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Do antibodies select a healthy microbiota?

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Abstract

Disruptions to the microbiota can have pathological consequences, which highlights the need to understand the factors that contribute to its stability. Although decades of research have focused on the importance of IgA during pathogenic infection, much of the IgA that is generated in the gut targets the resident commensal microorganisms. Despite this observation, the role of antibodies in regulating microbiota composition remains controversial and poorly understood. Here we propose that antibodies generated in response to microbial colonization of the gut shape the composition of the microbiota to benefit the health of the host through a process that we term antibody-mediated immunoselection (AMIS). Given the exquisite specificity of antibodies and an emerging interest in the use of immunotherapies, we suggest that understanding AMIS of the microbiota will highlight novel uses of antibodies to manipulate microbial communities for therapeutic benefit.

Antibodies in the gut target and control pathogenic infection, but they also target common resident commensal microorganisms. This suggests that immunoglobulins might influence microbiota composition during homeostasis. Although the immune system has evolved many ways to control microorganisms, the antibody response does so in an antigen-specific manner. Recent studies exploiting sequencing innovations together with immunological techniques and ecological theory have begun to demonstrate how host antibody responses might influence microbiota composition and function, a process that we refer to as antibody-mediated immunoselection (AMIS). Immunoselection refers to a process of natural selection within a host organism that is mediated by the immune system to influence microbial fitness, and consequently microbial ecology and evolution. All immune responses can influence microbial fitness, but different individuals do not respond to the same microorganism in the same way. In fact, emerging evidence suggests that even otherwise genetically identical individuals develop largely non-overlapping antibody repertoires[1]. We use the term AMIS to emphasize the potential importance of variability in immunoselection among individuals in shaping microbiota composition. Indeed, a central tenet of this article is that personalized antibody repertoires result in a dynamic pattern of immunoselection that leads to the

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establishment of unique microbial communities among individuals. We briefly review the available scientific literature pertaining to the role of adaptive immunity and IgA antibody responses in the gut to support our hypothesis that a process of AMIS shapes microbiota composition. We also describe how the microbiota can modulate this process, how AMIS promotes a ‘healthy’ microbiota, and how this process might be harnessed to treat diseases associated with dysbiosis (that is, unhealthy host–microbiota interactions).

The factors regulating microbiota composition are diverse and complex, and the hypothesis that adaptive immunity participates in this process is controversial[2]. Much of this debate stems from the difficulties in designing experiments that adequately control for confounding environmental variables that are known to influence microbiota composition. Co-housing of mice has been used in an attempt to address this concern but is insufficient because coprophagy in rodents has a homogenizing effect on microbiota composition. One potential solution to this issue might be to co-house wild-type and mutant animals in partitioned mouse cages that limits the transfer of bedding and faecal material but allows for the exchange of air. Another approach could be to design experiments in such a way that the effects of both the environment and host genetics can be quantified.

Generation of intestinal antibodies

In the gut, naive B cells are found in the immune cell-rich tissues immediately underlying the epithelium (the lamina propria), but they reach their highest concentrations in the specialized gut-associated lymphoid tissues, where they mature into long-lived memory B cells or antibody-secreting plasma cells.

Of the five known antibody isotypes, IgA, IgM and IgG are known to be secreted into the lumen of the gut under steady-state conditions, but IgA is by far the most abundant. Secretory antibodies can exist in polymeric (IgM, IgA) and monomeric forms (IgG). IgM and IgA bind the polymeric immunoglobulin receptor (PIgR), which is expressed on the basolateral surface of gut epithelium, and are transported across the epithelium to the apical surface. Proteolytic cleavage of the antibody-bound portion of PIgR, known as the secretory component, results in the release of secretory IgA and IgM. The secretion of IgG into the gut is mediated by a different transporter known as the Fc neonatal receptor[3]. Although IgA responses have been the primary focus of research into AMIS in the gut, other antibody classes may also influence this process.

IgA targets the microbiota.

Germ-free mice provide a powerful tool to understand the effects of the microbiota on the host[4]. It has long been appreciated that the absence of commensal microorganisms is associated with reduced antibody concentrations in the gut. When germ-free mice are colonized with a complex microbiota, plasma cell abundance rapidly increases in the lamina propria, and faecal IgA concentrations markedly increase[5–7]. Not surprisingly, much of this IgA directly targets the colonizing microorganisms. Various methods have been used to study the coating of particular commensals by IgA. Flow cytometry-based assays suggested that approximately 45% of the microbiota within human faeces is covered with IgA[8]. Using a simpler system, in which germ-free mice were mono-associated with a single bacterial organism, *Morganella morganii*, more than 90% of the bacteria were coated with IgA 70-days post-colonization[7]. Consistent with observations made in humans, animals colonized with a complex microbiota have 10–60% of the bacteria bound by IgA[9–13]. It seems that more bacteria are targeted by IgA within the small intestine than the colon in both mice and humans[12]. Thus, a large proportion of commensals within animals and humans are bound by IgA.

