from a nonhematopoietic precursor, which may include mesenchymal cells (Mueller and Germain, 2009). One of the main functions of FDCs is to facilitate the selection of high-affinity follicular B cells by antigen (Vinuesa *et al.*, 2009). Indeed, in the presence of CD40L from CD4⁺ T cells, immune complexes trapped on the surface of FDCs rescue high-affinity follicular B cells from apoptosis by engaging BCR (Allen *et al.*, 2007). Remarkably, FDCs can also deliver CSR signals to follicular B cells by promoting extensive BCR cross-linking through surface arrays of antigen complexes (El Shikh *et al.*, 2009). FDCs may provide additional CSR-inducing signals by releasing BAFF and APRIL, two TNF family members structurally related to CD40L (Badr *et al.*, 2008; Chiu *et al.*, 2007; El Shikh *et al.*, 2009; Gorelik *et al.*, 2003; Rahman and Manser, 2004). Interestingly, recent evidence shows that FDCs from Peyer’s patches are particularly efficient at inducing IgA class switching and production after sensing commensal bacteria through TLRs. Indeed, TLRs stimulate intestinal FDCs to upregulate specific matrix metalloproteases that induce secretion of mature TGF- β by promoting proteolytic cleavage of a LAP (latency associated peptide)-containing TGF- β

precursor (Suzuki *et al.*, 2010; reviewed by Suzuki *et al.* in Chapter 6). In addition to FDCs, intestinal lymphoid follicles contain a TNF- α - and inducible nitric oxide synthase (iNOS)-producing DC subset that seems to enhance TD IgA responses by upregulating the expression of TGF- β receptor type-II on intestinal follicular B cells via nitric oxide (Tezuka *et al.*, 2007).

3.4. TD signals for intestinal IgA CSR

In general, B cells from PPs require CD40L and TGF- β 1 to undergo IgA CSR (Cazac and Roes, 2000; Cerutti *et al.*, 1998; Defrance *et al.*, 1992; Fayette *et al.*, 1997; Islam *et al.*, 1991; Nakamura *et al.*, 1996; Shockett and Stavnezer, 1991; Zan *et al.*, 1998). As extensively discussed in previously published works (Bishop, 2004; Cerutti, 2008b; Van Kooten and Banchereau, 1996), CD40L elicits recruitment of multiple TRAF adaptor proteins to the cytoplasmic tail of CD40 on B cells (Fig. 2.2). This event is followed by activation of an I κ B kinase (IKK) complex that promotes phosphorylation and proteasome-dependent degradation of inhibitor of NF- κ B (I κ B), a protein that retains nuclear factor- κ B (NF- κ B) in the cytoplasm of resting B cells (Karin and Greten, 2005). The ensuing dissociation of NF- κ B from I κ B stimulates translocation of NF- κ B from the cytoplasm to the nucleus, where NF- κ B transcriptionally activates multiple B cell genes, including *AICDA* (Dedeoglu *et al.*, 2004). Although containing an NF- κ B-binding κ B site, the germline C α gene promoter does not require NF- κ B for its activation, but rather depends on signals from a heteromeric TGF receptor (TGFR) complex that activates mothers against decapentaplegic homolog (SMAD) proteins (Rubtsov and Rudensky, 2007; Stavnezer, 1995, 1996). In the presence of TGF- β 1, TGF β RII kinases phosphorylate TGF β RI, leading to the activation of TGF β RI kinases (Rubtsov and Rudensky, 2007). These kinases induce the phosphorylation of receptor-regulated SMAD (R-SMAD) proteins, thereby releasing them from the plasma membrane-anchoring protein SARA (SMAD anchor for receptor activation) (Rubtsov and Rudensky, 2007). After forming homo-oligomeric complexes, as well as hetero-oligomeric complexes with a 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 the germline C α gene promoter (Pardali *et al.*, 2000; Park *et al.*, 2001; Rubtsov and Rudensky, 2007). These SMAD complexes further associate with constitutive and TGF β R-induced cofactors, including RUNX3, cyclic AMP response element-binding protein (CREB) as well as PU.1 and Ets-like factor 1 (ELF1), which bind to RUNX-binding elements (RBEs), cyclic AMP response element (CRE), and Ets-binding sites, respectively (Cerutti, 2008b; Lin and Stavnezer, 1992; Shi *et al.*, 2001; Xie *et al.*, 1999). Of note, TGF- β 1 signals IgA CSR in B cells also with the help of microbial TLR ligands such as LPS (Cerutti, 2008b; Coffman *et al.*, 1989; Kaminski and Stavnezer, 2006, 2007; Tsuji *et al.*, 2008, 2009). TLRs activate B cells by recruiting the adaptor protein MyD88, which in turn activates IKK and triggers nuclear translocation of NF- κ B by interacting with interleukin-1 receptor-associated kinase 1 (IRAK1), IRAK4, TRAF6, and TGF β -activated kinase 1 (TAK1) (Medzhitov, 2001; Takeda *et al.*, 2003). After its induction by TLRs, NF- κ B may enhance IgA CSR and production by amplifying the expression of AID (Cerutti, 2008b; Cerutti and Rescigno, 2008). Consistent with this possibility, lack of MyD88 severely impairs IgA CSR in B cells from Peyer's patches (Suzuki *et al.*, 2010; Tezuka *et al.*, 2007).

3.5. TD signals for intestinal IgA secretion

The mechanisms underlying skewed IgA CSR and production at mucosal sites are incompletely understood, but the fact that TGF- β is very abundant in intestinal follicles certainly plays an important role (Cerutti and Rescigno, 2008; Coffman *et al.*, 1989; Craig and Cebra, 1971; Weinstein and Cebra, 1991). IL-2, IL-4, IL-5, IL-6, IL-10, IL-21, and VIP (vasoactive intestinal peptide) are also involved in intestinal IgA production and, together

with TGF- β , originate from multiple cell types, including DCs, FDCs, IECs, stromal cells, and mast cells (Cerutti, 2008b; Cerutti and Rescigno, 2008). While TGF- β predominantly triggers IgA CSR, IL-2, IL-4, IL-5, IL-6, and IL-21 augment the differentiation of IgA-expressing B cells into IgA-secreting plasma cells by signaling through STAT proteins, including STAT3 (Avery *et al.*, 2010; Bryant *et al.*, 2007; Cerutti and Rescigno, 2008). This transcriptional activator triggers the upregulation of B lymphocyte-inducing maturation protein-1 (Blimp-1), a transcriptional repressor that promotes plasma cell differentiation by turning off germinal center-retaining transcription factors such as B cell lymphoma-6 (Bcl-6) (Calame, 2001; Reljic *et al.*, 2000). Other cytokines such as IL-4 and VIP may enhance the formation of IgA class-switched B cells by stimulating naïve B cells to produce autocrine TGF- β in response to CD40L (Cerutti and Rescigno, 2008; Fujieda *et al.*, 1996; Zan *et al.*, 1998).

