Do the Microbiota Influence Vaccines and Protective Immunity to Pathogens?

Engaging Our Endogenous Adjuvants

Nicholas Collins[1] and Yasmine Belkaid[1,2]

[1]Mucosal Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

[2]NIAID Microbiome Program, National Institutes of Health, Bethesda, Maryland 20892

Correspondence: ybelkaid@niaid.nih.gov

The reliance of the immune system on constitutive microbial stimulation support the idea that both responsiveness to vaccines and vaccine design need to be considered in the context of host–microbiota interactions. Manipulation of microbe function or composition via diet alteration or microbiota engraftment may soon become a viable approach to control immunity and, as such, vaccine responses. Learning from our endogenous original adjuvants could be critical in overcoming the enormous hurdle of vaccine design against the numerous pathogens that cause chronic infection. Going forward, rationally designed vaccines that take advantage of the inherent adjuvant properties of the microbiota could have a major impact on the prevention of disease.

OUTLINE

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Humans are inhabited by a diverse commensal microbiota that consists of bacteria, fungi, viruses, and eukaryotic species (Ley et al. 2006). This relationship between host and commensals has evolved over millennia and is beneficial to both parties. For example, commensals are provided a nutrient-rich environment to inhabit, whereas the host gains energy-providing metabolites that would otherwise be unavailable (Tremaroli and Backhed 2012). However, in recent years it has become increasingly clear that the benefits derived by the host from commensals extend far beyond those that are metabolic, with the microbiota shown to be critical in many aspects of human physiology, health, and disease (Brestoff and Artis 2013; Belkaid and Hand 2014). Of relevance to the present discussion, the microbiota has been shown to play a central role in ensuring that cells of the immune system are appropriately regulated at steady state and in the context of infectious challenges (Honda and Littman 2016). The constitutive involvement of the microbiota in the regulation and function of the immune system supports the idea that vaccine design and trials should be approached in the context of the constant dialogue between microbes and the host, an area of research that has been given little attention to date (Ferreira et al. 2010; Valdez et al. 2014). In this review, we will highlight recent findings supporting the idea of a role for the microbiota in controlling vaccine responses before discussing the potential mechanisms of action and opportunities to harness such knowledge to increase vaccine efficacy.

INFLUENCE OF COMMENSAL MICROBIOTA ON THE IMMUNE SYSTEM AND RESPONSES TO INFECTION

The majority of infections occur at peripheral tissues such as the gut, skin, or lung. These sites contain a complex microbiota, as well as an intricate network of immune cells that are positioned to rapidly respond to invading pathogens (Sheridan and Lefrancois 2011). At these sites, the microbiota has been shown to control diverse aspects of immunity including the development of the immune system, the calibration of innate responses, and the induction of both effector and regulatory responses (Fig. 1) (Mazmanian et al. 2005; Belkaid and Hand 2014; Belkaid and Segre 2014; Trama et al. 2014; Jeffries et al. 2016; Kim et al. 2016; Zeng et al. 2016). As such, the microbiota represents a powerful adjuvant of the immune system that is able to promote adaptive responses against a wide range of bacterial, viral, fungal, and parasitic infections (Brandl et al. 2007; Hall et al. 2008; Ivanov et al. 2009; Zeng et al. 2016). Microbiota control of the immune system occurs via numerous mechanisms, including the generation of metabolites that activate lymphocytes or antigen-presenting cells, the activation of the inflammasome in innate cell populations, and by directly acting on epithelial cells (Belkaid and Hand 2014; Kim et al. 2016). In addition to its direct impact at barrier sites, the microbiota also influences the quality and amplitude of immune responses at distal sites (Belkaid and Hand 2014). One such example of systemic control is mediated by constitutive antibody responses to the microbiota that not only provide protection against gut microbiota translocation but also against infections with unrelated pathogenic bacteria via recognition of conserved outer-membrane molecules (Zeng et al. 2016). In this context, commensal-specific antibodies have been shown to also cross-react with HIV-1 (Trama et al. 2014; Jeffries et al. 2016), supporting the idea that preexisting commensal-specific antibodies could influence early responses (positively in the context of neutralization or negatively via enhanced infectivity) to a diverse range of microbes. How the preexistence of microbiota-specific antibodies controls the quality of vaccine responses and in particular those using "live" organisms remains to be established.