So, which bacterial organisms are targeted by IgA, and do they represent a specialized subset? Using high-throughput sequencing technologies, multiple investigations have shown that IgA binds to a distinct group of bacteria within the colon[10,12,14,15]. A recent study from our laboratory showed that when IgA-bound and IgA-unbound microorganisms are compared to the total community present within the mucosa or lumen, the IgA-bound fraction was more similar to the mucosa community than the faecal community[10], suggesting that IgA tends to target organisms that are more closely associated with host tissues. Supporting this, IgA preferentially targets organisms such as segmented filamentous bacteria, flagellated members of the microbiota belonging to Enterobacteriaceae, and mucus-degrading bacteria such as *Akkermansia muciniphila*, which are all known to localize to host tissue[12–14,16]. Although this is discussed in more detail later, IgA seems to bind organisms that are associated with inflammation and disease such as members of the Enterobacteriaceae[15]. Differences in the physical properties of mucus might influence IgA concentrations in the gut[17,18]. For example, the heavily glycosylated secretory component of dimeric IgA helps to anchor secretory IgA to the mucus lining of the gut[19]. Thus, the increased antibody binding to mucosa-associated organisms could be due to their localization in a site enriched for IgA antibodies. Alternatively, it may reflect a bias in the immune response towards targeting the tissue-associated microbial community, although there is currently no direct evidence to support this possibility.

Effects of antibodies on the microbiota

Antibodies secreted in the gut could directly affect microbial fitness through various effector functions, including agglutination of microbial cells, binding of surface epitopes to block the attachment of microorganisms to the host, opsonization of bacteria to enhance phagocytosis and antigen presentation by dendritic cells in the lamina propria, and neutralization of microbial toxins or other secreted factors. The myriad ways microorganisms have evolved to circumvent this immune defence[20] suggests that antibodies are a potent force of immune selection capable of influencing microbial ecology and evolution. Therefore, AMIS is predicted to have a significant effect on microbiota composition in the gut.

Adaptive immune deficiency alters microbiota composition.

The tremendous functional variability of antibody repertoires is generated by the processes of somatic recombination and receptor editing; the addition of non-templated nucleotides between V, D and J gene cassettes, somatic hypermutation (SHM) and class switch recombination (CSR). The absence of key enzymes involved in these processes causes complete loss of T cells and B cells (such as recombinase-activating gene (RAG) deficiency) and IgA deficiency (such as activation-induced cytidine deaminase (AID) deficiency), which has been shown to be associated with changes to the composition of microbial communities in the steady state.

Three recent studies by Dimitriu *et al.*[21], Zhang *et al.*[22] and Kawamoto *et al.*[23] characterized the importance of adaptive immunity in shaping microbiota composition. All of these studies demonstrated that RAG-deficient mice had significantly altered microbiota composition compared with their respective wild-type controls. However, they identified subtly different effects on the microbiota. Kawamoto *et al.* reported that a lack of adaptive immunity led to a marked reduction in the diversity of microorganisms present in the gut. However, Zhang *et al.* did not report a difference in diversity between wild-type and RAG-deficient animals, but they did show outgrowth of a mucosa-associated organism, *A. muciniphila*. Importantly, when RAG-deficient animals were given a bone marrow transplant to restore adaptive immunity, the levels of *A. muciniphila* were markedly reduced. Dimitriu *et al.* identified increased variation of the microbial communities between RAG-deficient mice, suggesting that adaptive immunity may contribute to stabilizing inter-individual variation around a 'core microbiota'. In particular, organisms from the families Helicobacteraceae and Alcaligenaceae were increased in the absence of B cells and T cells. The families Helicobacteraceae and Alcaligenaceae include members such as *Helicobacter* spp. and *Burkholderia* spp., respectively, which are known to have pathogenic potential. These studies suggest that adaptive immunity promotes diversity of the microbiota while limiting the levels of bacteria found at the mucosa.

Antibody deficiency alters microbiota composition.

Mouse models of B cell and immunoglobulin deficiency are the most direct way of testing the hypothesis that AMIS influences microbiota composition. Indeed, *Ighm*$^{-/-}$ mice, which lack B cells but have normal numbers of T cells, had an overall reduced microbial diversity compared with their *Ighm*$^{+/-}$ littermate controls, similar to what is observed in RAG-deficient animals[23]. Some of the first evidence that AMIS shapes microbiota composition came from studies of *Aicda* (the gene that encodes AID)-knockout mice, which cannot undergo class-switching to the IgA isotype and therefore do not have secretory IgA in the gut[24,25]. Fagarasan *et al.*[25] showed that these animals suffered from a severe lymphoid hyperplasia that was associated with the expansion of anaerobic members of the microbiota. In a follow-up study, secretory IgA-deficient *Rag2*$^{-/-}$ animals had a similar expansion of anaerobic bacteria, and their abundance could be restored to wild-type levels when bone marrow from AID-sufficient (*Aicda*$^{+/-}$) but not *Aicda*$^{-/-}$ mice was transferred to *Rag2*$^{-/-}$ hosts[26]. Finally, using a mouse model in which a knock-in mutation in the *Aicda* gene results in a polymorphism (AID^{G23S}) that allows CSR but severely reduces SHM, it was demonstrated that SHM as well as CSR were crucial for controlling microbiota composition[27]. Supporting these studies, IgA-deficient (*Igha*$^{-/-}$) mice have a unique microbiota composition compared with wild-type controls, in which Proteobacteria outgrowth is a typical feature[28]. Finally, Reikvam *et al.*[29] used 16S ribosomal RNA gene sequencing to profile steady-state microbiota composition across four sites in the gut (faeces, whole caecum, caecal luminal contents and caecal mucosa) between wild-type and PIgR-deficient mice (which are unable to secrete antibodies into the intestinal lumen). Significant differences in microbiota composition were observed across all four sites in PIgR-deficient mice compared with wild-type counterparts with notable decreases in putatively ‘beneficial’ bacteria, such as *Bifidobacterium* spp., and increases in known pathobionts, such as *Helicobacter* spp. Together, these studies indicate the importance of isotype switching and antibody secretion into the gut to prevent the outgrowth of potentially harmful organisms, such as Proteobacteria, and the maintenance of advantageous species.