3.6. Homing of intestinal IgA-producing B cells

The mechanisms underlying the homing of IgA class-switched B cells from the inductive sites of intestinal follicles to the effector site of the lamina propria are beyond the scope of the present review. Suffice to say that IgA class-switched follicular B cells acquire gut-homing properties upon exposure to retinoic acid (RA), an immunoregulatory factor released by specific intestinal DC subsets (Mora *et al.*, 2006, 2008). DCs as well as other cell types such as IECs produce RA from its precursor vitamin A (retinol) through a pathway involving the enzyme RALDH (retinaldehyde dehydrogenase) (Mora *et al.*, 2008). In the presence of RA, IgA-expressing B cells upregulate the expression of $\alpha 4\beta 7$, a gut-homing receptor that interacts with the adhesion molecule MadCAM-1 (mucosal adhesion cell-associated molecule-1) expressed by high endothelial venules in the intestinal lamina propria. Interaction of $\alpha 4\beta 7$ with Mad-CAM-1 permits IgA-expressing B cells emerging from Peyer's patches to colonize the intestinal lamina propria (Mora *et al.*, 2008). Furthermore, RA upregulates the expression of the chemokine receptor CCR9, which allows IgA-expressing B cells to respond to the IEC chemokine CCL25 (Mora *et al.*, 2008). Once they reach the intestinal lamina propria, IgA-expressing B cells receive more signals from IEC, DC, macrophage, and stromal cytokines, such as BAFF, APRIL, IL-6 and IL-10, and RA itself, which promote the formation, maturation, and survival of IgA-secreting plasma cells (Cerutti, 2008b; Cerutti and Rescigno, 2008). In addition to inducing gut-homing receptors on IgA-producing B cells and enhancing plasma cell differentiation and IgA secretion, RA further facilitates IgA-dependent intestinal homeostasis by promoting the generation of Tregs and Th17 cells. Treg cells trigger IgA CSR in intestinal follicles, whereas Th17 cells might facilitate IgA transcytosis across IECs in the intestinal lamina propria (Cong *et al.*, 2009; Jaffar *et al.*, 2009; Murai *et al.*, 2010; Tsuji *et al.*, 2009; Weaver and Hatton, 2009).

4. MUCOSAL IgA PATHWAYS NOT INVOLVING T CELLS

4.1. Function of intestinal TI antibody responses

Conventional TD antigens require 5–7 days to develop systemic IgG responses. Intestinal IgA responses may have a more prolonged latency, because IgA-producing B cells need to migrate from the inductive site of Peyer's patches to the effector site of the lamina propria in order to become plasma cells and release IgA. To compensate for this limitation, the intestinal mucosa has developed a faster TI pathway that generates IgA in response to highly conserved antigenic determinants on commensal bacteria and pathogens (Macpherson *et al.*, 2008; Suzuki and Fagarasan, 2009). This TI pathway involves specialized subsets of B cells such as peritoneal B-1 cells, which can rapidly produce IgA in the absence of help from CD4⁺ T cells via CD40L, at least in mice (Fagarasan and Honjo, 2000). In general, TI antibody responses lead to the generation of unmutated IgA (and IgM) that have low affinity

but high avidity for antigen (Macpherson *et al.*, 2008). In the intestine, these low-affinity antibodies are thought to provide a first barrier against commensals as well as limited protection against some pathogens (Cerutti and Rescigno, 2008; Macpherson *et al.*, 2008). Although lacking canonical B-1 cells, humans may have B cell subsets functionally equivalent to B-1 cells such as IgM⁺IgD^{low}CD27⁺ B cells (Weller *et al.*, 2004). These B cells are typically present in the circulation and marginal zone of the spleen, which is a lymphoid area highly responsive to blood-borne TI antigens, but might also colonize other lymphoid districts, including the subepithelial dome of Peyer's patches (Weill *et al.*, 2009). Similar to intestinal IgA-producing B cells from rabbits, IgM⁺IgD^{low}CD27⁺ B cells are prediversified in that they express somatically mutated V(D)J genes that encode Ig proteins with variable degrees of affinity for multiple types of antigens (Lanning *et al.*, 2005; Weller *et al.*, 2001, 2004). It remains to be established whether IgM⁺IgD^{low}CD27⁺ B cells or other similar B cell types can participate in TI IgA responses.

4.2. TI IgA production in intestinal follicles

Peyer's patches may comprise alternative pathways for the induction of IgA in addition to the canonical TD pathway involving cognate T-B cell interaction. Indeed, Peyer's patches from mice lacking BCR, HLA-II, CD28 (a co-stimulatory molecule critically involved in cognate T-B cell interaction and essential for the generation of functional germinal centers in systemic lymphoid follicles), or CD40 completely or partially retain their ability to undergo IgA CSR and production, at least in mice (Bergqvist *et al.*, 2006, 2010; Casola *et al.*, 2004; Gardby *et al.*, 2003). As already discussed, stimulation of FDCs by local commensal bacteria triggers production of innate IgA-inducing factors such as TGF- β , BAFF, and APRIL (Badr *et al.*, 2008; Chiu *et al.*, 2007; El Shikh *et al.*, 2009; Gorelik *et al.*, 2003; Rahman and Manser, 2004; Suzuki *et al.*, 2010). By cooperating with signals from BCR, complement receptors and possibly, TLRs generated by antigen exposed on the surface of FDCs, TGF- β , BAFF, and APRIL would initiate IgA CSR in B cells (Suzuki *et al.*, 2010). An additional follicular site that likely supports TI IgA CSR is that of isolated lymphoid follicles (Tsuji *et al.*, 2008). These solitary structures appear throughout the intestine immediately after birth in relationship to intestinal colonization by bacteria, require lymphoid tissue-inducer cells for their formation, and consist of solitary B cell clusters built on a scaffold of stromal cells with rare, interspersed T cells and abundant perifollicular DCs (Hamada *et al.*, 2002). Stimulation of TLRs on lymphoid tissue-inducer cells, stromal cells, and DCs by TLR ligands from commensal bacteria appears to trigger a complex cross talk that ultimately leads to TI IgA CSR via a pathway that requires matrix metalloprotease-dependent processing of LAP into active TGF- β (Tsuji *et al.*, 2008; Suzuki *et al.*, 2010).

