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Diverse regulatory pathways for IgA synthesis in the gut

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Immunoglobulin (Ig) A is the most abundant antibody isotype in mucosal secretions. In this study we summarize recent advances in our understanding of the compartments and mechanisms of intestinal IgA synthesis. We discuss the pathways leading to the generation of IgA⁺ B cells in follicular and extra-follicular structures, by T-cell-dependent and T-cell-independent mechanisms.

In mammals, the gut is populated with an extremely dense and diverse bacterial community. The colonization of the intestine with bacteria is invariably associated with a prompt and abundant generation of immunoglobulin A (IgA) from the B cells present in the gut-associated lymphoid tissues. Upon secretion into the intestinal lumen, IgA provide protection against pathogens, and has a key role in selection and maintenance of gut microbiota. Thus, from studies in mice, it is thought that hypermutated high-affinity IgA neutralize toxins and drive diversification of bacterial communities, whereas nonmutated natural IgA limit the penetration of commensal bacteria below the intestinal epithelial surface.^{1,2}

GENERATION OF IgA⁺ B CELLS IN PEYER'S PATCHES

IgA⁺ B cells are mostly generated in germinal centers (GCs) of Peyer's patches (PPs) on activation of B cells and induction of activation-induced cytidine deaminase (AID), a key enzyme required for class-switch recombination (CSR) and somatic hypermutation. The formation

of GCs as well as AID-dependent genetic alterations in Ig genes require the help of T cells.³ PP GCs have several unique characteristics. First, GCs are found continuously in PPs because of constant stimulation by gut bacteria. Second, GCs in PPs but not in peripheral lymph nodes can be induced in the absence of antigen-specific recognition through B cells receptors, given that B cells are activated by bacterial antigens through innate immune receptors (Toll-like receptor (TLR)-MyD88 signaling pathway) and also receive T-cell help. Third, some of the helper T cells in PP GCs but not peripheral lymph node GCs are derived from the "suppressor" Foxp3⁺ T cells that lost the Foxp3 expression and differentiated into T_{FH} cells.⁴ Fourth, GC B cells in PPs undergo CSR almost exclusively to IgA. The skewed CSR to IgA is thought to be the result of metabolic products and cytokines generated by activated epithelial cells (ECs), dendritic cells (DCs), B cells, and T cells (extensively reviewed by Cerutti and Rescigno²).

Transforming growth factor (TGF)- β 1 is an essential cytokine that directs IgA

switching, and mice rendered deficient for TGF- β RII have extremely few IgA⁺ B cells in PPs. Many cells in PPs, i.e., Foxp3⁺ T cells, CXCR5⁺ T_{FH} cells, DCs, or B cells express TGF- β 1 transcripts. However, this cytokine is embedded as inactive form in a large latent complex. Therefore, for its function, TGF- β 1 requires liberation from a large latent complex and activation, by several molecules, such as matrix metalloproteinase (MMP)-9 and MMP13, integrins (α v β 8), or thrombospondin-1. Nevertheless, which cell types and how they generate active TGF- β 1 in PP GCs remained unclear.

For generation of antigen-specific IgA⁺ B cells in PPs, TGF- β 1 cooperates with CD40 ligand (CD40L), a tumor necrosis factor (TNF) family member expressed by T_{FH} cells in GCs (**Figure 1a**). The recent findings that Foxp3⁺ T cells differentiate into T_{FH} cells almost exclusively in the PPs raise the possibility that T_{FH} cells derived from Foxp3⁺ T cell may be superior to other T_{FH} cells for helping IgA switching. How exactly Foxp3⁺-derived T_{FH} cells help generation of IgA⁺ B cells in PPs is not known. Interestingly, T_{FH} cells secrete interleukin (IL)-21, and a recent *in vitro* study indicated that IL-21 in synergy with TGF- β 1 enhances both the proliferation and differentiation of precursors for IgA plasma cells.⁵ These processes appear to be negatively regulated by IL-4, a cytokine produced by CD4⁺ T cells. Accumulating evidence indicate that DCs, conditioned by ECs, critically contribute to IgA synthesis in PPs.² PP DCs activated by bacteria and by ECs through thymic stromal lymphopoietin (TSLP) and retinoic acid (RA) produce TGF- β 1 and IL-6 that facilitate CSR and generation of IgA plasmablasts, respectively. After upregulation of gut-homing receptors, such as CCR9 and integrin α 4 β 7 (RA effect) and type 1-sphingosine 1-phosphate receptors, and