T cell subsets differentially influence AMIS.

T cells have a central role in promoting antibody responses against commensals. Indeed, $Cd3e^{-/-}$ mice (which possess B cells but lack T cells) had significantly reduced microbiota diversity compared with wild-type controls[23]. B cells can mature into antibody-secreting cells with or without the help of T cells, and these two pathways of terminal B cell differentiation are referred to as T cell-dependent (TD) and T cell-independent (TI) responses (FIG. 1). In general, IgA responses generated in Peyer’s patches involve B2 cell–T cell interactions, which result in the generation of high-affinity and specific IgA responses[30]. B1 cells are thought to contribute to the majority of TI IgA responses that generate low-affinity polyreactive responses within the lamina propria. B1 cell-derived IgA is considered an ‘innate’ form of IgA because its generation is independent of T cell help, and it is the main contributor to the ‘natural’ antibody pool[5,31]. The generation of TD and TI IgA responses in the gut has been extensively reviewed elsewhere[32–34]. However, the relative importance of these two pathways of IgA synthesis on AMIS in the gut has only very recently begun to be appreciated.

Kawamoto *et al.*[23] used adoptive-transfer experiments to show that the quality of the TD IgA response is important for promoting microbiota diversity. Although $Cd3e^{-/-}$ mice had a significantly reduced microbial diversity compared with wild-type mice, the transfer of total naive $CD4^+$ T cells led to an even further decrease in bacterial diversity. However, the transfer of regulatory T (T_{reg}) cells to these mice completely restored the diversity of the microbiota. In addition, the IgA molecules produced in the absence of T_{reg} cells had a low-affinity maturation index, suggesting defective affinity selection within the follicle, but led to an increase in the amount of bacteria that was bound by IgA. We believe these data indicate that forkhead box P3 $(FOXP3)^+$ T_{reg} cells promote the production of more specific IgA molecules that target a narrower range of bacterial species in the gut, in a process similar to that described in FIG. 1. In a subsequent study, Bunker *et al.*[12] compared the composition of IgA-bound bacteria between wild-type and T cell-deficient ($Tcrb^{-/-}Tcrd^{-/-}$) animals and found that TI and TD IgA responses in the gut targeted distinct subsets of commensal microorganisms. Although wild-type and T cell-deficient mice had similar levels of IgA-coated bacteria throughout the intestine, *Mucispirillum* spp. and segmented filamentous bacteria were highly enriched within the IgA-coated fraction. Both of these organisms are known to localize close to the mucosa, which might suggest that TD IgA responses are important for mediating IgA against invasive commensals.

T follicular helper (T_{FH}) cells are one of the most important T cell subtypes involved in TD IgA induction[35]. Using programmed cell death 1 (*Pdcd1*)-knockout animals that have an abnormally high abundance of T_{FH} cells, Fagarasan and colleagues explored how T_{FH} cells influence the structure of the microbiota[9]. $Pdcd1^{-/-}$ mice had undetectable levels of

Bifidobacteria spp. and *Bacteriodes* spp. and markedly higher levels of Enterobacteriaceae compared with wild-type controls. Thus, a deficiency in *Pdcd1* leads to the loss of bacterial members that are associated with a homeostatic environment and the outgrowth of organisms that are associated with pathology. Although these mice had higher levels of free IgA in the gut, the IgA bound to significantly fewer luminal bacteria. *Pdcd1*$^{-/-}$ mice had increased T_{FH} cells and a higher turnover of IgA-producing B cells. Collectively, these results suggest that altering the contribution of TD and TI IgA responses in the gut has important consequences for microbiota composition and host health.

Maternal antibodies define the initial stages of host–microbiota symbiosis.

Maternal antibodies that are passed in milk from a mother to her offspring may also influence microbiota composition. Maternal antibodies control bacterial growth in the neonatal gut and delay the development of antibody responses in the offspring[36]. Indeed, most of the IgA that binds to faecal bacteria during the first few weeks of life comprises passively transferred maternal antibodies. During lactation, plasma cells dramatically increase in number in mammary glands, and secretory IgA antibodies are delivered into the milk duct via PIgR-mediated transport. CC-chemokine receptor 10 (CCR10), which is important for plasma cell recruitment to the gut, is also involved in plasma cell recruitment to the mammary glands, suggesting a common route of migration of plasma cells between the gut and mammary tissues[37]. Indeed, a recent study showed that the IgA-producing plasma cell pool in mammary glands has tremendous overlap with the gut-associated IgA-producing plasma cell repertoires, which suggests that most of the antibodies that are passively transferred in mother’s milk are derived from maternal B cell responses generated against gut antigens[38]. Current research is focused on understanding how maternal antibody transfer influences microbiota community assembly. A recent study demonstrated that maternal antibodies may influence AMIS in the newborn gut[39]. Using a simple but elegant breeding strategy to manipulate maternal IgA transfer, Koch *et al.*[40] crossed *Pigr*$^{+/-}$ females (that are PIgR sufficient) with *Pigr*$^{-/-}$ males, and *Pigr*$^{-/-}$ females with *Pigr*$^{+/-}$ males. *Pigr*$^{+/-}$ and *Pigr*$^{-/-}$ offspring passively received IgA antibodies from the milk of *Pigr*$^{+/-}$ mothers but not *Pigr*$^{-/-}$ mothers. The authors observed markedly different microbiota composition in weanling *Pigr*$^{+/-}$ animals that did and did not receive maternal antibodies. Moreover, animals that received maternal antibodies were less likely to suffer systemic invasion by an opportunistic pathogen and had lower colitis scores in the dextran sodium sulfate (DSS) model of microbiota-induced inflammatory bowel disease. Maternal transmission of IgG can also function to maintain homeostasis within the infant gut by dampening immune responses[40].