4.3. TI IgA production in the intestinal lamina propria

Mice lacking Peyer's patches, mesenteric lymph nodes, and isolated lymphoid follicles retain variable amounts of IgA-producing plasma cells in the lamina propria, suggesting that organized lymphoid structures are not absolutely required for intestinal IgA production (Fagarasan and Honjo, 2003; Kang *et al.*, 2002; Yamamoto *et al.*, 2000, 2004). As extensively discussed in other reviews (Cerutti, 2008b; Cerutti and Rescigno, 2008; Fagarasan and Honjo, 2003; Fagarasan *et al.*, 2010), an alternative site for the induction of IgA is the nonorganized lymphoid tissue of the intestinal lamina propria (Fig. 2.3). Indeed, the intestinal lamina propria contains molecular hallmarks of ongoing IgA CSR, including S α -S μ switch circles, *AICDA* transcripts, and AID protein, albeit to a lesser degree than Peyer's patches (Crouch *et al.*, 2007; Fagarasan *et al.*, 2010; Shang *et al.*, 2008). Furthermore, the intestinal lamina propria contains IgM⁺ B cells that can undergo IgA CSR in response to appropriate signals, including TGF- β , BAFF, and APRIL from local DCs and stromal cells (Fagarasan *et al.*, 2001; He *et al.*, 2007; Shang *et al.*, 2008; Tezuka *et al.*, 2007). BAFF and APRIL activate IgA CSR by engaging calcium-modulating cyclophilin-

ligand interactor (TACI) on B cells (Castigli *et al.*, 2005a,b; He *et al.*, 2007, 2010a; Litinskiy *et al.*, 2002; von Bulow *et al.*, 2001). The existence of TI pathways for IgA production is consistent with the persistence of intestinal IgA in mice and humans lacking CD40L or CD40 (Bergqvist *et al.*, 2006; Castigli *et al.*, 1994; Ferrari *et al.*, 2001; He *et al.*, 2007; Xu *et al.*, 2008). Interestingly, intestinal IgA is conserved in humans lacking CD4⁺ T cells as a result of HIV-1 infection (He *et al.*, 2007; Xu *et al.*, 2009). Conversely, intestinal IgA is depleted in a strain of mice lacking APRIL (Castigli *et al.*, 2004). Furthermore, IgA responses to TI antigens are impaired in mice lacking TACI and humans harboring mutations in the gene encoding TACI (Castigli *et al.*, 2005a,b; von Bulow *et al.*, 2001). Finally, the presence of TI IgA CSR in the intestinal lamina propria has been recently confirmed in AID-green fluorescent protein reporter mice (Crouch *et al.*, 2007). Consistent with its inductive role, the human intestinal lamina propria contains B cells that exhibit traces of local clonal expansion and express γ -H2AX, a nuclear protein that targets dsDNA breaks introduced in S regions by AID during CSR (He *et al.*, 2010b; Yuvaraj *et al.*, 2009). The presence of IgA CSR in the lamina propria is also in general agreement with the presence of CSR and even SHM in extrafollicular areas of systemic lymphoid organs (Herlands *et al.*, 2008; MacLennan and Vinuesa, 2002; MacLennan *et al.*, 2003). In humans, lamina propria CSR allows B cells emerging from Peyer's patches to replace IgA1 with an IgA2, which has a hinge region shorter than that present in IgA1 (He *et al.*, 2007). This structural feature would make IgA2 more resistant than IgA1 to degradation by IgA-targeting proteases released by bacteria, which are particularly abundant in the distal intestine (Cerutti, 2008b; Kett *et al.*, 1986, 1995; Macpherson and Harris, 2004; Plaut *et al.*, 1974).

4.4. TI signals for intestinal IgA CSR and secretion

As discussed earlier, BAFF and APRIL trigger CSR by engaging TACI on B cells. Similar to CD40, TACI undergoes ligand-induced oligomerization and thereafter recruits TRAF adaptor proteins, which elicit IKK activation and NF- κ B nuclear translocation (Fig. 2.4). This pathway would be critical for the induction of *AICDA* transcription, but is unlikely to play a major role in germline C α gene transcription, which seems to require additional signals from cytokines such as TGF- β or IL-10 (He *et al.*, 2010a; Litinskiy *et al.*, 2002; Suzuki *et al.*, 2010; Tsuji *et al.*, 2009). Induction of IgA CSR and production by BAFF and APRIL is further enhanced by microbial TLR ligands (He *et al.*, 2007; Xu *et al.*, 2007, 2008). Indeed, B cell-intrinsic signals from TLRs play an important role in both TD and TI antibody responses, including IgA responses (Bernasconi *et al.*, 2002; Han *et al.*, 2007; He *et al.*, 2004; Herlands *et al.*, 2008; Lin *et al.*, 2004; Pasare and Medzhitov, 2005). The mechanism underlying TLR-mediated IgA CSR and production remains poorly understood, but induction of NF- κ B by TLRs likely plays an important role (He *et al.*, 2007; Xu *et al.*, 2007, 2008). Similar to DCs and macrophages, B cells recruit the adaptor protein MyD88 to a cytoplasmic Toll-interleukin-1 receptor (TIR) domain of TLRs (Takeda *et al.*, 2003). MyD88 forms a signaling complex with multiple downstream elements, including IRAK1, IRAK4, and TRAF6, thereby causing activation of the IKK complex, phosphorylation and degradation of I κ B α , and nuclear translocation of NF- κ B (Takeda *et al.*, 2003). Once in the nucleus, NF- κ B binds to and transactivates the *AICDA* gene promoter, thereby initiating AID expression (Cerutti, 2008b). NF- κ B also binds to a κ B site on the C α gene promoter, but this site has little or no role in C α gene transcription (Cerutti, 2008b; Shi *et al.*, 2001). In addition to enhancing IgA CSR, TLR signals cooperate with IL-4, IL-5, IL-6, and IL-10 to induce plasma cell differentiation of IgA class-switched B cells and IgA secretion (Cerutti, 2008b). Plasma cell differentiation and IgA secretion may be also enhanced by BAFF and APRIL through a pathway involving B cell maturation antigen (BCMA) and possibly also TACI, BAFF receptor (BAFF-R, also known as BR-3), and heparan sulfate proteoglycans

(Avery *et al.*, 2003; Benson *et al.*, 2008; Castigli *et al.*, 2007; Huard *et al.*, 2008; O'Connor *et al.*, 2004; Sakurai *et al.*, 2007).

