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INSIGHTS

Mutations make gut antibodies promiscuous

Emilie K. Grasset[1] and Andrea Cerutti[1,2,3]

In this issue, Kabbert et al. (https://doi.org/10.1084/jem.20200275) show that intestinal antibodies from healthy subjects or patients with Crohn's disease cross-target diverse but distinct communities of the gut microbiota through a mechanism involving somatic hypermutation but not germline-encoded polyreactivity.

Kabbert et al. demonstrated that mutations of the antigen-binding Fab domain of gut antibodies from control donors or patients with Crohn's disease (CD) confer cross-species reactivity to the commensal microbiota (Kabbert et al., 2020). Intestinal antibodies include immunoglobulin A (IgA) dimers and IgM pentamers, which accumulate in the gut lumen as a result of a close cooperation between plasma cells and epithelial cells (Chen et al., 2020b). Indeed, binding of the plasma cell–derived joining (J) chain from polymeric IgA and IgM to the epithelial polymeric Ig receptor (pIgR) is followed by IgA and IgM trafficking across the epithelium and leads to the intraluminal accumulation of secretory IgA (SIgA) and SIgM. These antibodies include a secretory component (SC) that derives from the intraepithelial processing of the pIgR (Chen et al., 2020b).

In mice, SIgAs—SIgMs are rare compared with humans (Chen et al., 2020b; Magri et al., 2017)—emerge from complementary T cell–dependent and T cell–independent pathways that generate mutated and unmutated antibodies, respectively (Bunker and Bendelac, 2018). By accumulating mutations in V(D)J genes encoding the antigen-binding Fab domain, SIgAs increase their reactivity to aggressive mucus-penetrant commensals (Bunker and Bendelac, 2018). In contrast, unmutated SIgAs with germline-encoded V(D)J genes display polyreactivity to unrelated soluble antigens in addition to broad microbial reactivity (Bunker and Bendelac, 2018). In humans, SIgAs and SIgMs are mostly mutated, raising questions as to the existence of polyreactive gut antibodies (Pabst and Slack, 2020).

One possibility is that the human gut generates unmutated antibodies only early in life (Chen et al., 2020a; Chen et al., 2020b). These antibodies would progressively hypermutate in response to signals from the intestinal microbiota, which is richer and more complex in humans than in mice (Pabst and Slack, 2020). Of note, gut B cells generate pro-inflammatory IgGs in the presence of inflammatory bowel diseases (IBDs) such as CD and ulcerative colitis (Castro-Dopico et al., 2019; Chen et al., 2020b). These IgGs likely reflect a defensive gut humoral response to invasive pathogenic microbes. But what is the role of antibody mutations in gut homeostasis or inflammation?

To address this question, a well-established methodology (Tiller et al., 2008) was used to generate recombinant monoclonal antibodies from the terminal ileum of control or CD donors (Kabbert et al., 2020). These antibodies were obtained by PCR-amplifying Ig variable heavy chain (VH) and variable light chain (VL) gene products from single-cell sorted gut IgA^+ or IgG^+ plasma cells (see panel A in the figure). Next, VH and VL transcripts were ligated into a heavy chain–encoding plasmid expressing the Cγ1 region and a light chain–encoding plasmid expressing either κ or λ (see figure, panel A). Finally, equal amounts of these plasmids were transfected into human embryonic kidney 293T cells to generate recombinant monoclonal antibodies, which were affinity purified from supernatants (see figure, panel A).

