IgA as a Promoter of Symbiosis Between a Host and Its Gut Microflora Through Homeostasis of Gut Microbe Populations

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Abstract

Secretory IgA (sIgA) is one of the key regulators of symbiosis between a human host and the host's gut microbiota. sIgA does this through multiple mechanisms that hinge on the unique immune responses that the gut microbiota elicits. Unlike most antibodies, sIgA can signal to other immune cells to neutralize, eliminate, ignore, increase, or even decrease bacterial populations to maintain a bacterial homeostasis that benefits its human host. This homeostasis is the result of coevolution between the human host and the gut. This paper will serve as a review of the known mechanisms of sIgA and will discuss the interactions between the host and the gut microbiota involving sIgA that help to achieve this mutually beneficial relationship.

Introduction

Humans are so much more than just human. While a human is just a single organism, there are trillions of microorganisms that make their home on and within a single human. The various communities of microorganisms that occupy different habitats within an organism are known as microfloras. This paper will focus specifically on the microflora within the human gut, where the gut, in the case of this paper, is defined as the small intestine and the large intestine. The gut microflora serves many essential roles in the human body, from aiding in digestion to shaping how the brain develops. However, the relationship between the gut microflora and the host immune system is significant, as its unique balance of give-and-take shows that they have evolved together and are intricately related. This relationship is remarkably complex as both the gut microflora and the host's immune system shape each other in a complex form of symbiosis and homeostasis.

One of the key regulators of this complex symbiotic relationship is an antibody called immunoglobulin A, which is also known as IgA. There are two main forms of IgA in the body, serum IgA and secretory IgA, where the latter has been shortened to sIgA. Although IgA cannot kill bacteria itself, it acts as a signal to other immune cells to eliminate bacteria through various means, such as phagocytosis and the release of cytotoxins and perforin. Secretory IgA, or sIgA, is typically found in the oral, gastric, and intestinal tracts, whereas serum IgA circulates in the blood. sIgA is actually the most common form of IgA as it is found in such large quantities in the gut and other mucosal membranes. In this paper, IgA and sIgA will be used interchangeably since the only form of IgA that is found in the gut is sIgA. In contrast, serum IgA will be referred to specifically as serum IgA.

IgA binds to bacteria and essentially tags the bacteria to be neutralized, eliminated, ignored, or promoted. Pathogenic bacteria that are recognized as part of the gut have specific IgA plasmablasts that create antigen-specific IgA that binds to surface proteins of the associated antigen. This prevents colonization by the bacteria and essentially neutralizes the bacteria. Conversely, pathogenic bacteria that are not recognized as part of the host's gut microflora trigger the generation of antigen-specific IgA that binds to these pathogenic bacteria and that signals immune cells to eliminate the bacteria.

IgA can also bind to the pathogen-associated molecular pattern molecules (PAMPs) that bacteria have. In an experiment performed using monoclonal antibodies derived from IgA plasma cells in the gut and the spleen, Bunker *et al.* found that IgA derived from the gut is polyreactive and binds with significantly higher affinity to multiple types of PAMPs (Figure 1). Normally, in cases of infection, PAMPs will bind to immune cells and trigger an immune response against them. However, since IgA binds with PAMPs associated with commensal bacteria, this prevents PAMPs from associating with any immune cells, which, in turn, prevents an immune response from being raised. As a result, these bacteria are ignored by the host immune system (Bunker *et al.,* 2017). Commensal bacteria can be promoted because IgA alters the gene expression of several key bacterial species that have co-evolved with the human species. While not all bacterial species are bound to IgA, the percentage that are bound can have large ecological impacts on the overall gut microbiota, increasing or decreasing bacterial species not bound to IgA. If a species of bacteria, for example, *Bacteroides thetaiotaomicron*, is promoted, the population of the species that feed on *B. theta* increases. Species that share the same niche as *B. theta* are out-competed and will decrease in population. The opposite occurs when a bacteria species is neutralized or a portion of the population is eliminated (Bäumler et al., 2016). Understanding other mechanisms and processes involving the gut microflora shows promise in improving human health. For example, by understanding the tolerance that the host develops from commensals, it might be possible to improve the body's ability to fight infection.

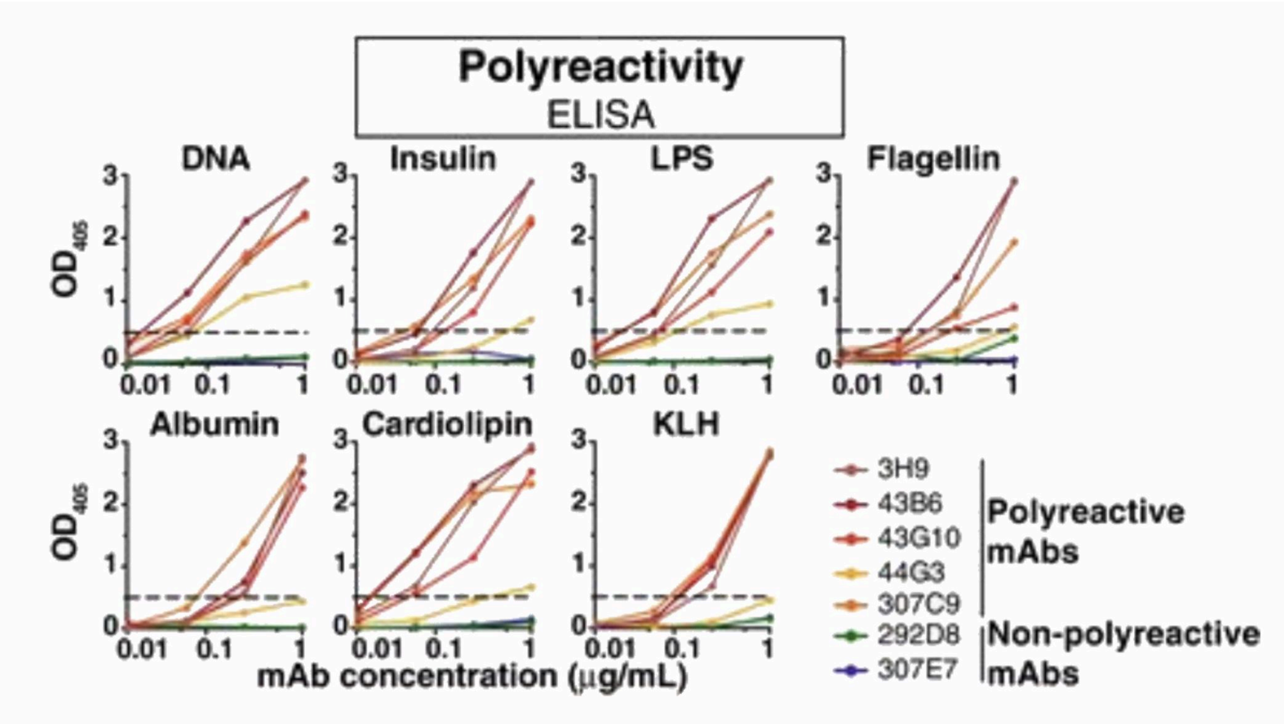

Figure 1: Polyreactivity of IgA to PAMPs
Polyreactive monoclonal antibodies derived from plasma cells found in the guts of mice. Non-polyreactive monoclonal antibodies derived from plasma cells found in the spleens of mice. Consistently, IgA derived from the gut is bound with higher affinity.