These studies provide evidence supporting the hypothesis that maternal antibody transfer represents a vertically transmissible programme of AMIS in the gut. By facilitating the engraftment of microorganisms that are favoured in the maternal gut, this phenomenon could in part account for the high degree of similarity in the microbiota between mother and offspring. Vertical transmission of AMIS may favour the establishment of a beneficial microbiota that has been ‘pre-screened’ by maternal antibodies. Lack of this selective screen could increase the chances of a harmful microbiota developing. This might explain why formula-fed infants have been shown to have a higher incidence of juvenile inflammatory bowel disease and enteric infection than breast-fed infants[41,42].

Immune recognition pathways in AMIS

Commensal bacteria have many ligands and antigens that are recognized by the immune system. Two immune recognition pathways that may have important roles in coordinating antibody responses in the gut and controlling microbiota composition are the Toll-like receptor (TLR) pathway and the antigen presentation pathway, which is mediated by MHC molecules.

Commensal ligands can induce AMIS through TLRs.

TLRs are generally thought to elicit inflammatory responses against pathogenic infection, but a seminal study using mice that lack the TLR signalling molecule MYD88 (myeloid differentiation primary response 88) identified that these animals developed more severe colitis[43]. This was a surprising result given that deletion of *Myd88* should reduce inflammation and therefore theoretically ameliorate intestinal autoimmunity. The observation that *Myd88*$^{-/-}$ animals developed enhanced inflammation suggested a role for TLRs in the maintenance of tolerance. Since then several studies have demonstrated that part of this regulatory role involves the induction of antibodies.

B cells express TLRs, and therefore B cell migration and antibody production can be directly influenced by microbial ligands. For example, TLR ligands present in the serum are important for the recruitment of B1 cells from the peritoneum into the gut[44]. A role for TLR-mediated recognition of microbial ligands by B cells has recently been evaluated by deleting *Myd88* from B cells (B-*Myd88*$^{-/-}$ mice)[45]. These mice are more susceptible to systemic bacterial invasion in response to DSS treatment compared with wild-type animals, and antibiotic treatment ameliorates disease. Sequencing of the bacterial 16S rRNA gene from the livers of B-*Myd88*$^{-/-}$ mice implied an overgrowth of members of the phylum Proteobacteria. These data suggest that loss of B cell-intrinsic TLR signalling altered the microbiota, resulting in a more pro-inflammatory composition that may have contributed to the observed patterns of disease. However, the adaptor protein MYD88 is also important for the TACI (transmembrane activator and calcium-modulating cyclophilin ligand interactor) signalling pathway in B cells, which promotes CSR and TI antibody responses[46]. This was not addressed in this study so it is not known how much of the observed effect by Kirkland *et al.*[45] was due to TLR or TACI deficiency. Also, although it was not shown that the dysbiosis observed in B-*Myd88*$^{-/-}$ animals was due to a deficiency in B1 cell recruitment to the gut, a study by Ha *et al.*[44] supports this conclusion.

Results from experiments in our laboratory demonstrated that animals with a T cell-specific deficiency of *Myd88* (termed T-*Myd88*$^{-/-}$ mice) had a marked reduction in T_{FH} cell numbers in the gut[10]. This led to a loss of germinal centre B cell responses and a failure to target commensal bacteria with IgA. The loss of IgA binding of bacteria in these models correlated with the overgrowth of mucosa-associated bacteria including Desulfovibrionaceae, *Mucispirillum* spp. and *Ruminococcus* spp. These observations further support a role for TD AMIS in controlling communities at the mucosa.

Interestingly, the communities found in T-*Myd88*$^{-/-}$ animals had greater inter-individual variability, suggesting that AMIS functions to constrain and stabilize the resident bacterial communities. The percentage of germinal centre B cells within these animals positively correlated with increased phylogenetic diversity, further suggesting that AMIS also functions to diversify the microbiota.