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Figure 1 Pathways for the generation of IgA in the gut. **(a)** T-dependent generation of IgA⁺ cells in germinal centers (GCs) of Peyer's patches (PPs). Dendritic cells (DCs) located beneath M cells uptake luminal bacteria and migrate to the T-cell zone, where they activate T cells. Activated CD4 T cells express CXCR5 and migrate toward CXCL13 into the FDC network, where they provide help for activated B cells (T_{FH} cells). Some T_{FH} cells are derived from Foxp3⁺ T cells that lost the Foxp3-expression. Conventional B–T interactions through major histocompatibility complex (MHC) II–T-cell receptor (TCR) and CD40–CD40L is critical for the activation of B cells, induction of activation-induced cytidine-deaminase (AID), and class-switch recombination (CSR)/somatic hypermutation in GCs of PPs. Preferential generation of immunoglobulin (Ig)A⁺ cells in PPs is due to the abundant production of activated transforming growth factor (TGF)- β 1 and interleukin (IL)-21 in this environment. Alternatively, B cells can be activated in the absence of cognate B–T interactions, through toll-like receptors (TLRs) and co-receptors (such as CD40). **(b)** T-independent generation in isolated lymphoid follicles (ILFs). Unlike within the follicles of PPs, generation of IgA⁺ cells within ILFs does not require T cells. Tumor necrosis factor (TNF) α is abundantly produced by activated DCs and also by LT α cells. Upon TNF α stimulation, stromal cells (SCs) and DCs express high levels of matrix metalloproteinases (MMPs), which mediate conversion of TGF- β 1 from inactive to active form. Together with BAFF and APRIL produced by DCs, active TGF- β 1 facilitates preferential switching of TLR-activated B cells from IgM to IgA. **(c)** T-independent generation in lamina propria (LP). IgM⁺ B cells (naïve B2 or peritoneal B1 cells) attracted in gut LP by chemokines produced by TLR-stimulated epithelial cells (ECs), are activated by polyclonal stimuli or by antigens presented by LP DCs. In the presence of TGF- β 1, BAFF, and APRIL (the later produced also by the ECs, upon bacteria/thymic stromal lymphopoietin (TSLP) stimulation), activated B cells expressing AID undergo preferential switching to IgA. TLR5⁺ DCs help IgA switching through their production of retinoic acid (RA). Factors produced by SCs and LP DCs (IL-6, IL-10, BAFF, and APRIL) also facilitate differentiation of IgA⁺ B cells into plasma cells.

downregulating the CXCR5 expression (IL-21 effect), IgA⁺ plasmablasts migrate from the PPs to the gut lamina propria (LP), where they further differentiate into IgA plasma cells.

GENERATION OF IgA⁺ B CELLS IN ISOLATED LYMPHOID FOLLICLES

Although important, PPs are not the only site for intestinal IgA production. Small follicular structures scattered throughout the intestine, called isolated lymphoid follicles (ILFs), have been documented in both mice and humans. At least in mice, ILFs serve as inductive sites, where precursors for IgA plasma cells are generated. ILFs represent perhaps the most dynamic compartment of the gut that function as

sensor of intestinal bacteria. Not only that the development of ILFs requires bacteria, but also the size, the cellular composition, and the IgA synthesis in ILFs depend on the bacterial load in the intestine. Hyperplasia of ILFs correlates with the expansion of Gram-positive anaerobes in the gut of AID-deficient mice that lack IgA. The ILF hyperplasia is abolished by antibiotic treatment or when AID^{-/-} mice are reconstituted with IgA-plasma cells. The contribution of ILFs to the generation of IgA in the absence of PPs was recently shown by studies in retinoic acid-related orphan receptor- γ t (ROR γ t)^{-/-} mice. In ROR γ t^{-/-} mice that lack PPs and ILFs, the frequency of IgA plasma cells increased considerably on reconstitution

with ROR γ t⁺ LT α cells, which induced the formation of ILFs. Many B cells in newly induced ILFs expressed AID, as well as the surface and cytoplasmic IgA, thus providing direct evidence for class switching of B cells to IgA-producing cells *in situ* in ILFs.⁶ Interestingly, the B-cell activation and generation of IgA⁺ B cells within ILFs do not require T cells (**Figure 1b**).

How are the B cells activated in ILFs and what factors direct their switching from IgM to IgA even in the absence of T cells or GCs?

The epithelium that covers ILFs contains M cells capable of up-taking and presenting intestinal bacteria to ILF DCs. ILF DCs may also sample bacteria directly from the lumen. Activated gut DCs

produce IL-6, TNF α , B-cell-activating factor of the TNF family (BAFF), and a proliferation-inducing ligand (APRIL), as well as cytokines capable of inducing T-cell-independent IgA production in the gut. Unlike peripheral lymph node DCs, gut DCs express high levels of MMP9 and MMP13, and at least *in vitro*, can activate TGF- β 1.⁶ The major source for TGF- β 1 in ILF is unknown, although we speculate that stromal cells (SCs) may produce most of the TGF- β 1, whereas ILF DCs together with ROR γ t⁺ LTi cells (conspicuously found in ILFs) enhance its activation through secretion of TNF α and MMPs. Recently, a subset of DCs expressing inducible nitric oxide synthase (iNOS) was found to enhance IgA production in murine gut. Nonetheless, the presence of iNOS⁺ DCs in the ILFs and their relationship with other DCs subsets or SCs present in and outside the ILFs remained unclear.