Regardless of the mechanisms involved, a consistent theme across numerous studies is that commensals influence the immune system in a way that the threshold required to respond to infection is lowered, thereby allowing for more rapid and efficient responses to pathogens. Such reliance of the immune system on constitutive microbial stimulation indicates that both responsiveness to vaccines and vaccine design need to be considered in the context of host–microbiota interactions.

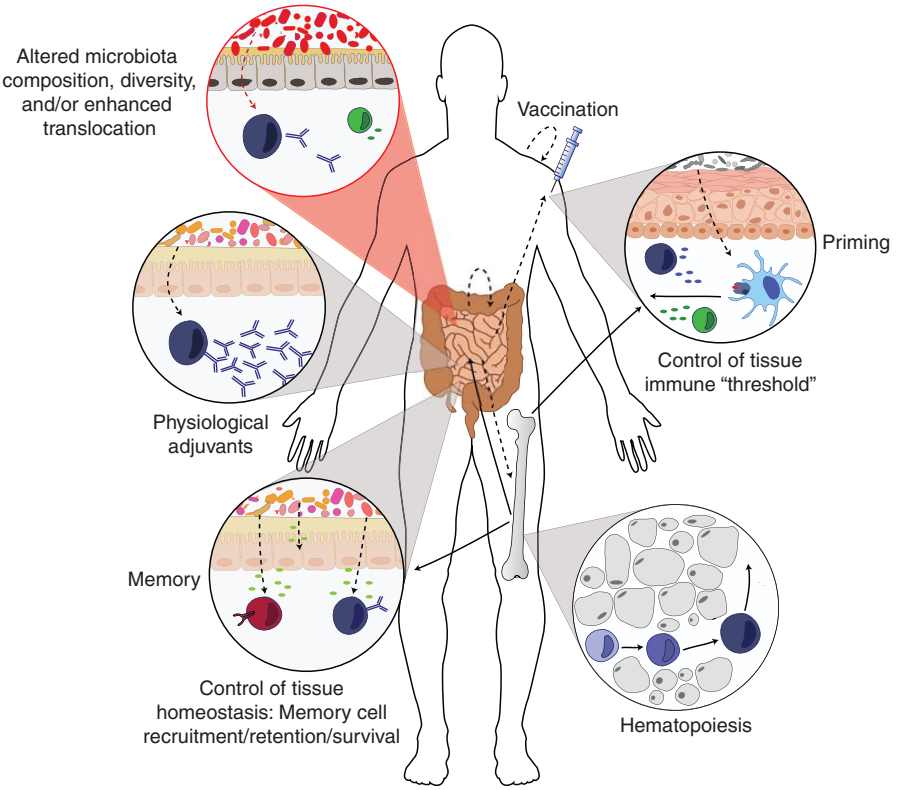

Figure 1. Influence of commensals on vaccine responses. Commensal bacteria influence the immune response in a number of ways, with these effects mediated either locally or from distal sites. Such influences include the promotion of hematopoiesis in bone marrow progenitor cells, which can then migrate throughout the body. In peripheral tissues such as the skin and gut, the microbiota can regulate the "immune threshold" of innate cells such as antigen-presenting cells or epithelial cells, allowing for rapid and efficient responses on activation. During a response to vaccination, commensals can directly promote the function of the adaptive immune system, such as by providing a source of physiological adjuvants in the gut that promote the production of antibody by B cells. Following the peak of a response, commensals may also be involved in creating an environmental niche within tissues that allow for the recruitment, development, and survival of long-lived memory cells. In addition (red circle), an altered relationship with the microbiota (e.g., decrease in microbial diversity or increase microbial translocation) of the gut microflora in settings of malnutrition and/or chronic infections could result in heightened systemic inflammation leading to blunted immune responses to vaccines. Solid lines, immune cell movement; dotted lines, microbiota effect.

INFLUENCE OF COMMENSAL MICROBIOTA ON VACCINE EFFICACY: MICROBIOTA STRATIFICATION AS A MEANS TO PREDICT VACCINE EFFICACY?