5. DENDRITIC CELL AND MACROPHAGE SIGNALS IN MUCOSAL IgA PRODUCTION

5.1. DC role in intestinal homeostasis

DCs are at the center of virtually all signaling networks underlying immune protection and homeostasis in the intestinal mucosa (Iwasaki, 2007). DCs sense the presence of commensals and pathogens by recognizing highly conserved microbial signatures through multiple families of pattern-recognition receptors, including TLRs (Kelsall and Rescigno, 2004; Takeda *et al.*, 2003). Signals emanating from TLRs stimulate mucosal DCs to initiate both innate and adaptive immune responses against invasive bacteria in a non-redundant manner (Banchereau and Steinman, 1998; Coombes and Powrie, 2008; Rescigno and Di Sabatino, 2009). However, TLR signals are also critical for the intestinal innate and adaptive immune systems to acquire information as to the type and composition of the local microbiota (Uhlig and Powrie, 2003). This information is processed and integrated with signals originating from phagocytic, epithelial, stromal, and neuroendocrine cells to generate a noninflammatory mucosal environment capable of supporting a peaceful and mutually beneficial relationship with the local microbial ecosystem (Rescigno *et al.*, 2008). The preservation of homeostatic conditions involves continuous TLR-dependent activation of DC programs that stimulate immune protection while attenuating inflammation (Laffont and Powrie, 2009; Reis e Sousa, 2006). Indeed, inflammation can disrupt the delicate epithelial barrier separating the contents of the intestinal lumen from the sterile environment of the intestinal lamina propria, an event that would put at risk the survival of the host. A recently identified DC strategy to generate intestinal immunity without causing inflammation consists in dampening the survival of commensal bacterial species with higher inflammatory potential through innate TNF-dependent mechanisms activated by the T-box transcription factor T-bet (Garrett *et al.*, 2007). Another important DC strategy involves the stimulation of massive amounts of IgA antibodies through multiple follicular and extrafollicular B cell pathways that operate with or without help from T cells (Cerutti, 2008a; Cerutti and Rescigno, 2008; Macpherson, 2006).

5.2. DC sampling of intestinal antigens

Intestinal DCs comprise distinct subsets with different phenotype, localization, and function (Iwasaki, 2007; Kelsall and Rescigno, 2004). A key role of intestinal DCs is to initiate high-affinity IgA responses in Peyer's patches through a process involving activation of antigen-specific CD4⁺ T cells endowed with B cell helper function (Macpherson *et al.*, 2008). Intestinal DCs accomplish this task after capturing antigen in the subepithelial dome of Peyer's patches, a follicle-associated mucosal area specialized in "controlled" antigen entry (Neutra, 1999). Indeed, the sub-epithelial dome is topped by a follicle-associated epithelium that comprises numerous M cells (Neutra, 1999). These epithelial cells filter bacteria through a highly specific glycocalyx and eventually sample them through receptor-dependent mechanisms that remain largely unknown (Neutra and Kozlowski, 2006). Poorly defined IgA receptors would allow M cells to sample IgA-coated commensals (Kadaoui and Cortes, 2007), whereas a glycoprotein 2 (GP2) receptor enables M cells to sample IgA-free commensals as well as some pathogens (Hase *et al.*, 2009). Sampled antigen is eventually transferred to DCs, which occupy large invaginations of the basolateral membrane of M cells (Neutra and Kozlowski, 2006). The details of M cell-DC interaction remain unclear, but it is likely that these cell types mutually influence their functions. In addition to capturing antigen from M cells, DCs directly sample antigen from the intestinal lumen by forming dynamic transepithelial projections that establish intimate contacts with

the surrounding IECs through tight junctions (Chieppa *et al.*, 2006; Rescigno *et al.*, 2001). Antigen-loaded DCs migrate from the subepithelial dome into the perifollicular area of Peyer's patches, where they present antigen to CD4⁺ T cells (Cerutti, 2008b). A similar antigen-presenting process may take place in areas of intestinal villi occupied by isolated lymphoid follicles, as also these structures are capped by a specialized epithelium rich in M cells (Fagarasan *et al.*, 2010; Hamada *et al.*, 2002). Finally, there is the possibility that M cells and DCs from isolated lymphoid follicles directly present antigen to B cells to initiate IgA responses in a TI fashion (Batista and Harwood, 2009; Batista *et al.*, 2001; Bergtold *et al.*, 2005; Tsuji *et al.*, 2008).

5.3. DC induction of intestinal IgA via Th2 cells

Antigen-sampling DCs prime perifollicular CD4⁺ T cells without promoting their differentiation into inflammatory Th1 effector cells (Cerutti, 2008b; Coombes and Powrie, 2008; Rescigno and Di Sabatino, 2009). Consistent with this notion, noninflammatory Th2 cytokines like IL-4, IL-6, and IL-10 are more abundant than inflammatory Th1 cytokines like IFN- γ in Peyer's patches (Gonnella *et al.*, 1998; Okahashi *et al.*, 1996; Xu-Amano *et al.*, 1993). In general, Th2 cytokines such as IL-10 inhibit macrophage release of inflammatory mediators, including TNF- α and IL-23, whereas Th1 cytokines such as IFN- γ have the opposite effect (Coombes and Powrie, 2008; Mazmanian and Kasper, 2006). Moreover, Th2 cytokines are also more effective than Th1 cytokines in inducing production of noninflammatory antibody isotypes such as IgA (Cerutti, 2008b; Fagarasan *et al.*, 2010). Conversely, Th1 cytokines are more effective than Treg and Th2 cytokines in inducing production of inflammatory antibody isotypes such as IgG (Stavnezer, 1996). The intestinal mucosa maintains a homeostatic balance between noninflammatory IgA-inducing and inflammatory IgG-inducing T cell subsets by delivering "conditioning" signals to DCs via thymic stromal lymphopoietin (TSLP) from IECs (Soumelis *et al.*, 2002). This IL-7-like molecule mediates an IEC-DCs cross talk that is critical for the establishment of mucosal homeostasis (Ziegler and Liu, 2006). Of note, IECs likely release TSLP upon sensing TLR ligands from commensals (Rimoldi *et al.*, 2005b). Signaling from TSLP leads to increased DC production of IL-10, which in turn promotes differentiation of IgA-inducing Th2 cells by suppressing DC release of IL-12 (Cerutti, 2008b; Rimoldi *et al.*, 2005a,b).