(Bunker *et al.,* 2017)

Since the health of the gut impacts several aspects of overall human health, research over the years into the action of IgA in the human gut has shown how increased understanding of the mechanisms of symbiosis and homeostasis can have a potentially huge impact on human health and the treatment of illness in humans. For example, understanding how the mechanisms of symbiosis in the gut microflora have allowed the human gut and bacteria to co-evolve and coexist, has given insight on how homeostasis is modulated by the host through IgA. Using this knowledge, IgA could be used to modulate microbial populations as a form of treatment, or could be used to measure microbial populations and thus serve as a diagnostic tool.

Section 1: Pathways that Make Secretory IgA

There are multiple pathways that produce IgA in the gut's immune system. In this paper, only two of the main pathways will be discussed, namely, the T-dependent pathway and the T-independent pathway, specifically the version of these pathways that takes place in the Peyer's patches.

In the T-dependent pathway (Figure 2), follicular dendritic cells (fDCs) passively pick up the antigen that has been transcytosed by the M cells. Meanwhile, dendritic cells (DCs) entering the T-cell zone activate naive T cells by means of major histocompatibility complex class II (MHC class II). This, in turn, presents the antigen to the TCR of the naive T-cell, while simultaneously the CD80 or CD86 binds to CD28. This combined action results in the T-cell differentiating into a T-effector cell. These T effector cells then express CD40L and enter the follicular mantle where they signal to a naive B-2-cell's CD40 in the germinal center. Then the B-cell receptor (BCR) binds to the antigens present on the fDC. This activates B-cells to upregulate activation-induced cytidine deaminase (AID) and triggers class switch recombination (CSR). Expression of the transforming growth factor-β receptor (TGFβR) on the B-cells is upregulated by nitric oxide (NO). These B-cells further differentiate into IgA plasma cells through CSR, and secrete IgAs with somatic hypermutation (SHM) in their VH gene (Neutra *et al.*, 2001; Pabst, 2012). Since the T-dependent pathway involves B-cells generating antibodies based on T-cells interacting with an antigen, the resulting antibodies are highly antigen-specific. T-dependent generated IgA is also known as "Classical" IgA, and the T-dependent pathway is also known as the "Classical Pathway".

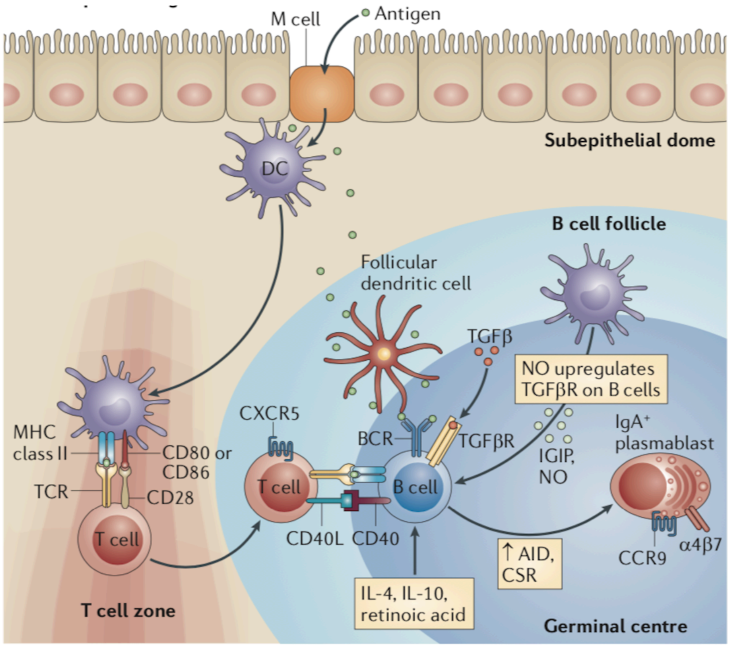

Figure 2: The T-dependent pathway
This pathway shows how "Classical" IgA is made in Peyer's patches. Antigen is taken in from the intestinal lumen by the M cells, where a DC passes the antigen on to a T-cell, which is then passed to a B-cell and which then becomes an IgA plasmablast that, in turn, generates a high affinity, antigen-specific IgA

(Pabst, 2012)

In T-independent pathway (Figure 3), DCs directly signal to B cells. The toll-like receptors (TLRs) on the B-cells are activated, and they respond directly through their BCRs to antigens that are transported by the M cells. Then the fDCs present the antigens to other B-cells in the area, and the B-cells release CD40L-related cytokines, such as B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), which are also produced by DCs and plasmacytoid DCs (pDCs). The release of cytokines then triggers the expression of AID through innate immune mechanisms, including Toll-like receptor (TLR) signalling, and this ultimately causes CSR in IgA+ plasmablasts (Pabst, 2012). T-independent generated IgA is also known as "Innate" IgA, and the T-independent pathway is also known as the "Innate Pathway".