Vaccination has drastically lowered the incidence rate of infections with pathogens such as polio and measles. However, there are no commercially available vaccines that currently exist for numerous life-threatening diseases caused by infections such as tuberculosis, AIDS, or malaria (Holmgren and Czerkinsky 2005). Whether the microbiota can be harnessed to improve the efficacy of vaccination against these pathogens remains an open question. A possible link between the microbiota and vaccine efficacy was first shown in a model of oral vaccination using heat-labile enterotoxin of enterotoxigenic *Escherichia coli* as adjuvant (LT R192G/L211A) (Hall et al. 2008; Norton et al. 2011). In this

setting, depletion of the gut microbiota was associated with profoundly depressed Th1 and Th17 responses to the antigen (Hall et al. 2008). Similarly, optimal antibody responses to the seasonal trivalent influenza vaccine (TIV), as well as to the polio vaccine (IPOL) required the presence of gut commensals (Oh et al. 2014). Recent studies have suggested that the gut microbiota could influence vaccine efficacy in human and nonhuman primates (Valdez et al. 2014). For instance, a study using Cynomolgus macaques supports the idea that a stable and more diverse gut microbiota correlates with a better response to vaccination and subsequent immunity (Seekatz et al. 2013). This finding is consistent with another study involving a small human cohort, in which the majority of individuals that responded to vaccination had greater community richness and diversity among their gut microbiota (Eloe-Fadrosh et al. 2013). However, we cannot exclude the possibility that, as for many other microbiota-related observations, a lack of microbial diversity may be a "red herring" and in fact results from underlying immune defects in the less responsive groups. Nonetheless, although these studies remain limited in number and in scope, their findings raise some intriguing possibilities. In the context of future vaccine trials, it may be important to stratify individuals based on their microbiota profile and, more importantly, microbiota metabolism as well as host-genetic influences. Such lines of research should provide insight into the link between host-genetic variation in shaping both immunity and the composition of the human microbiome (Goodrich et al. 2004; Blekhman et al. 2015) and provide a starting point toward understanding factors that predict vaccine success.

RESIDENT COMMENSAL MICROFLORA IN ALTERING THE TISSUE ENVIRONMENT TO PROMOTE THE FORMATION AND SURVIVAL OF MEMORY CELLS

Because virtually all aspects of the immune system, ranging from hematopoiesis to lymphocyte function, can be controlled by the microbiota, the response to vaccination, as well as the intensity of the response and memory formation, are likely controlled directly or indirectly by these microbial partners (Fig. 1). Of interest, the tonic action of the microbiota on tissue immunity could promote the formation of a niche within the tissue microenvironment that results in enhanced recruitment, survival, and maturation of both T and B cells. For instance, an absence of commensals is associated with decreased amounts of T-cell chemoattractants as well as the survival factors interleukin (IL)-7 and IL-15 (Vonarbourg et al. 2010; Fink et al. 2012; Jiang et al. 2013). Another systemic control mediated by the microbiota occurs via the manipulation of the metabolic landscape. For instance, fatty acids that are regulated by gut commensals are also important in the development, survival, and function of memory T cells (Martin et al. 2007; Pearce et al. 2009; van der Windt et al. 2013; Cui et al. 2015). Furthermore, microbiota-dependent fermentation of plant-derived polysaccharides into short chain fatty acids also promotes the differentiation of B cells into plasma cells (Kim et al. 2016). Thus, it is conceivable that the commensal microbiota could be exploited to create an environment better equipped to not only attract memory cells but also to promote a metabolic niche compatible with enhanced memory formation and survival.

HOST-MICROBIOTA DYSREGULATION AS AN UNDERLYING CAUSE OF VACCINE FAILURE?