Finally, the effects on the microbiota have also been well studied in mice that lack TLR5, which is activated by bacterial flagellin. Loss of TLR5 alters microbiota composition[47] and leads to the production of more gut IgA than wild-type controls[16]. However, the IgA produced in *Tlr5*$^{-/-}$ animals fails to target commensal flagellin, which implies that defects in TLR5-mediated IgA responses against the microbiota might contribute to the observed community differences between *Tlr5*$^{-/-}$ and wild-type animals. Interestingly, meta-transcriptomic analysis of the microbial communities from *Tlr5*$^{-/-}$ and wild-type animals showed that flagella-related gene expression was completely absent in wild-type animals but highly expressed in the microbiota of *Tlr5*$^{-/-}$ mice. Moreover, IgA produced in TLR5-sufficient animals directly downregulated the expression of flagellin and immobilized bacteria *in vitro*. Thus, sensing of flagellar ligands by TLR5 promotes an IgA response that induces the downregulation of epitopes that prevent invasion and movement of commensal bacteria. This is consistent with earlier findings showing a role for IgA in regulating epitope expression by commensal microorganisms[48].

Despite the aforementioned evidence, it is still unclear as to what extent TLR signalling influences microbiota composition in the gut. Ubeda *et al.*[1] compared the microbiota composition of wild-type and TLR-deficient littermates from several mouse models of TLR deficiency (that is, *Myd88*$^{-/-}$, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Tlr5*$^{-/-}$ or *Tlr9*$^{-/-}$ animals) and found no detectable effect of host genotype on microbiota composition, and any compositional differences appeared to be solely due to cage and litter effects. This is in stark contrast to many experiments demonstrating differences in some of these deficiency models. The authors suggest that discrepancies between their study and earlier work could be due to differences between mouse colonies reared at different institutions. Alternatively, it is possible that the homogenizing effect of coprophagy during cohousing from birth masked the effect of the host genotype in this study. Clearly, more work is needed to determine whether TLR-mediated immune responses control microbiota composition.

Variable antigen presentation influences IgA responses and microbiota composition.

MHC genes are some of the most polymorphic loci in vertebrates, and thus there is marked inter-individual variation with respect to the set of MHC molecules that are expressed. We recently explored whether natural variation in MHC genes among individuals can drive differences in microbial community structure[11]. In this study, we used three different lines of MHC-congenic animals that have the same genetic background but express different types of MHC molecules and had differing microbiota composition. Differences in the expressed MHC genes led to unique IgA repertoires in each MHC-congenic line, resulting in differential AMIS of the microbiota. Thus, the unique MHC repertoire of individuals might influence, in part, the unique community of commensal organisms found in the gut. This is one of the first studies to demonstrate that naturally occurring genetic variation relevant to

IgA responses in the gut can influence microbiota composition and potentially contribute to the development of a microbiota that is unique to the individual.

How AMIS promotes a healthy gut

The antibody response benefits host physiology in many ways. Broadly, antibody responses in the gut promote health by limiting bacterial dissemination, regulating bacterial virulence factor expression and managing microbiota composition to maintain benign communities. It is not clear what constitutes a ‘benign’ microbial community, and this is an ongoing focus of research in many laboratories. This is complicated by the fact that some commensal microorganisms may have evolved ways to circumvent or exploit antibody responses to facilitate their own colonization or inhibit that of others (FIG. 2). However, numerous studies have now clearly demonstrated that defective or variable antibody responses result in alterations to microbiota composition that can influence an individual’s susceptibility to disease.

Antibody responses limit the inflammatory potential of gut-resident microorganisms by prohibiting their dissemination into the systemic compartment. The first study of *Pigr*$^{-/-}$ mice reported that these animals have a leaky gut phenotype with markedly elevated serum levels of *Escherichia coli*-targeted IgG antibodies[49]. This implies aberrant activation of systemic immunity and outgrowth of potentially disease-promoting members of the Enterobacteriaceae. Supporting this idea, Reikvam *et al.*[29] demonstrated that the altered microbiota community observed in *Pigr*$^{-/-}$ animals was associated with a more severe colitis following DSS treatment compared with wild-type controls and could be ameliorated by antibiotic treatment. Increased dissemination of bacteria into the systemic compartment has also been observed in mouse models of B cell deficiency[27] and defective TD IgA responses[11], and in *Aicda*$^{-/-}$ mice[50]. Collectively, these studies indicate that deficient antibody responses in the gut result in bacterial invasion of host tissues.

Another beneficial role of antibody responses in the gut is the regulation of microbial virulence factor expression. Antibodies can protect host tissues by directly neutralizing microbial toxins in the gut or by downregulating the expression of pro-inflammatory epitopes. For example, J chain-deficient mice that are unable to make secretory IgA cannot neutralize cholera toxin and are significantly more susceptible to diarrhoeal disease[51]. In another study, IgA responses in the gut reduced the expression of inflammation-promoting surface proteins in the commensal microorganism *Bacteroides thetaiotamicron*[48]. Antibodies can also directly influence the expression of virulence factors that promote attachment and invasion of the gut epithelium. IgA responses against *Salmonella* surface antigens have been shown to limit attachment and SPI-1-mediated invasion of gut epithelial cells[52,53]. More recently, it was shown that lower levels of flagellin-specific antibody in *Tlr5*$^{-/-}$ mice increase flagella expression by various commensal Proteobacteria species, leading to increased motility and penetration of flagellated bacteria into the systemic compartment[16]. IgG responses in the gut have also recently been shown to directly limit virulence factors expressed by *Citrobacter rodentium* that facilitate attachment of the bacterium to the gut epithelium[54]. These studies demonstrate that antibody responses in the gut limit infection by enteric pathogens by blocking the effects of virulence factors that promote pathogen colonization. They also demonstrate that antibodies reduce the inflammatory potential of normally occurring commensal species by regulating the expression of pro-inflammatory surface epitopes.