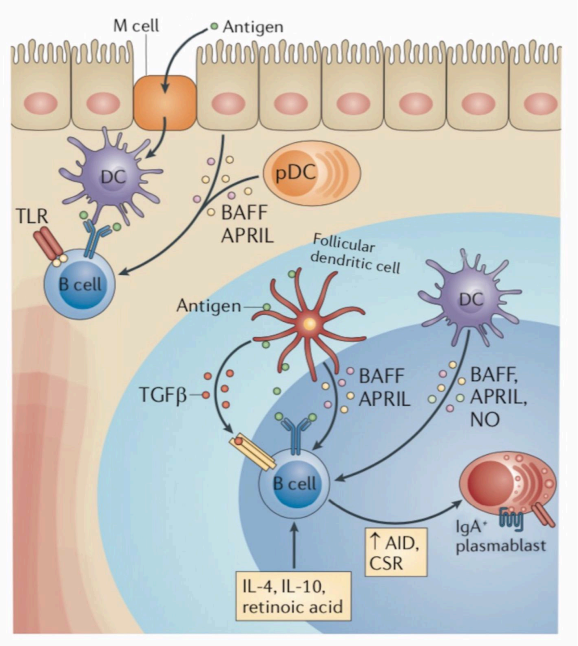

Figure 3: The T-independent pathway
This pathway shows how "Innate" IgA is made in Peyer's patches. Antigen is taken in from the lumen by the M cells where a DC passes the antigen to a B-cell which becomes an IgA plasmablast that generates highly polyreactive IgA

(Pabst, 2012)

The IgA produced by each pathway is different as a result of how they were produced. Moreover, there is also a genetic component to IgA diversity (Fransen *et al.*, 2015) as CSR is impacted by the genetics of the host. This was seen in a study of the two different strains of mice, BALB/c and C57BL/6 (Figure 4). In the study, both strains were healthy and taken care of the same way. To measure the diversity of bacteria in the guts of these mice, fecal samples were taken and sequenced. The control group (NT) were raised separately, the second group (Coh) were co-housed together, and the third group (FT) received a treatment of antibiotics and a fecal transplant of the other strain. BALB/c mice were found to have more IgA diversity in both the NT and Coh group. This was determined to be caused simply by the fact that BALB/c mice were genetically predisposed to have more varied VDJ regions. Since the Coh group had no significant differences in diversity, the mice's environment was found to have little effect on overall diversity. This study found that this diversity resulted from a positive feedback loop involving the generation of polyreactive IgA. The feedback loop is kicked off by the initial genetic predisposition, where the host initially generates highly polyreactive IgA, and then subsequent generations of IgA will recombine into more diverse and polyreactive IgA, allowing for a more diverse variety of microbes to inhabit the gut. Additionally, even after the homeostasis of the gut microbiota of these mice were disturbed by the FT treatment, the original diversity returned, with the exception of the Firmicutes. This indicated that there are strong genetic ties to the diversity of the gut that even strong environmental changes impact very little.

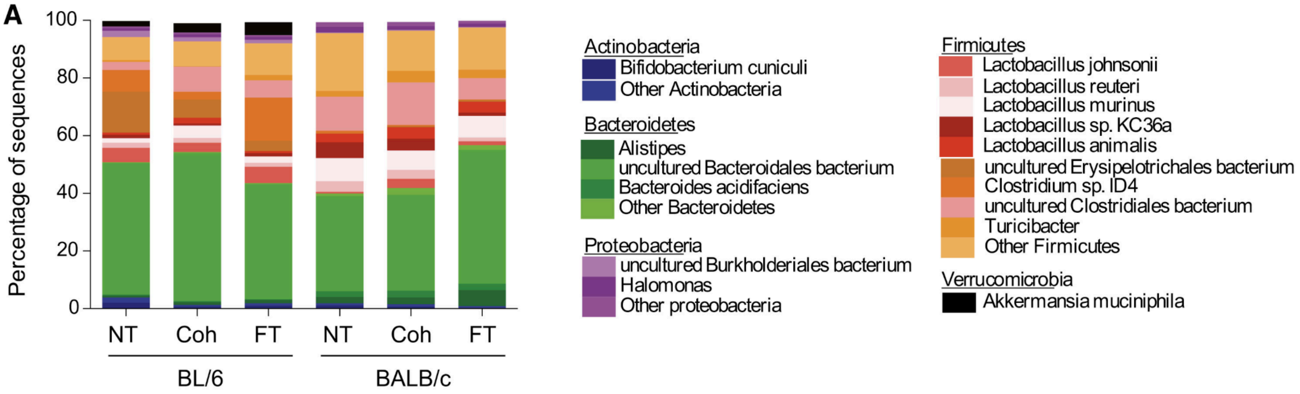

Figure 4: Microbial diversity comparision of C57BL/6 and BALB/c mice
In this figure, the C57BL/6 strain of mice is labeled as BL/6. This graph shows the microbial diversity of mice found through fecal samples. The NT group was not treated, the Coh group was the group where both strains of mice were co-housed together. There is no significant difference when comparing the NT and Coh diversity within each strain. The FT group were given a round of antibiotics and received a fecal transplant of the other strain. The FT group saw significant reduction of the proportion of Firmicute found in BALB/c mice, while the proportion of Firmicutes increased in C57BL/6 mice.

(Fransen *et al.*, 2015)

After the action of these pathways is complete, the mechanism that changes serum IgA into sIgA involves the cooperative behavior of plasma cells and mucosal epithelial cells in the gut (Tsuji *et al.*, 2008). The B-cells in the gut tissue space produce polymeric IgA (pIgA), while the gut mucosal epithelial cells express an immunoglobulin receptor called the polymeric Ig receptor (pIgR). When pIgA is generated by the B cells, pIgA binds to the pIgR which transports pIgA across the epithelial wall. Once across the epithelial wall, the pIgA cleaves from the pIgR, along with the extracellular domain of pIgR. This extracellular domain is known as the secretory component which covalently binds to pIgA and generates the sIgA complex (Figure 5). Since this all takes place on the epithelial wall, sIgA is concentrated on the outer layer of the gut mucosal membrane. In the gut mucosal membrane, the presence of IgA serves as a secondary defense against bacteria, while the mucosal membrane acts as the primary defense by being a physical barrier for the epithelial cells of the gut (Rogier *et al.*, 2013).

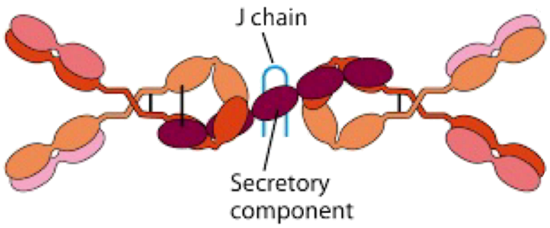

Figure 5: Illustration of sIgA
Diagram of IgA showing the covalently bonded pIgR that generates the secretory component of IgA.