5.4. DC induction of intestinal IgA via Treg and Tfh cells

In addition to inducing differentiation of noninflammatory Th2 cells, IEC-conditioned DCs promote the formation of noninflammatory Treg cells. Indeed, peripheral naïve T cells differentiate into Treg cells upon exposure to intestinal CD103⁺ DCs (Coombes and Powrie, 2008). Of note, CD103 is an α E β 7 integrin that functions as a receptor for the epithelial molecule E-cadherin, suggesting that CD103 facilitates the cross talk between IECs and DCs (Coombes and Powrie, 2008; Rescigno and Di Sabatino, 2009). Mucosal DCs induce Treg cell differentiation by releasing TGF- β 1 and RA, two mediators also involved in the induction of IgA (Cerutti, 2008b; Coombes and Powrie, 2008; Mora and von Andrian, 2008; Sun *et al.*, 2007). Consistent with this, Treg cells stimulate intestinal production of noninflammatory IgA in addition to suppressing inflammatory T cells, again underscoring the intertwined nature of the signaling networks mediating intestinal homeostasis (Cong *et al.*, 2009; Tsuji *et al.*, 2009). The mechanisms by which Treg cells initiate IgA CSR and production are the object of intense investigation. One study shows that intestinal Treg cells migrate to Peyer's patches, where they differentiate into Tfh cells through a CD40-dependent process that requires collaboration with local DCs and/or B cells (Tsuji *et al.*, 2009). The resulting Tfh cells would induce GC formation, IgA synthesis, and plasma cell differentiation through a mechanism that could involve release of IL-21 and TGF- β 1 (Dullaers *et al.*, 2009; Spolski and Leonard, 2010). Another study shows that antigen-

specific Treg cells stimulate intestinal IgA production by directly activating B cells via TGF- β 1 (Cong *et al.*, 2009).

5.5. DC subsets with B cell-activating function

In addition to activating B cells through Th2 and Treg cells, intestinal DCs release powerful B cell-stimulating factors such as BAFF and APRIL upon sensing microbial products through TLRs (He *et al.*, 2007; Shang *et al.*, 2008). BAFF and APRIL not only promote the survival of B cells and plasma cells but also activate IgA CSR and production in the absence of any help from CD40L (Castigli *et al.*, 2004, 2005b; Dillon *et al.*, 2006; Hardenberg *et al.*, 2008; He *et al.*, 2007; Litinskiy *et al.*, 2002; O'Connor *et al.*, 2004; Schneider, 2005; Schneider *et al.*, 1999). It remains unclear whether BAFF and APRIL are released by an intestinal DC subset specialized in providing help to B cells. Recent studies show that commensal bacteria induce intestinal iNOS⁺/TNF⁺ DCs phenotypically and functionally similar to systemic iNOS⁺/TNF⁺ DCs induced by infection with *Lysteria monocytogenes* (Serbina *et al.*, 2003; Tezuka *et al.*, 2007). In the intestinal lamina propria, iNOS⁺/TNF⁺ DCs would promote TI IgA responses by releasing large amounts of BAFF and APRIL in response to nitric oxide (Tezuka *et al.*, 2007). iNOS⁺/TNF⁺ DCs would also colonize Peyer's patches, where they enhance TD IgA responses by upregulating the expression of TGF- β receptor type-II on follicular B cells through a mechanism that, again, implicates nitric oxide (Tezuka *et al.*, 2007). Additional intestinal DCs with potential IgA-inducing function include CD11b⁺ DCs from Peyer's patches, which release IgA-inducing cytokines such as IL-10 and TGF- β in response to microbial TLR ligands or T cell CD40L (Iwasaki, 2007; Sato *et al.*, 2003). Similar DCs release IL-6, a cytokine implicated in the differentiation of IgA class-switched B cells into IgA-secreting plasma cells (Jego *et al.*, 2003; Sato *et al.*, 2003). Of note, mucosal DCs are involved not only in the inductive phase, but also in the effector phase of intestinal IgA responses (Cerutti, 2008b). Indeed, DCs from Peyer's patches can upregulate the expression of specific gut-homing receptors such as $\alpha_4\beta_7$ integrin and CCR9 on IgA class-switched B cells by producing RA (Mora *et al.*, 2008). This process enables IgA class-switched B cells to migrate from Peyer's patches and mesenteric lymph nodes to the intestinal lamina propria via the thoracic duct and circulation under the influence of chemokines released by IECs, including CCL25 (Mora *et al.*, 2008). During this migration, IgA class-switched B cells become IgA-secreting plasmablasts and plasma cells upon exposure to maturation signals generated within mesenteric lymph nodes and lamina propria (Cerutti, 2008a; Cerutti and Rescigno, 2008).