Differences in the quality of the IgA response and their effect on microbiota composition can have important implications for health. As mentioned above, we showed that deficiencies in T_{FH} cell development and IgA responses (in T-*Myd88*$^{-/-}$ animals) resulted in altered IgA targeting of commensals and was associated with the establishment of a more pro-inflammatory microbiota[10]. In a second study, we showed that the naturally occurring polymorphisms in MHC genes resulted in differences in microbiota composition between different lines of MHC-congenic mice, and this influenced colonization resistance against an enteric pathogen[11]. Both of these studies used microbiota transplantations in germ-free mice to demonstrate that defects or naturally occurring variability in AMIS among individuals lead to the establishment of unique microbial communities that can influence host susceptibility to inflammatory and infectious disease in the gut.

An important question is whether antibody responses generated against the microbiota can protect hosts from more dangerous bacterial species through the generation of cross-reactive antibodies. Nunez and colleagues recently addressed this question and demonstrated that the generation of serum IgG antibodies against a widely conserved epitope (murein lipoprotein) from commensal Gram-negative bacteria in the gut conferred protection against systemic invasion by these commensal species as well as against systemic infection by the Gram-negative pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium, which also expresses this epitope[50]. The mechanisms by which the microbiota can influence AMIS are summarized in FIG. 2.

Implications for AMIS in immunotherapy

Currently, there is great interest in the potential of harnessing the immune response to treat a variety of human diseases (such applications are termed immunotherapies). Given the potential importance of antibodies for selection of the microbiota together with the significance of maintaining an appropriate bacterial community structure, we propose that future endeavours could be directed at developing immunotherapies that specifically function to manipulate the microbiota (FIG. 3). The most obvious immunotherapy is direct modulation of the antibody response in the gut through oral vaccination. Oral vaccines could be designed to limit the growth of pathogens or to favour colonization by microbial species that are antagonistic to a pathogen. Alternatively, monoclonal antibodies could be generated that target pathogen virulence factors like flagella or type I pili and could be used therapeutically to treat immune-compromised patients that are unable to mount an effective antibody response to these antigens. The microbiota is known to generate unique metabolites and secrete molecules that positively influence intestinal homeostasis. It is possible that the body generates antibodies to these molecules that interfere with their function or availability. Therefore, blocking antibodies against these novel molecules might also prove to be a useful therapy. As we continue to better understand the function of different organisms within the gut, commensal-targeted antibodies might be used as biomarkers for susceptibility to or diagnosis of certain diseases, similarly to the use of anti-*Saccharomyces cerevisiae* antibodies (ASCAs) in the diagnosis of Crohn disease[55]. A detailed understanding of the nature of antigen selection on developing B cell repertoires is necessary to assess the feasibility of exploiting these potential therapies, and should remain a focus in the IgA field.

Collectively, we believe these studies support an important role for AMIS of the microbiota to promote diversity while also constraining fluctuations that might result in loss of beneficial microorganisms. AMIS of the microbiota also functions to prune the expression of potentially harmful epitopes of commensals and prevent lethal dissemination by controlling mucosa-associated communities. Thus, we believe AMIS of the microbiota is a critical host mechanism, which mediates symbiosis that could be exploited therapeutically to improve health.

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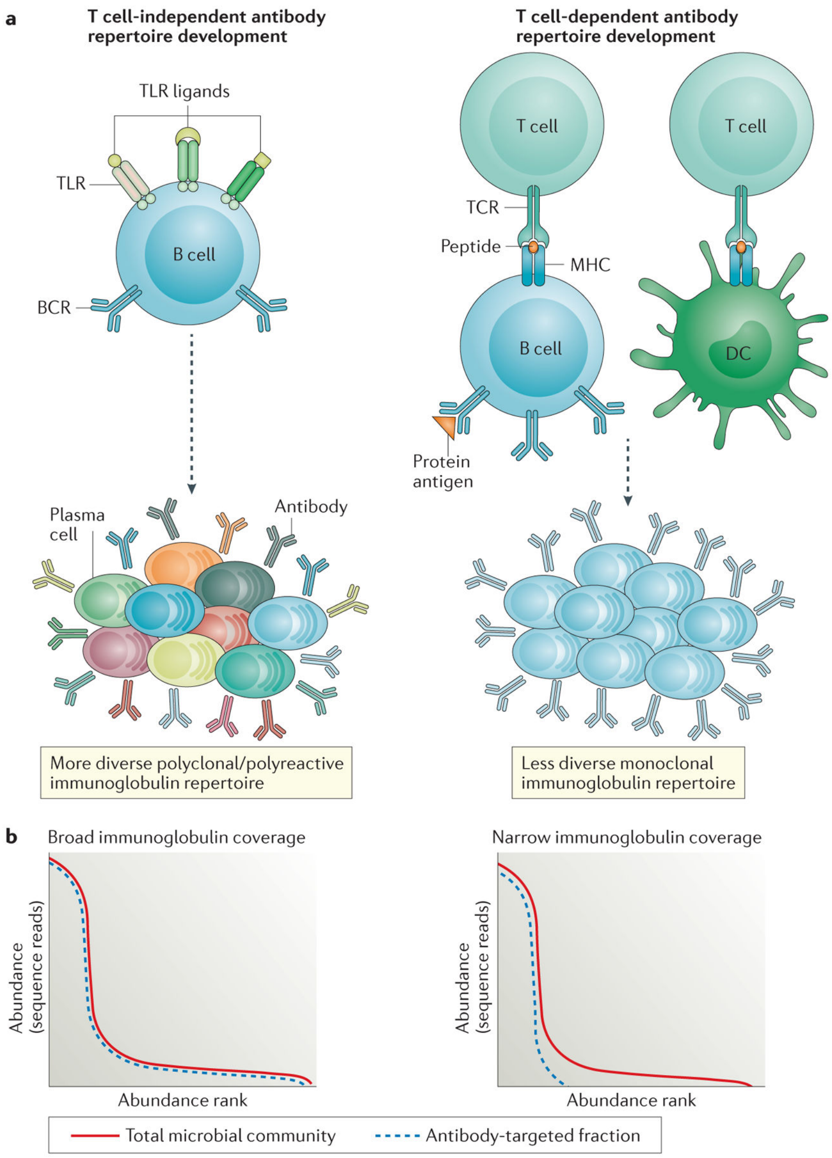