The role of the commensal microbiota could be particularly important when considering vaccine-induced immune responses in low- to middle-income countries, which are typically some of the worst affected by infection (Valdez et al. 2014). Studies on the effectiveness of vaccination in these areas have consistently shown blunted responses when compared to that of high-income countries. These defects are particularly striking when considering oral vaccines. For instance, oral vaccines for Rotavirus, Poliomyelitis, *Vibrio cholerae*, and *Shigella* ad-

ministered to children in low- to middle-income countries often fail to protect with the same degree of efficacy as in high-income countries (Hallander et al. 2002; Grassly et al. 2009; Jiang et al. 2010; Levine 2010; Valdez et al. 2014). Several interrelated conditions including persistent infections, gut inflammation, malnutrition, and environmental enteropathy have been proposed to contribute to this important public health issue (Levine 2010; Korpe and Petri 2012). Aberrant relationships with the microbiota under these extreme settings could also contribute to these defects. Indeed, children from defined low-income areas of the world have a distinct gut microbiota compared to those in high-income countries with the "malnourished microbiota" enriched in microbes with invasive and/or inflammatory properties (Yatsunenko et al. 2012). Another phenomenon contributing to microbiota-induced vaccine deficiencies could be associated with immune defects imposed by defined infections and sustained by the microbiota. For instance, acute infections can have dramatic and long-term consequences for tissue-specific immunity via tissue remodeling, a process that can be sustained by the microbiota in the gut (Fonseca et al. 2015). Chronic exposure to pathogens and malnutrition is also associated with a phenomenon referred to as "leaky gut," a setting leading to increased systemic microbial translocation and subsequent inflammation (Brenchley and Douek 2012; Valdez et al. 2014). Such a heightened inflammatory tone caused by both invasive microbes and enhanced microbial translocation may play a negative role in the proper orchestration of adaptive immune responses to vaccines and preclude the establishment of a healthy memory pool (Fig. 1). While resolving the environmental challenges underlying immune suppression in parts of the world deprived of basic infrastructure remains the priority, ongoing work currently exploring the microbiota of these vulnerable populations (Humphrey 2009) may allow for the development of interventions aimed at tackling defined classes of microbes, metabolites, or pathogens to improve vaccine efficacy.

LEARNING FROM THE ENDOGENOUS ADJUVANTS: THE COMMENSAL MICROBIOTA AND THEIR PRODUCTS AS A SOURCE OF PHYSIOLOGICAL ADJUVANTS?

A critical aspect in vaccine design is that the antigen administered is able to induce a potent immune response. However, antigens administered alone are not immunogenic, and so must be coupled to an immunostimulatory component, referred to as an adjuvant. The choice of an appropriate adjuvant is critical, as it can dramatically impact the long-term protective effects of the vaccine (Galli et al. 2009). A concern and major challenge going forward for the design of future vaccines is adjuvant safety (Mutsch et al. 2004). This may provide a unique opportunity to exploit the resident commensal microbiota and their products, which have been shown to possess natural adjuvant properties (Fig. 1). For example, the DNA of commensals is critical in regulating effector T-cell responses in the gut (Hall et al. 2008). Optimal antibody responses to the seasonal influenza TIV vaccine as well as to the IPOL polio vaccine require the presence of gut commensals (Oh et al. 2014). Specifically, the presence of *E. coli* species expressing flagellin were essential, with this component able to directly promote plasma cell differentiation as well as stimulate lymph node macrophages to produce plasma cell growth factors (Oh et al. 2014). It therefore appears that defined commensals and their products can promote the development of vaccine-induced immunity. While the identification of novel molecular determinants and the underlying mechanisms of microbiota–host interactions remains in its infancy, such lines of investigation appear promising. Indeed, because the microbiota has coevolved with its host to finely tune the unique requirements of the gut, these microbes may provide highly adapted tissue-specific adjuvants. Uncovering these pathways and the microbiota-derived molecules (Donia and Fischbach 2015; Medema and Fischbach 2015) involved in these processes may allow for the development of novel classes of adjuvants capable of boosting local immunity while preserving tissue homeostasis.

FUTURE PERSPECTIVES

Manipulation of microbe function or composition via diet alteration or microbiota engraftment may soon become a viable approach to control immunity and, as such, vaccine responses. This is not only true for the gut microbiota but also for all barrier tissues. For instance, at sites such as the skin or lung, which are characterized by low microbial biomass, subtle alterations in defined nutrients (Scharschmidt and Fischbach 2013) may have a dramatic impact on the microbiota composition. Going forward, rationally designed vaccines that take advantage of the inherent adjuvant properties of the microbiota could have a major impact on the prevention of disease. To the initial question "Can the microbiota be exploited to improve the efficacy of vaccines?" the answer is that learning from our endogenous original adjuvants could be critical in overcoming the enormous hurdle of vaccine design against the numerous microbes that cause chronic infections.