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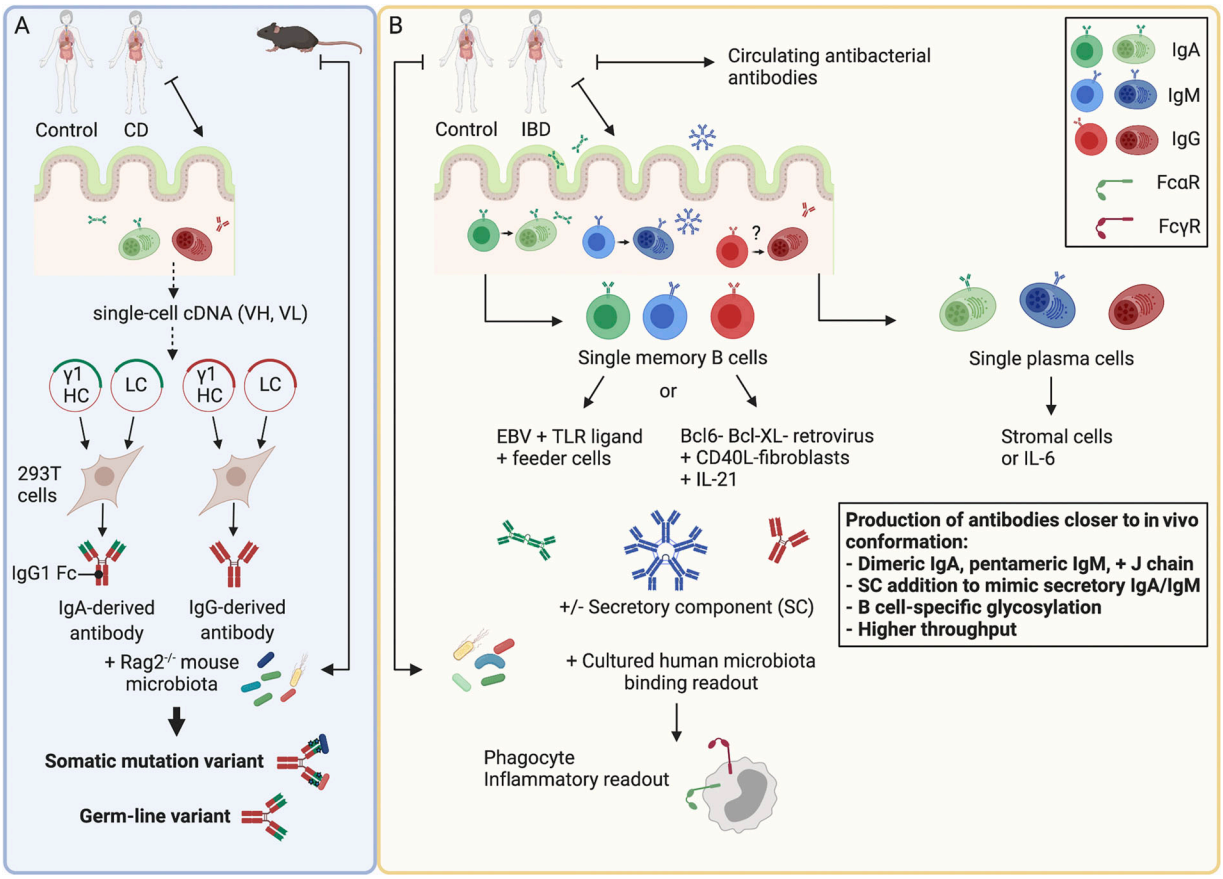
This strategy allowed authors to measure the Fab-mediated binding of IgA or IgG clones to gut bacteria from lymphocyte-depleted *Rag2*-deficient mice (Kabbert et al., 2020). Such mice lack antibodies, which avoided microbiota binding competition between exogenous recombinant and endogenous gut-derived antibodies. DNA sequencing combined with flow cytometry demonstrated that all microbiota-binding antibodies displayed mutated V(D)J genes (Kabbert et al., 2020). In addition, these antibodies showed cross-species reactivity, as they could recognize a diverse set of commensals from nonrelated taxa (Kabbert et al., 2020). Of note, most antibodies lost microbiota reactivity when their mutated

[1]The Immunology Institute, Department of Medicine, Mount Sinai School of Medicine, New York, NY; [2]Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain; [3]Catalan Institute for Research and Advanced Studies, Barcelona, Spain.

Andrea Cerutti: andrea.cerutti@mssm.edu.



Isolation and functional characterization of IgAs and IgGs from human gut. (A) Strategy used by Kabbert et al. (2020) to isolate human IgAs and IgGs from noninflamed or inflamed terminal ileum. (B) Additional strategies for the isolation and functional characterization of human gut IgAs and IgGs. HC, heavy chain; LC, light chain.

V(D)J genes were reverted into a germline configuration (Kabbert et al., 2020). These findings indicate that, in humans, mutations are indispensable to generate cross-species reactivity.

Furthermore, germline-reverted antibodies showed no polyreactivity (Kabbert et al., 2020), which characterizes the unmutated fraction of mouse gut antibodies (Bunker and Bendelac, 2018). Moreover, mutated antibodies retained cross-species reactivity after being deglycosylated (Kabbert et al., 2020), suggesting that glycans do not mediate cross-species reactivity (Chen et al., 2020b; Pabst and Slack, 2020). In general, these results provide strong evidence that, in adult humans, the cross-species reactivity of gut antibodies stems from V(D)J gene mutations.

Consistent with earlier studies (Lindner et al., 2015), pre-existing mutated memory IgA$^+$ or IgG$^+$ B cells may recirculate into preformed mucosal germinal centers to edit their antigen-binding Fab segments. Combined with plasma cell differentiation (Chen et al., 2020b), this process may allow IgAs and IgGs but also IgMs to adjust their Fab-dependent reactivity to small changes of the microbiota, at least in adults (Lindner et al., 2015; Magri et al., 2017). Aside from increasing mutations, more dramatic antigenic changes brought about by CD would bias gut antibodies toward pro-inflammatory IgG instead of non-inflammatory IgA (Chen et al., 2020b).