Figure 1 |. Role of immune recognition pathways in antibody-mediated immunoselection of the microbiota.

a | Microbial products can differently influence the development of the antibody repertoire in the gut. This schematic shows how Toll-like receptor (TLR)- and MHC class II-mediated signalling influence T cell-independent and T cell-dependent antibody responses, respectively. T cell-independent antibody responses are promoted by the mitogenic activity of TLR engagement on B cells, and this leads to the generation of relatively low-affinity antibodies with polyreactive specificities. Peptide–MHC class II–T cell receptor (TCR) interactions place more stringent selection on maturing B cells, which reduces the overall diversity of antibody repertoires but results in the generation of high-affinity antibodies with high epitope specificity. **b** | Two models using species rank abundance curves predict the effect on the microbiota of differential immune signalling in T cell-independent and T cell-dependent antibody responses. The red lines in both panels represent the typical distribution of species in microbiota communities from the gut. A small subset of species numerically dominate the community, but most of the community diversity resides in the long-tailed distribution of rare species. Low-affinity polyreactive antibody repertoires are predicted to bind a wider array of species — that is, have greater antibody coverage of the total microbial community (blue dashed line in left panel) — whereas high-affinity monoreactive antibody repertoires are predicted to bind a more limited array of species — that is, have a narrower range of coverage of the total microbial community (blue dashed line in right panel). By focusing the antibody response against the most abundant species, T cell-dependent antibody responses should favour diversity by constraining the growth of the fastest replicators, which maintains niches for rare and more fastidious microbial species. DC, dendritic cell.