GENERATION OF IgAs IN LAMINA PROPRIA

In the absence of PPs or ILFs, IgA⁺ B cells and plasma cells can be generated directly in LP. This is shown by the presence of IgA plasma cells in mutant mice lacking PPs and ILFs (i.e., ROR γ t^{-/-}, Id2^{-/-} mice). It should be noted that the frequency of IgA plasma cells in the gut-associated lymphoid tissue-deficient mice varies depending on the age, the mouse strain, and the bacterial load in the gut. Compared with wild-type mice, Id2^{-/-} mice have normal, if not more, gut IgA plasma cells, yet very few IgA plasma cells could be detected in the LP of Id2^{-/-} MyD88^{-/-} mice.⁷ Thus, recognition of bacteria through the TLR-MyD88 pathway is essential for IgA generation in the absence of follicular structures.

The fact that B cells undergo CSR in the LP was first suggested by the detection of several molecular markers, such as C α germ line, AID, and α -circle transcripts (α CT-short-lived transcripts initiated from the I α promoter located in the circular DNA that is looped out during class switching to IgA) in gut B cells outside PPs. However, other studies failed to detect these markers in LP. These contradictory results suggest that the efficiency of CSR to IgA⁺ cells in LP

might be low (indeed, in a clean facility it took 5 months for ROR γ t^{-/-} mice to reach the IgA levels equivalent to those in 2-month-old wild-type mice) and that the interpretation of the results depends on the experimental procedures (i.e., whether sorting of B cells were performed or not). The detection of α CTs seems to be the most problematic, and indeed, reliable PCR products of these short-lived transcripts can be obtained only when at least few hundred cells have just completed CSR. However, the expression of AID by the LP B cells was convincingly shown recently, using AID-GFP reporter mice or by *in situ* AID-staining methods in both mice and humans.^{6,8,9}

T-CELL-INDEPENDENT IgA GENERATION IN LP

Although activated CD4⁺ T cells from LP may help IgA class switching through their co-stimulatory molecules and secretion of cytokines (i.e., CD40L, IL-10, TGF- β 1), growing evidence indicate that T cells are not absolutely necessary for the generation of IgA responses in the gut (Figure 1c). Indeed, intestinal IgA responses are retained in mice and humans lacking CD40 or T cells.²

Toll-like receptor signaling in the intestinal ECs promotes the recruitment of B cells to the LP through the increased production of chemokines, such as CCL20 and CCL28.¹⁰ These B cells are naive B2 cells or peritoneal B1 cells, mobilized from the peritoneal cavity upon downregulation of CD9 and integrins in response to TLR stimulation. Furthermore, ECs, LP SCs, and peritoneal B1 cells stimulated through TLRs secrete APRIL and BAFF. In addition, TSLP (possibly nitric oxide) derived from TLR-stimulated ECs augments production of BAFF and APRIL by LP DCs. The engagement of the receptor transmembrane activator and CAML interactor (TACI) may be sufficient to induce CSR in LP B cells that are activated by antigens presented by CX₃CR1⁺ LP DCs or by polyclonal stimulation by microbes.² Consistent with this idea, mice lacking APRIL, TACI, or iNOS and humans expressing mutant TACI molecules exhibit selective IgA deficiency. BAFF and its receptors, B-cell maturation antigen and BAFF receptor

(BAFF-R), may be involved in survival of IgA⁺ B cells and plasma cells. Indeed, overexpression of BAFF results in B-cell hyperplasia and a considerable increase in IgA plasma cells in the gut LP.

Recent data indicate that a subset of LP DCs expressing TLR5 may help the generation of IgA⁺ B cells *in situ*, through their production of RA.⁷ These TLR5⁺ DCs were also shown to facilitate differentiation of T_H17-producing cells in the small intestine LP. Nevertheless, if the RA-producing LP DCs are similar to the CD11c⁺CD70⁺CX₃CR1⁺ DC subset, inducing T_H17 cells in response to bacteria is currently unknown. Also, whether T_H17 cells contribute to IgA synthesis in gut remains to be investigated. At least in mice, most of the T-independent pathways for generation of IgA⁺ B cells in the small intestine apparently require TGF- β 1. LP SCs and ECs appear to be the main source of TGF- β 1 in gut, although CD103⁺ DCs may also contribute. In humans, and in the murine large intestine, T-cell-independent stimuli, such as BAFF, APRIL, and TLR ligands, might be sufficient to drive CSR to IgA even in the absence of TGF- β 1.

CONCLUSION

It is becoming increasingly clear that co-evolution of mammals with bacteria has contributed to the development of multiple pathways for the generation of IgA responses. The role of IgA in homeostasis of gut flora, which is essential to prevent overstimulation of the whole immune system is well established. We still know little about the role of individual bacterial species in the generation of the gut IgA and their role in induction of immune competence of the systemic immune system. Furthermore, we have a very limited knowledge about how the IgA diversity and quality impact on the composition of commensal bacteria. Addressing these questions will help to understand the basis for the development and function of the immune system, and to manipulate the system and reinforce its fitness.

DISCLOSURE

The authors declare no conflict of interest.

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