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Do the Microbiota Influence Vaccines and Protective Immunity to Pathogens?

If So, Is There Potential for Efficacious Microbiota-Based Vaccines?

Dan R. Littman[1,2]

[1]Kimmel Center for Biology and Medicine of the Skirball Institute, New York University School of Medicine, New York, New York 10016

[2]The Howard Hughes Medical Institute, New York University School of Medicine, New York, New York 10016

Correspondence: dan.littman@med.nyu.edu

The gut-resident constituents of the microbiota protect the mucosa from invasive pathogens through engagement of both innate and adaptive branches of the immune system. They are also likely to provide systemic protection from pathogens, by enhancing host robustness and tolerance to the invasive microbes and by inducing immune responses that prevent their growth. These properties of commensal microbiota, particularly the capacity of some bacteria to induce diverse types of antigen-specific immune responses, raises the prospect that they could be deployed as vaccine vectors to generate effective local and systemic immunity to viral and bacterial pathogens.

OUTLINE

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It has long been known that the commensal microbiota contributes to development and priming of immune system cells. During the past decade, there have been major advances in our understanding of how gut-resident microbes communicate with both innate and adaptive arms of the immune system (Hooper et al. 2012). Much of the dialogue ensures that commensal species can replicate within their niches without causing harm to the host (Ayres 2016). This process of host tolerance to microbes often provides the added benefit of resistance to pathogens, providing a first line of innate defense until adaptive responses can be mustered to clear the offending organisms. For example, commensal microbes in the gut induce innate lymphoid cells to produce interleukin (IL)-22, which, in turn, promotes barrier function through epithelial cell regeneration and production of antimicrobial peptides, limiting attachment and growth of adherent-invasive *Escherichia coli* or *Citrobacter rodentium* (Fig. 1) (Sonnenberg et al. 2011; Hooper et al. 2012; Longman et al. 2014). Commensal microbes also induce B- and T-cell responses that regulate the levels of different microbial constituents, either through direct interactions (e.g., by way of opsonized secreted IgA or T-cell killing of infected cells) or, indirectly, by antigen-specific responses that engage downstream innate effector functions (e.g., recruitment of neutrophils that kill pathogenic and nonpathogenic organisms) (Fig. 1) (Honda and Littman 2016). Engagement of both innate and adaptive immune responses by microbiota offers the potential for utilizing individual bacterial species or consortia of select strains to develop microbiome-based vaccination strategies. In this article, I will speculate on how the microbiota may provide protection from pathogenic microbes and will propose applications toward infection control.

INTESTINAL BACTERIAL CROSS TALK WITH THE HOST IMMUNE SYSTEM

It is currently thought that only a small subset of gut-resident bacteria has direct influence on the immune system. Many of these bacteria can readily be identified because they are coated with IgA or IgG. These are likely bacterial species that can be engaged by the antigen presentation machinery in the lamina propria, after which they elicit diverse T-cell response programs. Flavell and colleagues showed that mice colonized with IgA-coated bacteria from inflammatory bowel disease patients are rendered sensitive to chemically induced colitis (Palm et al. 2014). However, the IgA^+ fraction of bacteria from healthy donors did not have such propensity. Similarly, the IgA^+ microbiota from undernourished children was enriched for IgA-coated species (particularly Enterobacteriaceae) that were colitogenic in gnotobiotic mice, but the IgA^+ fraction from healthy control donors was largely protective (Kau et al. 2015). These results are consistent with the notion that gut bacteria can elicit diverse immune responses, and indicate that their ability to induce bacteria-specific IgA does not need to reflect either pro- and anti-inflammatory barrier immune responses.