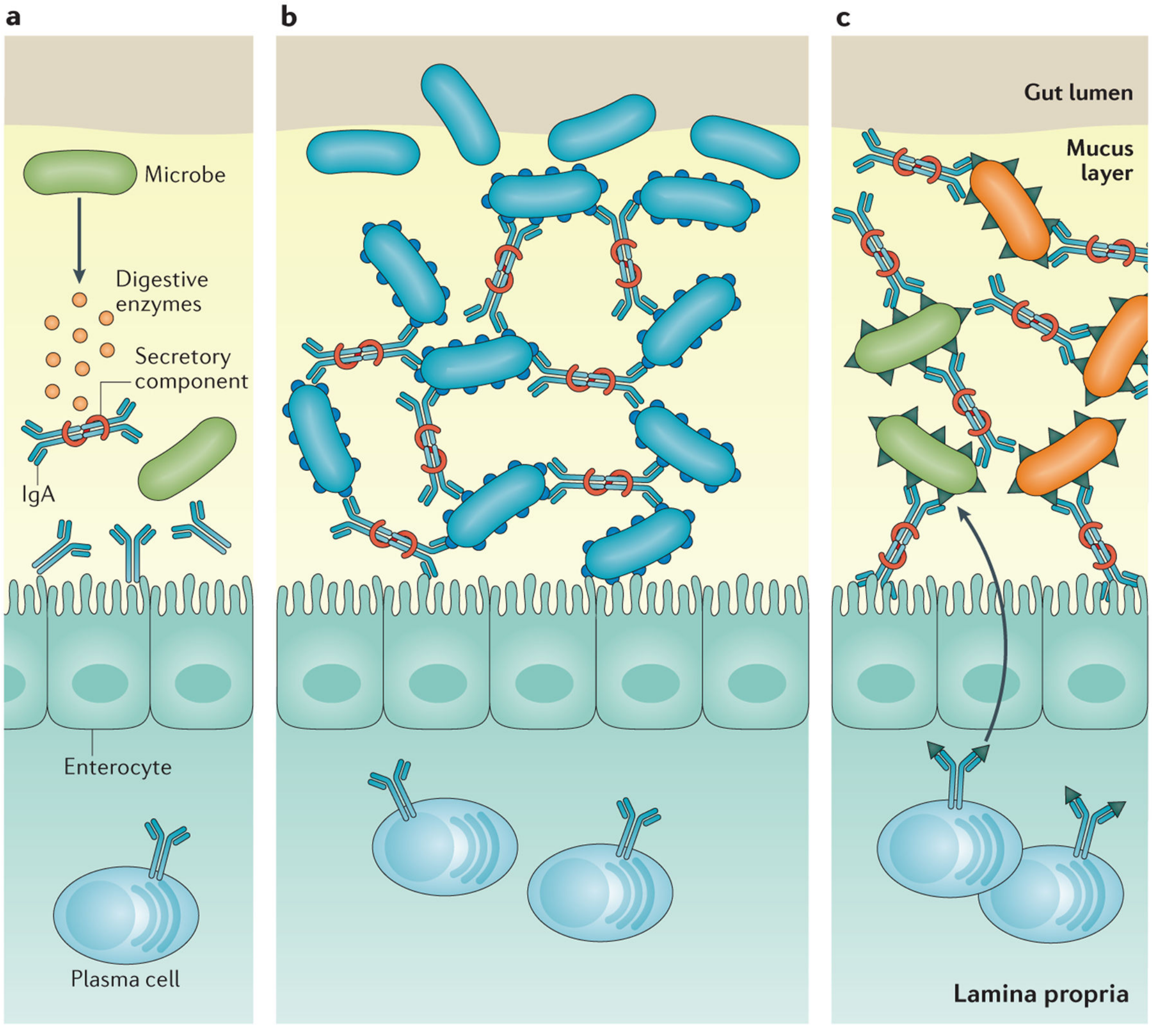

Figure 2 |. Antibody–microorganism interactions that influence antibody-mediated immune-selection of the microbiota.

a | Some microorganisms can secrete enzymes that degrade the secretory component of secretory antibodies, which disrupts the stability of the antibody molecule, rendering it ineffective. **b** | Some microorganisms may express surface epitopes that bind secretory IgA and facilitate colonization of the mucus lining in the gut. **c** | Some microorganisms express surface epitopes that are very similar to those on other microorganisms. Antibodies generated against one microorganism can lead to the generation of antibody specificities that cross-react with similar epitopes found on other microorganisms.

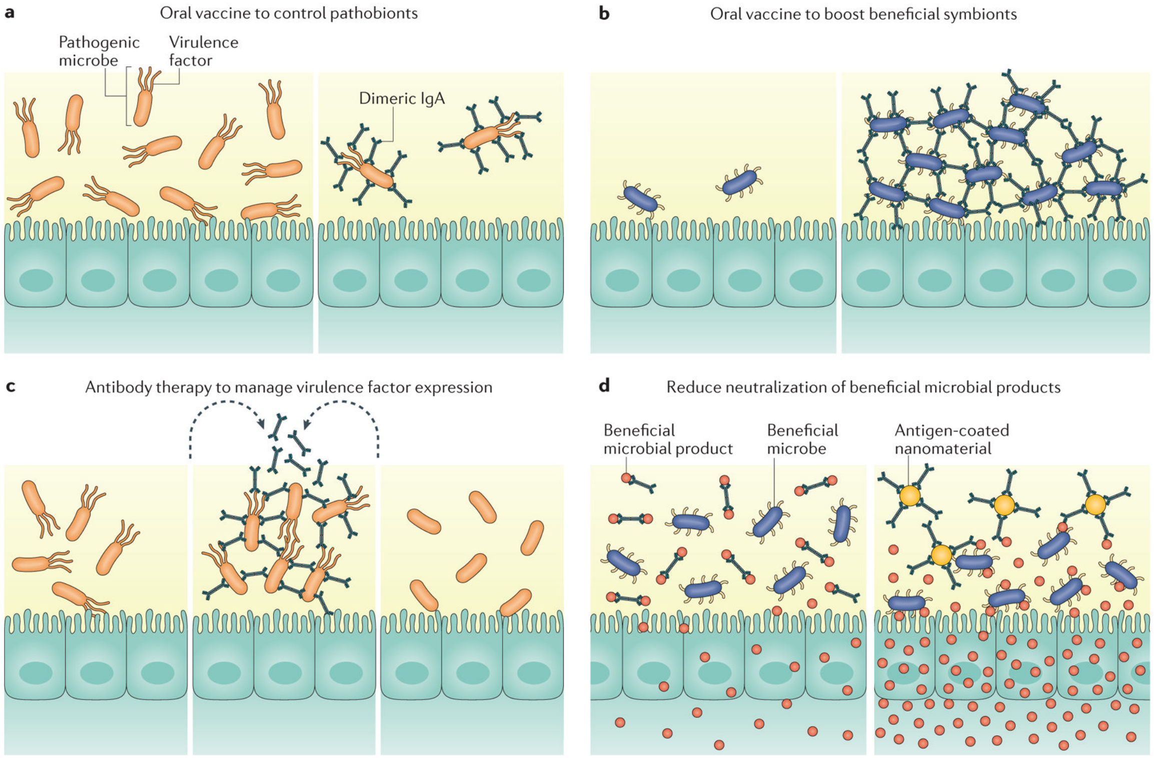

Figure 3 |. Potential immunotherapies to modulate antibody-mediated immunoselection in the gut.

Manipulating antibody-mediated immune-selection (AMIS) to establish a healthy gut can be achieved by enhancing or limiting antibody responses to desired antigens. Oral vaccination could be used to specifically limit pathobiont expansion (part **a**) or to promote biofilm-formation by beneficial species (part **b**). Exogenous antigens could be delivered into the gut to manage virulence factor expression by pathobionts (part **c**). Sequestration of antibodies against beneficial microbial products could increase their availability to the host or to other beneficial microorganisms (part **d**).