(Brimeyer, 2016)

There is, however, some contention about the avidity and affinity of T-independent generated IgA. T-independent generated IgA is typically commensal-reactive, and, as previously mentioned, is known as the ‘Innate’ IgA (Cerutti, 2008). Bunker *et al.* used knockout mice to determine the avidity and affinity of IgA generated by both T-independent and T-dependent generated IgA with commensals in the gut. Bunker found that most commensals triggered a strong T-independent IgA response with high affinity from B1b and B2B cells. Commensals with atypical cell wall structures triggered a strong T-dependent IgA response with high avidity and affinity from an orphan B1b lineage (Bunker *et al.*, 2015). This is likely because the researchers used protocols that only achieve T-independent responses. In order to generate only a T-independent response, Bunker *et al.* used knockout mice that had no T-cells or germinal centers. As a result, no somatic hypermutation could occur. It is entirely possible the reason these knockout mice had such high affinity in T-independent generated IgA was that the immune system was trying to compensate for the lack of a T-dependent response. On the other hand, in an experiment performed by Slack *et al.,* monoclonal mice were used. As a result, the initial genome for the B-cells was limited which, in turn, limited the affinity of the IgA generated. Based on these experiments, currently, only speculations can be made about the true affinity of T-independent IgA (Slack *et al.*, 2012). Another consideration that may apply is that immunity is the culmination of multiple pathways occurring simultaneously. However, in order to study the T-independent pathway specifically, all other pathways are inhibited. We currently do not know what possible downstream effects this restriction has on the T-independent pathway, since the T-independent and T-dependent pathways work in tandem. Notably, both pathways may intersect to shape a micro milieu that directs class-switching towards IgA.

Section 2: Regulation of IgA Pathways

The cells that we will be focusing on in the T-dependent pathway are Th17 cells because they produce multiple important cytokines and, as such, are a key regulator of IgA production in this pathway (Cao *et al.*, 2012). Interleukin 17 (IL-17) is one important secretion of Th17 cells, as IL-17 generates signals that can both upregulate and downregulate intestinal epithelial pIgR expression (Figure 6a-b). By modulating pIgR expression, there is, in turn, control over the overall generation of sIgA in the gut. As mentioned earlier, a portion of the pIgR is secreted with IgA, during transcytosis, and covalently linked to create sIgA. T_h17 cells also secrete IL-21 which induces the naive B-cells class switch recombination of B-2 cells to IgA+ cells (Cao *et al.*, 2015). This class switch recombination is also mediated by transforming growth factor β1 (TGFβ1) and can accelerate IgA class switch recombination. This process enhances IgA+ CSR, IgA production, and B cell trafficking into the intestine, and this results in a diverse and robust level of IgA in the gut.

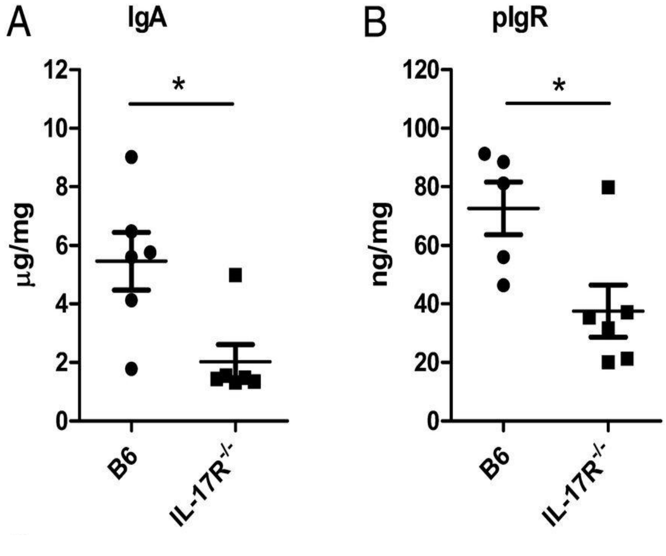

Figure 6a: Comparing IgA quantity in B6 mice versus IL-17 knockout mice
Fecal samples of a B6 mice were used as a control for baseline quantity of IgA. The fecal samples of IL-17 knockout mice have significantly lower amounts of IgA

Figure 6b: Comparing pIgR quantity in B6 mice versus IL-17 knockout mice
Fecal samples of a B6 mice were used as a control for baseline quantity of pIgR. The fecal samples of IL-17 knockout mice have significantly lower amounts of pIgR

(Cao *et al.*, 2012)

In the T-independent pathway, plasmacytoid dendritic cells (pDC) are a key regulator of IgA class switch recombination (Tezuka *et al.*, 2011). Although the mechanism is not completely elucidated, a strong positive correlation between the pDC expression of APRIL and BAFF and the production of IgA has been observed. This has important implications because pDC expression of APRIL and BAFF is dependent on stromal cell-derived Type I IFN signaling. It should be noted that Type I IFN signalling is activated when cells are infected but is not triggered by commensals. Thus, the presence of Type I IFN signalling strengthens the idea that T-independent IgA is also produced in response to pathogenic bacteria and not just by commensals. Another possible theory for why Type I IFN signalling triggers the release of APRIL and BAFF, is that these compounds also signal the T-dependent pathway. As a result, pDCs could trigger both pathways, as cell infection can be triggered by commensals or by pathogenic bacteria. To study the correlation between pDCs and T-independent response, only pDCs were stimulated with type I IFN to observe the amount of APRIL and BAFF produced. Then using this data, a mouse model was made (Figure 7), where it was found that there is a correlation between BAFF and APRIL and overall IgA production. A mouse model, as used in this study and in other studies discussed later in this paper, is helpful and can be meaningful when studying the human gut because the mouse gut is very similar to the human gut.

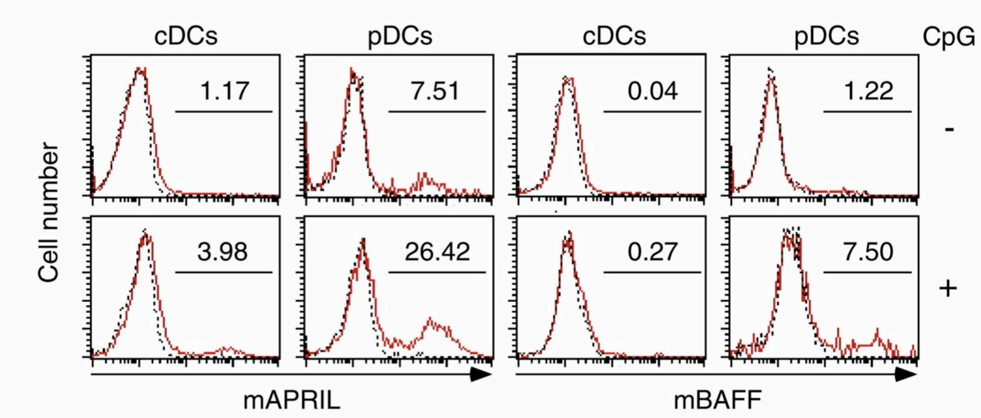

Figure 7: Average counts of cDCs and pDCs after an increase in APRIL and BAFF
A positive trend is observed, as an overall increase in both APRIL and BAFF results in overall higher cell counts of cDCs and pDCs.

