MZB1 folding and unfolding the role of IgA

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The immune system sustains a continuous dialogue with the endogenous microbial communities residing at the mucosal surfaces, mediated by many factors, including IgA, the most abundant antibody isotype. In PNAS, Xiong et al. (1) explore the role of a B cell-specific factor in regulation of IgA secretion in the gut.

IgA is crafted and secreted by plasma cells residing mostly in the lamina propria (LP) of the intestinal villi. Once released into the gut lumen, IgA coats bacterial surfaces and regulates the maintenance and function of the microbiota (2). In turn, the microbiota primes and fine-tunes immune cell function, affecting how the host responds to environmental stimuli. The generation and maintenance of the “pool” of IgA at the mucosal surfaces is critical for immune homeostasis. IgA deficiency disturbs the composition of the gut microbiota, leading to allergies, autoimmunity, and other inflammatory diseases in mice and humans (3).

Synthesis of T cell-dependent, adaptive IgA is a regulated, stepwise process requiring (i) activation of IgM⁺ B cells in the germinal center of Peyer’s patches to switch to IgA⁺, directed by activation-induced cytidine deaminase (AID); (ii) migration of IgA⁺ cells to the intestinal LP, directed by integrins and chemokines; (iii) differentiation of plasmablasts into IgA⁺ plasma cells capable of secreting large amounts of protein; and (iv) transport of IgA across the LP epithelium into the gut lumen. Humans secrete an extraordinary amount of IgA each day (40 mg/kg body weight) (3). However, T cell-independent, innate IgA can also be generated from other innatelike B cells, such as those residing in the peritoneal cavity (3). These alternative pathways are characterized by their relative speed and the polyreactivity of the resulting IgA. T cell-independent antibody appears within hours, bridging the lag time between the innate immune responses mediated by granulocytes and macrophages within minutes of antigen exposure and the acquired T cell-dependent immune responses that arrive after a week or more (4). Xiong et al. (1) unfold a new layer of complexity in gut IgA synthesis, identifying the marginal zone B and B-1 cell-specific protein (MZB1) as a molecular chaperone

facilitating the formation of polymeric IgA, especially during rapid responses.

MZB1 is an endoplasmic reticulum (ER)-localized protein constitutively expressed in innatelike B cells, such as marginal zone (MZ) B cells and B1 cells, and highly up-regulated during plasma cell differentiation. In the ER, MZB1 associates with antibody heavy and light chains (HC and LC, respectively) to promote the assembly and secretion of IgM polymers (5, 6). MZB1 also regulates calcium homeostasis and integrin-mediated cell adhesion in innatelike B cells (7). MZB1 is thus critical for the rapid recruitment of B cells and the secretion of polyreactive IgM antibodies during the first-line antibody response. Now, Xiong et al. (1) show that MZB1 is also involved in IgA responses. They begin with the striking observation that the amount of IgA and IgM, but not IgG, was significantly reduced in the serum of *Mzb1*^{−/−} mice. Cultured *Mzb1*^{−/−} splenocytes up-regulated AID, underwent class switch, and differentiated into IgA plasma cells normally, but secreted less IgA (and IgM) on a per-cell basis. These observations led the authors (1) to investigate the molecular mechanisms of MZB1 regulation of early IgA synthesis with elegant molecular cell biology techniques.

To uncover how MZB1 regulates IgA secretion at the cellular level, Xiong et al. (1) inactivated *Mzb1* in a plasmacytoma cell line using CRISPR/Cas9-mediated gene editing. These plasmacytomas were coerced to secrete antibodies after “Ig reconstruction”—retroviral transduction of antibody HC and LC. Like *Mzb1*^{−/−} splenocytes, MZB1-inactivated cell lines secreted significantly lower amounts of IgA compared with MZB1-sufficient cells. In contrast, MZB1-inactivated cell lines produced normal amounts of retrovirally transduced IgG1, suggesting that MZB1 specifically regulates IgA secretion from antibody-producing cells.

MZB1 was identified as part of a molecular complex with several ER-localized chaperones, including BiP and GRP94, and was previously demonstrated to aid the assembly of IgM subunits and the secretion of mature IgM polymers (5, 6). In their study, Xiong et al.