While elegant and informative, the approaches of this study could be further refined to achieve additional breakthroughs. For instance, cross-species reactivity was tested in gut commensals from *Rag2*-deficient mice (Kabbert et al., 2020). Studies with human gut microbes from culture collections (Palm et al., 2014) would be needed to validate and detail this cross-species reactivity. However, in vitro culture of gut bacteria may cause loss of epitopes linked to cross-species reactivity besides eliminating prebound endogenous antibodies. Stripping these antibodies from the fresh microbiota (Magri et al., 2017) may represent an alternative strategy.

Moreover, the recombinant IgA antibodies generated in this study differed from endogenous intestinal IgA antibodies with respect to some Fab-independent properties, including the presence of a monomeric instead of a dimeric Fc domain, the lack of both J and SC chains, the expression of Cγ1 instead of Cα1 or Cα2, and the induction of antibody glycosylation by 293T cells instead of plasma cells (Kabbert et al., 2020). All these differences could influence both function and reactivity of in vitro assembled IgA antibodies (Chen et al., 2020b; Pabst and Slack, 2020).

To more accurately define the microbiota-binding pattern of gut antibodies, individual memory B cells could be FACS sorted, immortalized, and differentiated into antibody-secreting plasmablasts (see figure, panel B). In this regard, it is worth noting that most gut plasma cells

from healthy adults originate from memory rather than naive B cells (Lindner et al., 2015; Magri et al., 2017). Immortalization of these memory B cells could be achieved through infection with Epstein-Barr virus (Traggiai et al., 2004) or transduction with a lentivirus coexpressing the pro-survival proteins BCL-6 and BCL-xL (Kwakkenbos et al., 2016). These approaches would further involve B cell stimulation with innate Toll-like receptor ligands or a combination of adaptive CD40 ligand and IL-21, respectively (Kwakkenbos et al., 2016; Traggiai et al., 2004). Alternatively, long-term culture of single-sorted plasma cells could be achieved on immortalized stromal cells. For shorter-term cultures, recombinant IL-6 would be sufficient to maintain plasma cell viability (Corti et al., 2011).

Ultimately, these methods would generate long-lived antibody-secreting cells, which could be selected for IgA (IgA1 or IgA2), IgM, or IgG release. Such gut antibodies would be decorated by physiological B cell–derived glycans, which are known to shape both Fab-dependent and Fab-independent facets of gut antibody reactivity (Pabst and Slack, 2020). Furthermore, these gut antibodies would have a physiological oligomeric structure, which also shapes antibody reactivity (Chen et al., 2020b; Pabst and Slack, 2020). Finally, the higher throughput of these methods would allow for the analysis of a broader set of gut antibodies and facilitate their comparison with circulating counterparts. This comparison could provide new insights as to the biology of the circulating antibacterial specificities that often emerge in CD patients (Rieder et al., 2010).

An additional approach would involve the assembly of secretory-like IgAs from polymeric IgAs and recombinant SCs (Longet et al., 2013). Moreover, the development of novel transwell-based co-cultures composed of gut IgA$^+$ plasma cells facing the basolateral surface of a polarized pIgR$^+$ monolayer of epithelial cells could permit the generation of physiological SIgAs. Compared to the monomeric IgAs generated in this study (Kabbert et al., 2020), secretory-like IgAs or bona fide SIgAs would provide more physiological insights into IgA interactions with both gut microbes and immune cells (see figure, panel B). Indeed, the IgA-binding Fcα type I receptor expressed by immune cells such as phagocytes can drive either immune-activating or immune-inhibitory signals when engaged by polymeric or monomeric IgAs, respectively (Chen et al., 2020b). Ultimately, it would be important to functionally evaluate monomeric and polymeric IgAs as well as monomeric IgG. The latter enhances gut inflammation via immune-activating Fcγ receptors (Castro-Dopico et al., 2019).

Aside from elucidating the significance of mutations in human intestinal antibodies, this work (Kabbert et al., 2020) went one step closer to generate the reagents needed to measure the affinity of such antibodies for bacterial antigens and dissect the relationship of this affinity with mutations. Indeed, the correlation of mutations with affinity has never been measured in purified gut antibodies (Pabst and Slack, 2020). Measuring this affinity and its disease-induced changes would advance our understanding of the impact of humoral immunity on the pathogenesis of IBD. It might also lead to the development of novel therapeutic strategies, including gut plasma cell-depleting strategies.

The present work (Kabbert et al., 2020) also suggests strategies for personalized interventions. Recombinant IgAs with known cross-species reactivity to pathogenic commensals could be used in personalized cocktails that may attenuate IBD by reinforcing immune exclusion via bacterial "clumping" (Pabst and Slack, 2020). Furthermore, immortalized IgA$^+$ memory B cells could be used to develop personalized IBD-screening assays involving diagnostic sets of fluorescent bacteria. Finally, B cell–bound fluorescent bacteria could facilitate the identification of dominant IBD-driving commensals.

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