(Tezuka *et al.*, 2011)

In both pathways, inhibitory co-receptor programmed cell death–1 (PD-1) regulates antibody diversification (Kawamoto *et al.*, 2012). PD-1 is not part of either pathway, but is upstream in the immune system where it regulates immune cell populations within the body. PD-1 has been observed to have a very profound impact on T follicular helper cell (T_{FH}-cells) populations. This is because T_{FH}-cells induce the expression of AID, which, in turn, induces class switch recombination (Fagarasan *et al.*, 2002). Because PD-1 regulates T_{FH}-cells by triggering cell death, T_{FH}-cell population are kept within a strict specificity of phenotypes. In mouse models, where PD-1 has been knocked out, T_{FH}-cells were generated in excess with altered phenotypes. This, in turn, resulted in dysregulated selection of IgA precursor cells in the germinal centers of Peyer's patches, which, in turn, led to lower affinity IgA (Figure 8b-c). As a result, it can be concluded that PD-1 regulates antibody diversification with high microbial binding ability.

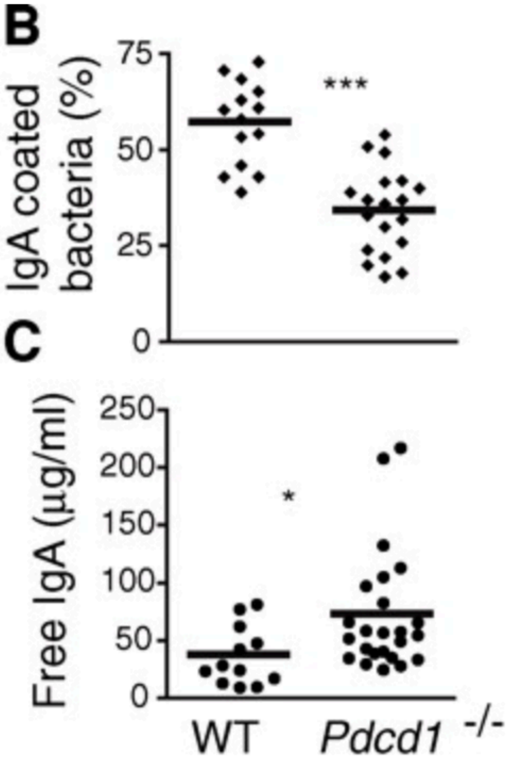

Figure 8b: Bacteria coated in IgA wild type vs PD-1 knockout
There is a significant drop in bacteria coated in IgA as PD1 is not selecting for IgA with high affinity

Figure 8c: Free IgA Wild type vs PD-1 knockout
There is a significant drop in free IgA as PD-1 is not selecting for IgA with high affinity and, therefore, less IgA is actually binding to bacteria

(Kawamoto *et al.*, 2012)

Section 3: IgA Interactions with Microbes

In this section, we will discuss the interaction between the host's immune system and the gut microflora. Before doing so, it is important to understand how this interaction takes place. Peyer's patches (PP) are patches of lymphoid tissue in the wall of the small intestine which are involved in the development of immunity to antigens (Jung *et al.*, 2010). Peyer's patches (Figure 9) are made of multiple sections.

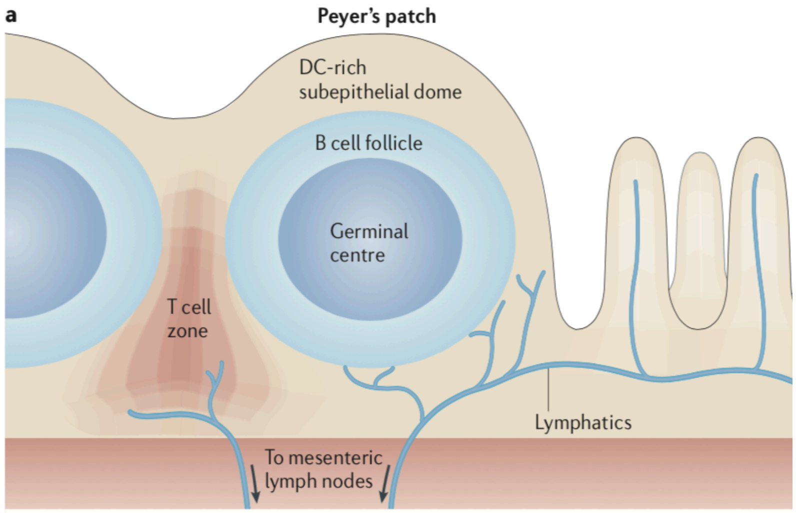

Figure 9: Anatomical model of Peyer's patches within the small intestine
Within the T-cell zone resides mature dendritic cells and, as the name suggests, a variety of T-cells in different stages of maturation. Within the germinal centers reside follicular dendritic cells and a variety of B cells in different stages of maturation.

(Pabst, 2012)

First is the subepithelial dome (SED) where immature dendritic cells, macrophages, T/B lymphocytes, memory cells, IgM+ plasma cells, and IgA+ plasma cells reside. Underneath this dome is the perifollicular area where there are two distinct types of zones, namely, T-cell zones and germinal centers. The T-cell zone makes up most of the space, and germinal centers are interspersed among this T-cell zone. These germinal centers are also surrounded by a follicular mantle which separates them from the T-cell zone. Within the T-cell zone resides mature dendritic cells and, as the name suggests, a variety of T-cells in different stages of maturation.

Within the germinal centers reside follicular dendritic cells and a variety of B cells in different stages of maturation. On the surface of the PPs, there are microfold cells (M cells) which transport antigens from the inside of the gut to the subepithelial dome through transcytosis (Rios *et al.*, 20015). The presence of these M cells is what allows the controlled cross-talk between the host's immune system and the gut microflora to take place (Neutra *et al.*, 2001) (Pabst, 2012).

Since one of the key jobs of the gut microflora is to aid with digestion, this interaction between the host and the gut microflora is the first one we examine. One of the interesting skills that the immune system has within the gut is the ability to sense metabolites (Wu *et al.*, 2017). In response to the metabolites sensed, the host can modulate an immune response in the intestines. A clear example of this is microbiota metabolite short-chain fatty acid acetate promoted intestinal IgA responses. GPR43, which can perform "metabolite-sensing," has been identified as a key modulator. Metabolism is an interesting area for future study since, by modulating metabolite sensing, it might be possible to increase or decrease basal metabolism, which has great potential for treating individuals that are both underweight and overweight.