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Fig. 1. MZB1 regulates IgA generation by plasma cells and defends the mucosal barrier during inflammation. (*Right*) In MZB1-sufficient IgA-secreting plasma cells, BiP binds IgA HC, and then MZB1 and J chain associate with the secretory tailpiece to stabilize the HC–LC complex. Upon DSS insult, bacteria infiltrate the LP and stimulate rapid innate IgA production. (*Left*) W27 ameliorates the severity of DSS-induced colitis in *Mzb1*^{−/−} mice. IgA HC–LC complexes are quickly degraded in MZB1-deficient plasma cells. Inefficient IgA secretion in MZB1-deficient mice is associated with the increased percentage of phyla *Proteobacteria* and *Verrucomicrobia* and with reduced *Bacteroidetes*. Dysbiosis in MZB1-deficient mice exacerbates DSS-induced colitis pathology. Oral administration of a monoclonal IgA (W27) restores the gut microbiota, increasing the percentage of *Bacteroidetes* and *Firmicutes* (particularly *Lactobacillus*), shifting the balance away from *Proteobacteria* and *Verrucomicrobia* such as *Akkermansia*. The regulation of gut microbiota composition by IgA might promote anchoring of *Bacteroidetes* in the mucus, allowing other beneficial bacteria members such as *Firmicutes* to thrive.

(1) found that MZB1 coprecipitated with IgA, but not IgG1, even when both shared an identical LC. Together with previous findings showing that MZB1 interacts with the IgM HC, these data point to a role for MZB1 as a chaperone for the formation of multimeric Ig (8). In contrast to MZB1, BiP bound to both IgA and IgG1, while GRP94 did not bind any subclass in these experimental settings, suggesting that each chaperone binds a unique site. Xiong et al. hypothesized that MZB1 might bind to the secretory tailpiece required for the polymerization of both IgM and IgA. Two different IgA mutants were generated, in which either the 18-amino acid secretory tailpiece or its key cysteine residue was disrupted. Immunoprecipitation analyses revealed that both mutant IgA proteins bound to BiP, but not to MZB1, confirming that the MZB1 binding site lies in the tailpiece region of the IgA HC. Interestingly, 10 times less MZB1 bound to IgA HC when it was expressed by cell lines in the absence of LC, whereas BiP strongly interacted with the IgA HC alone. Because IgA HC–LC complexes were degraded within 12 h in the absence of MZB1, Xiong et al. conclude that MZB1 stabilizes the assembled complex rather than its components.

The secretory tailpiece of IgA HC binds to the J chain, a 15-kDa polypeptide that promotes IgA polymerization and transepithelial secretion to the mucosal surfaces. Dimeric IgA and J-chain expression was greatly reduced in sera from *Mzb1*^{−/−} mice as well as in MZB1-deficient cell lines. Thus, MZB1 supports J-chain incorporation into IgA dimers by an as yet unknown mechanism at some point during antibody secretion. Although both MZB1 and J chain were critical for successful IgA dimerization, the 2 did not

coprecipitate. Xiong et al. (1) propose a model in which BiP, MZB1, and J chain bind sequentially to HC before (BiP) and after (MZB1, J chain) LC complex formation, to support the secretion of dimeric IgA (Fig. 1).

Having established the importance of MZB1 for the stability of dimeric IgA complexes, Xiong et al. (1) next asked whether MZB1 deficiency affected the mucosal IgA compartment. Unexpectedly, in steady-state conditions, *Mzb1*^{−/−} mice had normal fecal IgA levels. However, compared with *Mzb1*^{+/+} mice, the amount of fecal IgA was significantly decreased in *Mzb1*^{−/−} mice 1 d after lipopolysaccharide injection into the peritoneal cavity. This rapidly induced IgA is probably derived from peritoneal B1 cells which migrate into the gut LP and differentiate into IgA plasma cells (3). Furthermore, *Mzb1*^{−/−} mice were also more susceptible to chemical [dextran sodium sulfate (DSS)]-induced colitis. In normal animals, fecal IgA and IgM levels surged during colitis; however, no increase was seen in *Mzb1*^{−/−} mice. In line with these IgA measurements, microbiome analysis showed that fecal bacteria were similar between control mice and *Mzb1*^{−/−} mice in steady state, yet diverged during colitis.