It is becoming appreciated that commensal microbes induce dominant T_H1, T_H17, or Treg-cell responses in gut-associated mucosal tissues. In skin, CD8 T-cell responses are elicited by *Staphylococcus epidermidis* and, while similar responses have not been investigated in detail in the intestine, they clearly do exist, as shown recently in mice colonized with microbiota from pet store animals rather than from mice in specific pathogen-free facilities (Naik et al. 2015; Beura et al. 2016). In addition, some bacteria and helminths induce distinct cytokine responses by epithelial and myeloid cell subsets, resulting in activation of distinct subsets of innate lymphoid cells (Sano et al. 2015; Howitt et al. 2016). Together, these responses contribute not only to local barrier enforcement or wound healing, but also to systemic immune system modulation that can have either beneficial or detrimental outcomes. For example, bacteria that induce local production of IL-22 by type 3 innate lymphoid cells (ILC3) and the differentiation of T_H17 cells have important roles in protection of mucosal surfaces from pathogenic invasive microbes, but in some settings can provoke local or systemic autoimmune disease (Honda and Littman 2016).

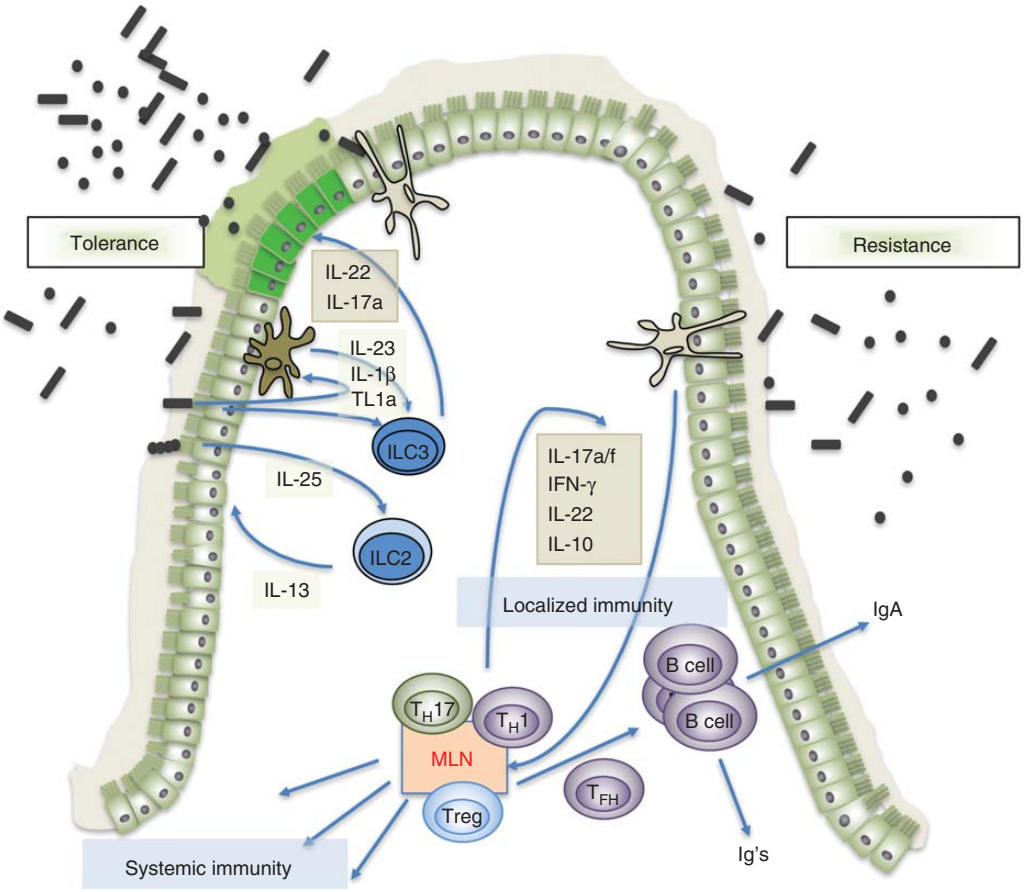

Figure 1. Microbiota-mediated strategies for host-mediated protection from pathogens. (*Left*) Tolerance induced by microbiota by way of innate signals mediated by epithelial cells, myeloid cells, and innate lymphoid cells. Cytokines and growth factors produced by cells such as ILC3 result in more robust epithelial barrier function and modifications in the mucin layer (e.g., allowing pathogenic bacteria to forage fucose, precluding invasion of host tissues despite high bacterial load). ILC2 may also participate in promoting tolerance, by responding to microbe-induced epithelial tuft cell–produced interleukin (IL)-25 with secretion of IL-13, which promotes epithelial robustness. (*Right*) Resistance to pathogenic bacteria through induction of microbiota-specific T- and B-cell responses. Myeloid cells (macrophages/dendritic cells) take up microbial products, transport them to draining lymph nodes (mesenteric lymph node [MLN]), and present microbiota antigen to naïve T cells, activating them and polarizing them (CD8 T cells are not depicted, but are likely also primed). The T cells then distribute throughout the body, including the intestinal lamina propria, and limit microbial growth through production of cytokines and recruitment of neutrophils and other phagocytic cells. The T cells also differentiate into follicular helper T cells (T_{FH}), which promote B-cell affinity maturation and isotype class switching, with production of secreted IgA and other classes of antibody (Ig) that can function systemically. IFN, Interferon.

The best-known and clinically validated example of microbiome-based therapy to protect from a pathogen is fecal microbiome transfer (FMT) for *Clostribium difficile*–mediated colitis. Pamer and colleagues used a mouse model for *C. difficile* pathogenesis to show that *Clostridial* species (e.g., *Clostridium scindens*), endowed with enzymatic capacity to modify primary bile acids (i.e., bile salt hydrolases), can prevent germination of *C. difficile* spores and thus reduce its vegetative growth. Whether this is the active principle of FMT remains to be