While the gut microflora has a significant impact on the digestion of the food eaten by the host, the host's diet also conversely has an important impact on the gut microflora population. By just looking at fecal samples of vegetarians and meat eaters, a marked difference in populations can be observed, as certain bacteria are better at digesting plant matter, while other bacteria are better at digesting meat. However with these shifts in diet, there are observable trends with inflammation, as in multiple studies, a diet high in red meats and lipids is found to be associated with inflammation (Turner *et al.*, 2017). By knowing what bacteria are associated with these diets, a better understanding of digestion can be achieved. An example of how this knowledge might be useful is in the treatment of lactose intolerance, where a possible therapy is to ingest lactose-digesting bacteria so the host itself will not need to make lactase.

The gut, in addition to containing varying types of IgA, also contains bacteria that are coated with IgA, where the level of coating depends on the bacteria's taxa. The amount of IgA that coats the bacteria is dependent on the immune response it elicits, such as inflammation and Toll-like receptors (TLRs). Colitogenic bacteria, such as *Prevotellaceae*, *Helicobacter*, and segmented filamentous bacteria (SFB), have been known to drive intestinal inflammation. Using a mouse model, fecal samples were collected and, using magnetic-activated cell sorting to measure relative IgA coating, colitogenic bacteria were found to be the most highly-IgA-coated bacteria (Palm *et al.*, 2014; Stephens & Round, 2014). This finding arises from the action of the T-dependent pathway in "Classical" IgA, where highly-specific IgA is produced to coat specific bacteria, with high affinity. The IgA coating of bacteria in the gut is also influenced by the genetics of the host, as the ability for IgA to recombine with high affinity is itself dependent on the genetics of the host. The genetic variability in the hosts results in coating pattern variations in the bacteria within these host subjects (Jung & Alt, 2004).

The location of the bacteria within the gut has also been identified as a major determining factor for the level of IgA coating on the bacteria. While a majority of the bacterial population of the gut microflora resides in the large intestine, as it is the more hospitable habitat for microorganisms, residency within the small intestine has been observed as a strong indicator of IgA targeting (Bunker *et al.*, 2015). This was measured by using flow cytometry to sort bacteria coated in IgA and by using 16S rRNA gene sequencing to identify typical location within the gut. The basis for why this occurs is that Peyer's patches are located in the small intestines. As previously discussed, Peyer's patches (PP) are patches of lymphoid tissue in the wall of the small intestine which are involved in the development of immunity to antigens (Jung *et al.*, 2010). IgA antibodies are produced by naive small intestinal plasma cells which are recirculated and enriched within PPs. As a result, IgA becomes polyreactive independently of the action of exogenous antigen and T cells innately recognizing the gut microbiota. IgA has many more properties other than coating that result from generations of coevolution between the human body and the gut microflora (Bunker *et al.*, 2017).

While IgA may seem like an irreplaceable antibody for human gut health, IgA deficiency has very few health effects and is quite common, with about twenty-five percent of the population having this condition. If this does occur, many people produce the antibody immunoglobulin M (IgM) as a substitute for IgA. In the gut, IgM uses a mechanism very similar to that used by IgA, where IgM complexes use pIgR to generate a secreted form of IgM that binds to antigens. Additionally, IgM is created using the same pathways, and these pathways result in a “Classical” IgM and an “Innate” form of IgM that have different binding capabilities. As a result, many individuals with IgA deficiency experience mild dysbiosis. IgA-deficient humans are good observational subjects as they have a healthy functioning immune response in the gut, while simultaneously lacking IgA naturally. By observing how IgA behaves compared to IgM, it is possible to study the nuances of symptoms or behaviors that IgA produces without compromising the entire gut and immune systems, as those can be confounding factors. Although human IgA deficiency is not associated with large perturbations in the gut microbial ecology, however, there are nevertheless observable shifts that are shared among most IgA-deficient individuals, indicating that these commonly observed shifts are tied directly to the ability of IgM to bind to specific taxa of bacteria (Catanzaro *et al.*, 2018)(Fadlallah *et al.*, 2018). As a result of this, diversity of the microflora in the gut is not as great because IgM has lower affinity and thus is not able to bind to the same variety of bacteria as IgA.

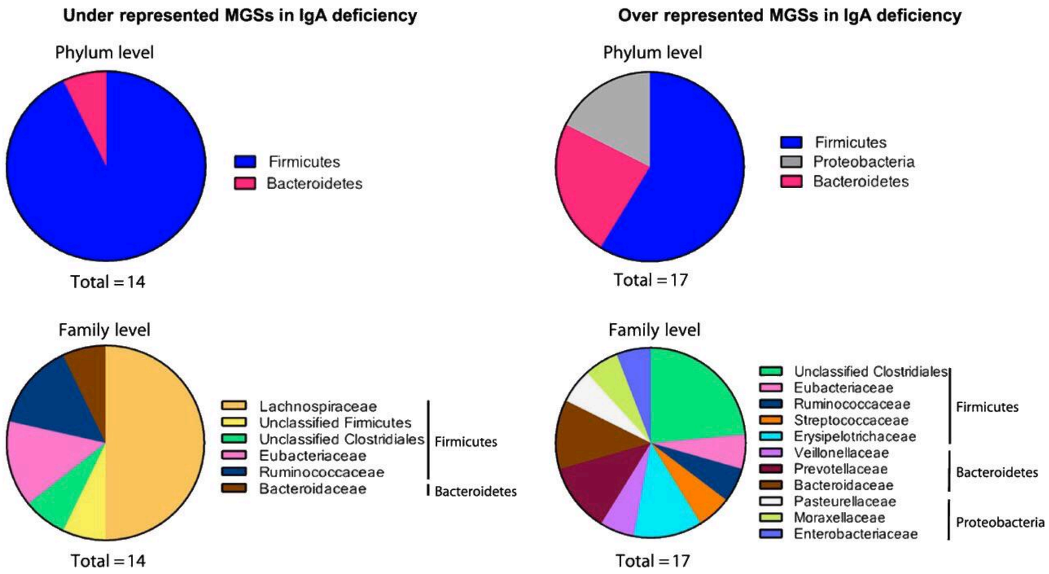

Figure 10: Effects of IgA Deficiency
A graph showing the average shift in the gut if an individual has IgA deficiency. On the left are the under-represented microbes, while on the right are the over-represented microbes.