5.6. DC induction of intestinal IgA with no help from T cells

Originally described for their ability to prime and differentiate T cells (Banchereau and Steinman, 1998), DCs can also activate B cells (Fayette *et al.*, 1997; Wykes *et al.*, 1998). Indeed, DCs can specifically take up blood-borne bacteria and subsequently enter the bridging channels of the spleen (Balazs *et al.*, 2002; MacLennan and Vinuesa, 2002). There, DCs trigger TI production of antigen-specific IgM antibodies by activating marginal zone B cells through BAFF and APRIL (Balazs *et al.*, 2002; MacLennan and Vinuesa, 2002). Similarly, DCs from lymph nodes can present antigen to specific follicular B cells, which then migrate to extra-follicular areas through a BCR-dependent process (Qi *et al.*, 2006). Antigen presentation to B cells would involve sampling of immune complexes through poorly characterized receptors, including Fc γ RIIB, and subsequent B cell internalization of these complexes into a nondegradative endocytic pathway (Bergtold *et al.*, 2005). Recycling of antigen-containing vesicles to the cell surface enables DCs to present intact TI antigens to B cells (Bergtold *et al.*, 2005). This process would lead to B cell activation and proliferation through a mechanism involving engagement of BCR and TLRs by antigen. DCs also acquire TI IgA-inducing functions upon receiving activating signals from microbial TLR ligands (He *et al.*, 2007; Litinskiy *et al.*, 2002; Macpherson and Uhr, 2004; Poeck *et al.*, 2004; Xu *et*

et al., 2007). Together with IECs and stromal cells, these IgA-inducing DCs would account for the induction of TI IgA responses in the intestinal lamina propria and perhaps the subepithelial dome of Peyer's patches (Cerutti, 2008b). In addition to BAFF and APRIL, gut DCs produce RA and IL-6 in response to microbial TLR ligands (Mora *et al.*, 2006; Sato *et al.*, 2003). Besides facilitating the migration of IgA class-switched B cells to the gut lamina propria (Mora *et al.*, 2006), RA and IL-6 enhance IgA production by increasing IgA CSR as well as plasma cell differentiation and IgA secretion (Mora and von Andrian, 2008; Sato *et al.*, 2003; Tokuyama and Tokuyama, 1999; Watanabe *et al.*, 2010). Accordingly, vitamin A-deficient or IL-6-deficient mice have less IgA-producing B cells in the lamina propria, but normal numbers of IgM-expressing B cells in PPs (Mora *et al.*, 2006; Ramsay *et al.*, 1994). Of note, a recently described subset of lamina propria CD11c^{hi}CD11b^{hi} DCs expressing TLR5 but not TLR4 can generate IgA⁺ plasma cells from naïve B cells through a TI pathway that does not seem to involve BAFF and APRIL (Uematsu *et al.*, 2008). This pathway involves DC production of RA and IL-6 in response to engagement of TLR5 by the bacterial protein flagellin (Uematsu *et al.*, 2008).

5.7. DC induction of intestinal IgA upon antigen sampling

Although the acquisition of IgA-inducing activity by DCs clearly requires the presence of commensals, the mechanism by which these commensals deliver activation signals to DCs remains poorly understood. One possibility is that these signals are generated as DCs sample commensals from the intestinal lumen. Indeed, nonmigratory DCs expressing the fractalkine receptor CX₃CR1 continuously sample antigens from the lower segment of the small intestine by extending transepithelial projections without disrupting interepithelial tight junctions. Of note, transepithelial sampling requires IEC expression of CX₃CL1 (fractalkine), the ligand for CX₃CR1 (Chieppa *et al.*, 2006). In this process, commensal bacteria play a fundamental role, because antibiotic treatment markedly reduces the number of transepithelial DC extensions (Chieppa *et al.*, 2006; Rescigno, 2009). After capturing commensal antigens, DCs may be capable of directly presenting them to subepithelial B cells (Batista and Harwood, 2009; Cerutti, 2008a). The ensuing stimulation of both somatically recombined (i.e., BCR) and germline gene-encoded (i.e., TLRs) antigen receptors would initiate TI IgA CSR and production, particularly in the presence of co-signals from appropriate cytokines (Cerutti, 2008b). In mice, intestinal DCs initiate TI production of low-affinity commensal-reactive IgA antibodies by presenting antigens from commensal bacteria to peritoneal and perhaps lamina propria B-1 cells (Macpherson and Uhr, 2004; Macpherson *et al.*, 2000). These B-1-activating DCs likely include TNF⁺iNOS⁺ DCs, which trigger IgA responses by stimulating B cells through BAFF, APRIL, and NO (Tezuka *et al.*, 2007). Of note, germ-free mice as well as mice lacking TLR2, TLR4, or TLR9 have no intestinal TNF⁺iNOS⁺ DCs and show severely decreased intestinal IgA production (Tezuka *et al.*, 2007), which demonstrates that signals from commensals are critical for DCs to induce IgA. Importantly, IgA production is also impaired in iNOS-deficient mice, but this impairment can be reversed by adoptively transferring lamina propria iNOS⁺ DCs from wild-type animals (Tezuka *et al.*, 2007), further underscoring the key role of intestinal DCs in IgA production. In humans, intestinal lamina propria DCs express IgA-inducing factors such as BAFF and APRIL through a pathway that likely involves activation of DCs by microbial TLR ligands and epithelial cytokines such as TSLP (He *et al.*, 2007; Xu *et al.*, 2007, 2008). As already discussed, DCs would trigger TI IgA production not only in the nonorganized lymphoid tissue of the lamina propria but also in isolated lymphoid follicles (Cerutti, 2008b; Tsuji *et al.*, 2008). B cells colonize these solitary lymphoid structures in response to TLR-dependent signals from commensals and locally undergo TI IgA CSR and production upon exposure to antigen and cytokines from local DCs and stromal cells, including TGF- β , APRIL, BAFF, and IL-6 (Tsuji *et al.*, 2008).

5.8. Macrophage involvement in induction of intestinal IgA

Similar to DCs, mucosal macrophages can be an important source of immunoregulatory molecules with IgA-inducing properties (Craxton *et al.*, 2003; Litinskiy *et al.*, 2002). Recently, published studies show that intestinal lamina propria CD11b⁺F4/80⁺CD11c⁻ macrophages are hyporesponsive to TLR stimulation and spontaneously produce large quantities of IL-10 (Denning *et al.*, 2007; Smythies *et al.*, 2005). Consequently, these macrophages suppress the differentiation of Th1 and Th17 cells, but promote the differentiation of IL-10-producing Treg cells (Denning *et al.*, 2007), which may contribute to the induction of IgA CSR and production in Peyer's patches and perhaps lamina propria (Tsuji *et al.*, 2008, 2009). The mechanisms by which intestinal macrophages acquire noninflammatory properties remain unclear, but IEC factors such as TGF- β may be implicated. TGF- β would impair macrophage secretion of inflammatory cytokines without compromising phagocytosis (Smythies *et al.*, 2005). It remains to be established whether macrophages can initiate commensal-specific IgA CSR and production as DCs do.