To understand the physiological importance of IgA up-regulation during acute inflammation, Xiong et al. (1) supplemented *Mzb1*^{−/−} mice with a monoclonal IgA (W27) in drinking water during the induction of colitis. W27 was previously shown (9) to bind to multiple bacteria and to suppress the growth of *Escherichia coli* in vitro, and oral administration can modulate gut microbiota composition to suppress chronic DSS-induced colitis. In Xiong et al., too, administration of W27 restored the tissue damage

and disruption of bacterial populations in DSS-treated *Mzb1*^{−/−} mice to wild-type levels. This observation supports the conclusion that MZB1 aids the rapid generation of polyreactive IgA, providing a critical defense of the mucosal barrier in inflammatory conditions.

It is puzzling that MZB1 deficiency affected innate IgA responses, but not homeostatic IgA production. MZB1 may stabilize IgA only under the transcriptional profile of innatelike B cells differentiating into plasma cells, and not of IgA⁺ B cells programmed within the germinal center microenvironment. The unique requirement for stimulation by Toll-like receptor (TLR) alongside the B cell receptor in MZ and B1 cells may be the key, as MZB1 can influence TLR signaling (7). Alternatively, MZB1 may influence both homeostatic and inflammatory IgA production, but measurement of fecal IgA levels is not sensitive enough to discern subtle changes in the *in vivo* secretory potential of IgA plasma cells. Indeed, IgA trapped within the intestinal mucus layer together with bacteria is not detected by this method. Similarly, fecal bacterial sequencing provides only partial information regarding the composition or function of microbes in the many physiological niches that exist along the length of the intestinal tract.

How MZB1-dependent IgA protects the mucosal barrier is still mysterious. Xiong et al. (1) found that the chemical-induced colitis, which was aggravated by MZB1 deficiency, associated with an expansion of *Proteobacteria* and *Verrucomicrobia* and a significant reduction of *Bacteroidetes* (Fig. 1). This shift at the phylum level points to an important role for innate-type MZB1-dependent secretory IgA in (i) maintaining *Bacteroidetes* in the colon and (ii) regulating the balance between bacteria belonging to other phyla such as *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia*. In this study, administration of W27 increased the percentage of *Bacteroidetes* and *Firmicutes*, particularly *Lactobacillus*, and decreased the abundance of *Proteobacteria* and *Verrucomicrobia* such as *Akkermansia*. This observation agrees with our recent study (10) showing that

a heavily glycosylated monoclonal IgA binds and “anchors” bacteria belonging to *Bacteroidetes* to the colonic mucus (Fig. 1). *Bacteroidetes* established in their preferred, nutrient-rich mucosal niche facilitate the expansion of *Firmicutes* via the release of metabolites such as short-chain fatty acids (10). These studies point to an unanticipated, complex role mediated by innate, polyreactive IgA for symbiosis and protection of the mucosal barrier.

Future studies that address these remaining issues will not only clarify the function of MZB1, but lead to a deeper understanding of the properties and function of innate versus adaptive IgA. One can speculate that innate IgA is selected to protect mucosal barriers from invasive agents, including bacteria, while adaptive IgA developed to facilitate symbiotic relationships with commensals. In other words, innate IgA eliminates pathogens, while adaptive IgA maintains indigenous bacterial communities within the intestinal tract. The PNAS study presented by Xiong et al. (1) and other recent work suggest that this hypothesis might be too simplistic. Innate IgA may initiate some symbiotic interactions in addition to providing protection against invasive commensals or pathogens. Natural IgA coating might serve as an important niche-targeting factor for certain bacteria, including *Bacteroidetes* that thrive in the mucus layer. In the absence of such primitive IgA, established communities could be less resilient to acute insults such as antibiotics treatment, immune inflammatory diseases, or chemically induced inflammation.

Many diseases of the modern world, such as autism, Parkinson disease, autoimmunity, inflammatory bowel diseases, and endocrine pathologies like diabetes, as well as responsiveness to cancer immunotherapy are influenced by changes in microbiota composition and function. Undoubtedly, this work, together with multiple recent studies not cited here, unfolds and advances our understanding of the role of IgA in health and diseases, with implications extending well beyond the intestine.

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