(Fadlallah *et al.*, 2018)

Section 4: Mechanisms that IgA Uses to Manipulate the Gut

After the complex process of producing IgA is completed, IgA is secreted into the mucosal membrane of the gut and the lumen of the gut. Active and sIgA-coated bacterial fractions are characterized by a higher diversity in non-IgA-deficient individuals when compared to IgA-deficient individuals (D'Auria *et al.*, 2013; see also Figure 10). In a study conducted by Nakajima, sIgA coating can be used as a measure of the most active bacterial fractions. Nakajima looked specifically at *B. theta*, as metabolic activity could be measured with flow cytometry (Figure 11). This study found consistently that metabolically active *B. theta* is coated with high amounts of IgA. The implication of this finding is that the host's immune system selects the survival of commensals, as IgA coating is a survival advantage in the gut (Nakajima *et al.*, 2018).

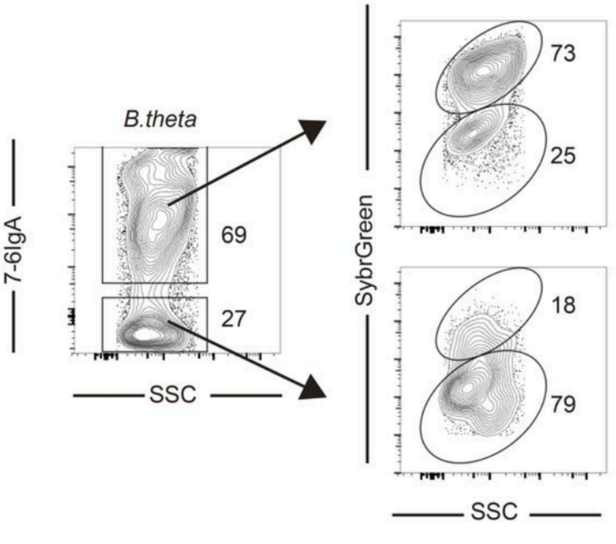

Figure 11. A flow cytometry side scatter of *B. theta*
The first graph shows the coating level of *B. theta*. In the graph, the top box represents the *B. theta* that is highly coated and the bottom box represents the *B. theta* with low coating. Each box becomes the corresponding graph of sybr green detected which was used to measure metabolic activity.

(Nakajima et al., 2018)

In addition to maintaining the homeostasis of the gut microbiota, IgA can change what is homeostatic equilibrium. An example of this change in homeostasis is the maturation of the gut microbiota, which is dependent on IgA (Mirpuri *et al.*, 2013). Typically, a host can develop a gut microflora in the womb. However, one of the major initial shifts the gut microbiota undergoes is when a mammal first consumes breastmilk, when antibodies in the breast milk are transferred from the mother to the baby. As a result of this transference during infancy, a unique microbiome in the infant is developed with a large population of γ-Proteobacteria. However, in a mouse study, mice deficient in IgA had persistent intestinal colonization with γ-Proteobacteria that resulted in sustained intestinal inflammation (Figure 12). This indicates that the microflora has difficulty acquiring a new homeostasis in the new infant host, as the host diet changed with maturation of the infant. This has implications of what causes the dysbiosis in IgA-deficient individuals, as bacterial colonies are not kept in check in the same way as they are for non-IgA-deficient individuals.

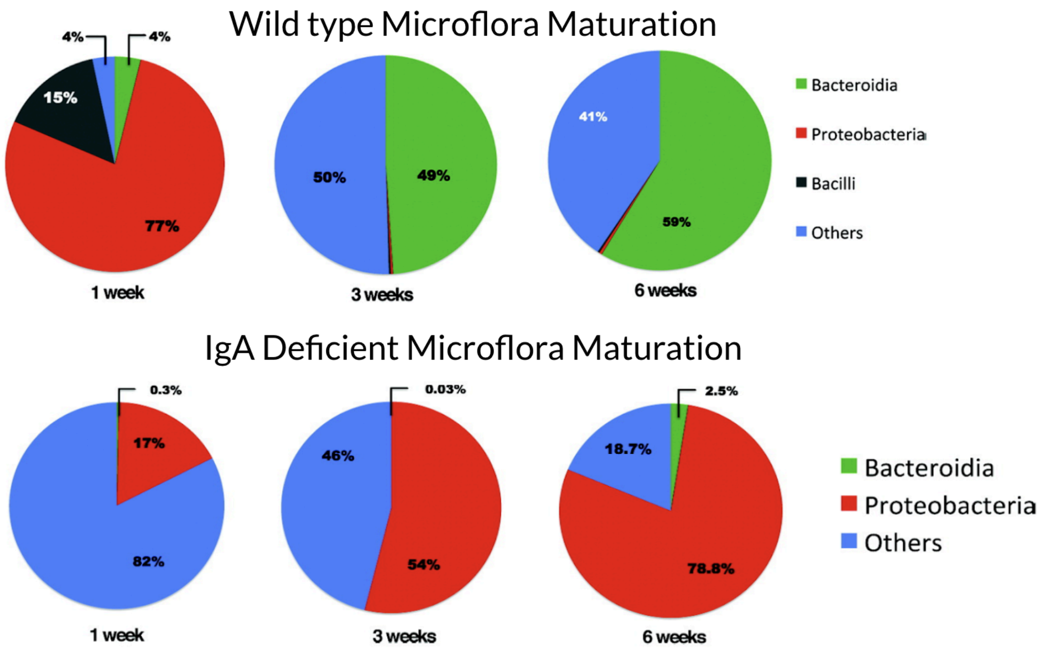

Figure 12: Maturation of gut microbiota in wild type mouse vs IgA-deficient mouse
Gut microbiota maturation of a wild type mouse depicted at the top, as the mouse matures, the amount of *Proteobacteria* decreases while the *Bacteroidia* increases. In the IgA-deficient mouse, *Proteobacteria* consistently make up a large portion of the gut microbiota.

(Mirpuri *et al.*, 2013)

The relationship that has developed between host and microbes is encoded in the genetics of not only the host, but also the microbes. *In vivo* IgA has been observed to alter the expression of polysaccharide utilization loci (PUL); this results in the expression of Mucus-Associated Functional Factor (MAFF) (Nakajima *et al.*, 2018). This PUL expression promotes adhesion to the mucosal layer in the gut, which, in turn, promotes symbiosis with other Firmicutes through generation of a new niche. This gene expression is only seen in environments similar to the gut, suggesting that this behavior is from coevolution of Firmicutes and the gut. In another mouse study, the regulatory system in place for *Bacteroides fragilis* modulates its surface architecture to promote binding of IgA in mice (Donaldson *et al.*, 2018). This generates a defined niche within the mucosal membrane that mediates stable colonization of the gut and excluded exogenous competitors. As a result, this shows cooperation within the host to support survival of specific bacteria that are deemed useful. In addition, this shows the coevolution of host and gut microflora, as these niches only exist within the gut, and are carefully regulated with homeostasis (Zitomersky *et al.*, 2011).