6. EPITHELIAL CELL SIGNALS IN MUCOSAL IgA PRODUCTION

6.1. IECs mediate frontline immunity

Originally thought to function only as a physical barrier against bacteria, IECs are now recognized as central players in the signaling networks required for the maintenance of intestinal homeostasis (Artis, 2008). Indeed, IECs continuously educate the intestinal immune system as to the composition of the local microbiota through a process that involves microbial sensing through multiple pattern-recognition receptors such as TLRs and Nod-like receptors (NLRs) (Bouskra *et al.*, 2008; Rakoff-Nahoum *et al.*, 2004). Surface and intracellular TLRs recognize microbial membrane structures and nucleic acids such as LPS, peptidoglycan, flagellin, and double- or single-stranded DNA and RNA (Takeda *et al.*, 2003), whereas intracellular NLRs detect microbial peptidoglycan components such as mesodiaminopimelic acid and muramyl dipeptide (Fritz *et al.*, 2006). Signals emanating from TLRs and NLRs enable IECs to collaborate with the intestinal immune system to promote both intestinal homeostasis and immunity (Abreu, 2010; Hill and Artis, 2010; Hooper and Macpherson, 2010). Remarkably, numerous immune mediators released by IEC can modulate IgA CSR and production either by directly activating B cells or by enhancing the B cell-stimulating function of DCs (Cerutti, 2008b; Cerutti and Rescigno, 2008).

6.2. IECs cross talk with DCs, macrophages, and T cells

The intestinal mucosa contains multiple DC subsets that drive noninflammatory responses, including IgA production, even upon exposure to classical inflammatory stimuli (Coombes and Powrie, 2008). This circumstance suggests that signals originating in the intestinal microenvironment shape the development of DCs and their precursors to generate mucosal DCs (Kelsall and Rescigno, 2004). Unlike any other lymphoid environment, the intestine contains sophisticated epithelial cells and therefore it is not surprising that epithelial signals are central to the functional reprogramming ("mucosalization") of the precursors of intestinal DCs (Iliev *et al.*, 2007). And indeed, DCs derived from mice with impaired IEC capacity to respond to bacteria show dysregulated expression of inflammatory cytokines and develop spontaneous intestinal inflammation (Nenci *et al.*, 2007; Zaph *et al.*, 2007). Remarkably, many of the IEC factors involved in intestinal homeostasis also enhance IgA CSR and production. One of these factors is TSLP, an IL-7-like cytokine that suppresses DC-mediated differentiation of IgG-inducing Th1 cells, but enhances DC-mediated differentiation of IgA-inducing Th2 and Treg cells (Cerutti and Rescigno, 2008; Iliev *et al.*, 2009a,b; Rimoldi *et al.*, 2005a,b; Soumelis *et al.*, 2002; Ziegler and Liu, 2006). TSLP further promotes the formation of an IgA-rich mucosal environment by stimulating DC production of BAFF, APRIL, and IL-10 (He *et al.*, 2007; Xu *et al.*, 2007). In addition to

TSLP, IECs release factors such as TGF- β , RA, and chemokines that stimulate the induction and recruitment of tolerogenic DCs, macrophages, and Treg cells (Butler *et al.*, 2006; Denning *et al.*, 2007; Iliev *et al.*, 2009a; Rimoldi *et al.*, 2005b). IECs may further enhance intestinal tolerance and IgA production by presenting antigen to intra- and subepithelial CD4⁺ T cells through MHC-II molecules (Hershberg and Mayer, 2000; Hershberg *et al.*, 1998; Kaiserlian, 1999; Kaiserlian and Vidal, 1993). Given that IECs lack the co-stimulatory molecules required for optimal T cell stimulation, such as CD80 (B7.1) and CD86 (B7.2), antigen presentation by IECs would promote preferential expansion of Treg cells, which, as discussed earlier, play an important role in the induction of IgA (Artis, 2008; Cerutti and Rescigno, 2008; Fagarasan *et al.*, 2010; Westendorf *et al.*, 2009).

6.3. IECs cross talk with B cells

In addition to delivering activating and conditioning signals to DCs, macrophages, and T cells via TSLP and RA, IECs can directly communicate with B cells by releasing BAFF and APRIL (He *et al.*, 2007; Kato *et al.*, 2006; Shang *et al.*, 2008; Xu *et al.*, 2007). These IEC cytokines may not only trigger IgA CSR in lamina propria B cells but also enhance the survival of lamina propria IgA-secreting plasma cells (Belnoue *et al.*, 2008; Cerutti, 2008a,b; Cerutti and Rescigno, 2008; Dillon *et al.*, 2006; He *et al.*, 2007; Huard *et al.*, 2008; Litinskiy *et al.*, 2002; O'Connor *et al.*, 2004; Sakurai *et al.*, 2007; Shang *et al.*, 2008). In humans, APRIL from IECs also elicits IgA2 CSR (He *et al.*, 2007). Consistent with this, APRIL is very abundant in the lamina propria of the distal intestine, which constitutes the major site of IgA2 production (Crago *et al.*, 1984; He *et al.*, 2007; Kett *et al.*, 1986, 1995). IECs release APRIL in response to bacteria and their products through a mechanism that requires TLR signaling via MyD88 (He *et al.*, 2007), which would explain the strong correlation between IgA2 and intestinal areas heavily colonized by bacteria such as the distal intestine. IEC production of APRIL would induce direct IgM-to-IgA1 CSR, IgM-to-IgA2 CSR as well as sequential IgA1-to-IgA2 CSR (He *et al.*, 2007). This latter would allow IgA1-expressing B cells arriving from PPs to acquire a novel C α 2 region, which is more resistant than C α 1 to degradation by bacterial IgA proteases (Plaut *et al.*, 1974). To exert their IgA CSR-inducing activity in an optimal fashion, BAFF and APRIL require co-signals from IL-10 (in humans) or TGF- β (in mice), two cytokines released by IECs, DCs, and macrophages (He *et al.*, 2007; Litinskiy *et al.*, 2002; Tsuji *et al.*, 2008). Additional signals from BCR or TLRs further enhance IgA CSR and promote expansion and differentiation of IgA-producing B cells, suggesting that BAFF and APRIL activate B cells as they interact with DCs loaded with commensal bacteria (He *et al.*, 2007; Macpherson and Uhr, 2004).