Another case of this coevolution behavior is that *in vivo* IgA mediated cross-linking enchains bacterial daughter cells (Moor *et al.*, 2017; Figure 13). This enchaining prevented daughter cell separation after division, which, in turn, caused aggregation correlated to division rate. This behavior significantly limits and controls bacterial growth and is typically seen in response to pathogenic species, such as *Salmonella*. Furthermore, IgA-enchained plasmid-donor and recipient clones were put in separate clumps, thus preventing conjugative plasmid transfer *in* *vivo*. This has very interesting implications about host-controlled evolution, since by preventing bacterial conjugation, pathogenesis is limited. Additionally, this could be used to prevent commensal bacteria from developing pathogenesis.

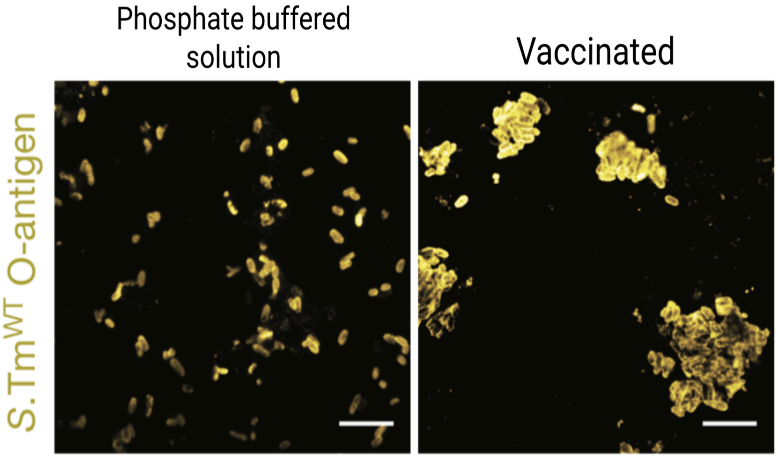

Figure 13: *Salmonella typhimurium* in a control mouse and in a vaccinated mouse
S. typhimurium in the control mouse (left) grows in a spread-out pattern. In the mouse that has been vaccinated with *S. typhimurium* previously, *S. typhimurium* grows in aggregated-like clumps.

(Moor *et al.*, 2017)

Discussion

Considerations: Mice versus Humans

While all this research about IgA in the gut is promising, almost all of this research is done on mouse models and the findings should not be directly translated to humans without further human studies to confirm. Even though the mouse gut is very similar to the human gut, mouse models are nevertheless not an ideal organism to study the gut microbes in humans or to study host interactions anatomically. One main reason is that Peyer's patches in mice are only located in the ileum portion of the small intestine, whereas, in humans, they are scattered throughout the entire small intestine. This affects the gut microbiota, as location has been a huge determining factor for the amount of IgA coating on bacteria. As a result, the IgA coating levels in mice are more selective for the bacteria that can survive in the more acidic part of the small intestine.

However, one of the reasons that mice are still used, is that germ-free mice are a very important experimental measure, whereas germ-free humans obviously do not exist and therefore are not something that can be studied. Germ-free mice are especially useful in understanding immune reactions to bacteria, as the gut microflora can be, in a way, curated to the specifics of an experiment to observe individual properties. Additionally, when studying the gut microbiota from humans, only fecal samples from the colon can be taken. As a result, it is hard to get an accurate picture of the gut microflora, as the colon has developed its own unique microflora that is similar, but different, from the gut microflora. Another consideration is that a mouse's diet is very different from a human's. Mice, in general, tend to have a mostly herbivorous diet, consisting of plant matter and occasional insects, while most humans are omnivores. These differences in diet play a huge role in the gut's microflora, as different bacteria are needed to digest different matter. In the cases of mouse experiments, diet is also extremely controlled, but that is something that does not translate for most humans,

Future Applications: Treatments and Therapies

Since IgA can be used to manipulate the homeostasis of the entire gut microbiota, introducing a new homeostasis is entirely possible. Normally the homeostasis of a host is consistent: even with the host constantly taking in a large multitude of bacteria through consumption of food, the shifts that these outside pathogens generate quickly are reversed back to homeostasis. In a well-regulated gut microflora, even large shifts generated by eating contaminated and spoiled food are reversed through IgA regulations. Even a change in diet easily and quickly generates a new homeostasis, thus allowing the body to adapt easily to changes in diet. However, as mentioned earlier, this only occurs because of the unique cross-talk that occurs between the gut microflora and the human host. Because of this, using fecal transplants to introduce bacteria that can generate controlled change in the gut microflora is possible. This controlled change in the gut microbiota is especially interesting, as it could potentially generate a new homeostasis which could, in turn, cause permanent beneficial improvements, such as reduced inflammation, to the gut microflora. The results of current studies show that fecal transplants as treatments for autoimmune diseases and chronic inflammation within the gut have varied and inconsistent results, more often than not, with no clear benefit to the host. However, using fecal transplants to increase the overall diversity of the gut microbiota has been a fairly successful treatment for *Clostridium difficile* infection (Lopez & Grinspan, 2016). With further studies though, a better understanding of gut microbes and host can possibly be achieved, with the hope that the success that fecal transplantation has for a *C. difficile* infection can be translated over to autoimmune diseases and chronic inflammation within the gut.

Another idea that has been experimented with is introducing vaccines through the mucosal membrane, as it is a location where bacteria are readily taken in. Especially in the context of a vaccine, this method would be very useful as the body could quickly form immunity and memory (Xiong *et al.*, 2015). However, this could also potentially interfere with the tolerance the body builds, as the vaccine could be recognized as a commensal and no immune response would be activated. Another unique possibility for vaccinations via the gut are live attenuated vaccines, since the immune response generated from the gut is very robust and has good longevity. This could also be a way to combat chronic gut inflammation because, if tolerance can be taught to the gut through a vaccine, people who suffer from Irritable Bowel Syndrome (IBS) and other gut diseases might be able to get some relief. Currently, there is no real treatment for IBS other than moderation of diet and this line of investigation seems hopeful for IBS sufferers. Overall, IgA is an important regulator of the entire immune response to the gut microflora and should continue to be studied.

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