7. CONCLUSIONS

IgA plays a key role in the establishment of intestinal homeostasis and immunity. Indeed, this mucosal antibody isotype can afford both immune protection and immune exclusion without causing a tissue-damaging inflammatory reaction. Several TD and TI mechanisms have been described for the induction of IgA responses in the intestine. These mechanisms implicate both follicular and extrafollicular B cell pathways that function in the presence or absence of help from CD4⁺ T cells and give rise to IgA antibodies with either high or low affinity for antigen. While high-affinity IgA is critical to neutralize pathogens, low-affinity IgA would play an important role in immune exclusion. Remarkably, growing evidence indicates that intestinal IgA responses require microbial activation of intertwined innate signaling networks linking IECs with DCs, macrophages, T cells, and B cells. In the presence of signals from the local microbiota, IECs instruct DCs and macrophages to initiate defensive innate and adaptive immune responses without causing inflammation. These responses include the differentiation of noninflammatory Th2, Treg, and Th17 cells that activate B cells and induce them to undergo IgA CSR and production. Alternatively, IECs

activate B cells in concert with DCs, FDCs, and macrophages to initiate IgA CSR and production through an alternative TI pathway. The precise mechanisms by which the innate immune system generates noninflammatory tolerance against commensals and inflammatory immunity against pathogens and the precise role of IgA in the discrimination between these opposite responses remain a major enigma in the field. Yet, there is little doubt that a better understanding of the innate immunoregulatory networks operating in the intestinal mucosa will open exciting opportunities for the design of novel vaccines against intestinal pathogens such as HIV and more effective therapies against intestinal inflammatory disorders such as Crohn's disease and ulcerative colitis.

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FIGURE 2.1.

TD induction of IgA CSR and production in intestinal Peyer's patches. DCs located in the subepithelial dome (SED) of Peyer's patches capture SIgA-bound or free microbial antigens by interacting with M cells or by extending dendritic projections into the lumen of the gut. While capturing antigen, DCs are exposed to "conditioning" factors such as TSLP and RA, which are produced by IECs in response to microbial TLR ligands. TSLP and RA prevent antigen-loaded DCs from inducing inflammatory Th1 responses. Instead, TSLP- and RA-primed DCs migrate to the perifollicular area to promote differentiation of naïve CD4⁺ T cells into Treg and Th2 cells. These noninflammatory T cells induce IgA CSR and production by activating B cells through CD40L and cytokines such as IL-4, IL-10, and TGF- β in the context of an antigen-driven cognate T-B cell interaction. TSLP- and RA-primed DCs further enhance IgA CSR and production by releasing IL-6, IL-10, and TGF- β 1. Of note, Treg cells can further differentiate into Tfh cells, which trigger IgA CSR and production through IL-21 and TGF- β 1. In the germinal center of Peyer's patches, B cells undergo SHM in addition to completing IgA CSR. In the presence of antigen-exposing FDCs, SHM facilitates the selection of B cells expressing high-affinity IgA. FDCs also enhance IgA CSR and production by releasing BAFF, APRIL, and TGF- β 1 in response to microbial TLR ligands. After emerging from the germinal center, high-affinity IgA class-switched B cells enter the circulation and migrate to the lamina propria of the intestine, where they differentiate into long-lived IgA-secreting plasma cells.

FIGURE 2.2.

Signaling events in TD IgA CSR and production. TGF- β 1 from T cells, DCs, FDCs, IECs, macrophages, and stromal cells forms a heteromeric TGF receptor (TGFR) complex on B cells that activates SMAD proteins. In the presence of TGF- β 1, TGF β RII kinases phosphorylate TGF β RI, leading to the activation of TGF β RI kinases. These kinases induce the phosphorylation of receptor-regulated SMAD (R-SMAD) proteins, 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 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 ($C\alpha$) gene promoters. These SMAD complexes further associate with constitutive and TGF β R-induced cofactors, including 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 together with PU.1. CD40L expressed on T cell surface elicits oligomerization of CD40 on B cells, recruitment of TRAFs to CD40, activation of the IKK complex, and phosphorylation and degradation of I κ B. The resulting I κ B-free NF- κ B proteins translocate to the nucleus to induce transcription of the *AICDA* gene promoter and AID expression.

FIGURE 2.3.

TI induction of IgA CSR and production in the intestinal lamina propria. IECs sense microorganism through TLRs and thereafter release APRIL, which triggers direct IgM-to-IgA1 CSR in lamina propria IgM⁺ B cells and sequential IgA1-to-IgA2 CSR in lamina propria IgA1⁺ B cells. TLR-activated IECs further amplify TI IgA CSR and production by stimulating DCs, including antigen-sampling CX3CR1⁺ DCs, iNOS⁺TNF⁺ DCs, and CD11c^{hi}CD11b^{hi}TLR5⁺ DCs, through TSLP and RA. Together with stromal cells, macrophages, and lymphoid tissue-inducing cells (not shown), DCs would elicit IgA CSR and production in the nonorganized lymphoid tissue of the lamina propria or organized isolated lymphoid follicles (not shown) by releasing BAFF, APRIL, IL-6, IL-10, and TGF- β 1, and by engaging BCR and TLRs through antigen. The resulting IgA class-switched B cells differentiate into short-lived IgA-secreting plasma cells.

FIGURE 2.4.

Signaling events in TI IgA CSR and production. TGF-β1 from T cells, DCs, FDCs, IECs, macrophages, and stromal cells forms a heteromeric TGF receptor (TGFR) complex on B cells that activates SMAD proteins. In the presence of TGF-β1, TGFβRII kinases phosphorylate TGFβRI, leading to the activation of TGFβRI kinases. These kinases induce the phosphorylation of receptor-regulated SMAD (R-SMAD) proteins, 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 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 *Ca* gene promoters. These SMAD complexes further associate with constitutive and TGFβR-induced cofactors, including 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 together with PU.1. BAFF and APRIL from IECs, DCs, FDCs, macrophages, and stromal cells elicit oligomerization of TACI on B cells, recruitment of TRAFs to TACI, activation of the IKK complex, and phosphorylation and degradation of IκB. The resulting IκB-free NF-κB proteins translocate to the nucleus to induce transcription of the *AICDA* gene promoter and AID expression.