determined and, indeed, intestinal epithelial cell–derived antimicrobial peptides, induced by ILC3-derived IL-22, as well as interferon γ (IFN-γ)-producing ILC1 may also have critical roles in restraining *C. difficile* growth (Abt et al. 2015). Modulation of the host immune response may have an influence in therapeutic efficacy of probiotics in blooms of *C. difficile* and other pathogenic bacteria, such as vancomycin-resistant enterococcus and antibiotic-resistant *Klebsiella pneumoniae,* but mechanisms of action have not been elucidated. A detailed discussion of probiotic therapies that induce colonization resistance through different mechanisms is found in a recent review (Pamer 2016).

Studies that specifically examined microbiota effects on pathogenic microbes have largely been confined to proximal interactions that can involve competition between microbes, as postulated for *C. difficile* control, activation of host-tolerance mechanisms, or activation of immune pathways that can contribute to resistance to the infectious agent. Examples of how microbiota induce tolerance to specific pathogens are scarce, and are largely confined to model systems. For example, a recent publication described how a peptidoglycan hydrolase produced by the commensal *Enterococcus faecium* can activate host cells to tolerate infection with *Salmonella* both in *Caenorhabditis elegans* and in mice (Rangan et al. 2016). This tolerance to high levels of pathogenic microbes was proposed to be the result of enhanced barrier integrity, which would likely provide protection from multiple invasive microbes (Fig. 1). This type of microbiota-triggered host-defense mechanism seems most likely to be effective at mucosal surfaces, where high titers of potentially pathogenic microbes can be sustained with minimal damage to the host. Microbiota-mediated tolerance has also been proposed to extend beyond intestinal niches, as a mouse commensal with invasive potential was found to protect animals from muscle wasting following systemic infection with pathogenic bacteria (Schieber et al. 2015). Tolerance is often mediated through activation of innate immune pathways (e.g., inflammasome or Toll-like receptor activation and production of cytokines such as IL-22) that reinforce epithelial barriers and/or provide a source of nutrients, such as fucose, to foraging bacteria (Ayres 2016).

HARNESSING ADAPTIVE IMMUNE RESPONSES TO PROTECT FROM PATHOGENS

Although mechanistic characterization of commensal microbe-guided immune responses is in its infancy, it is becoming clear that microbial antigen-specific responses can be matched to effector functions of the responding cells. This feature of microbiota-dependent immune modulation may be amenable to manipulation for therapeutic or prophylactic vaccination to provide immunity to pathogens, for enhancing antitumor immunity, or for reducing autoinflammatory responses.

In contrast to innate responses that contribute to tolerance, resistance to pathogens by way of their targeting and elimination is most effectively achieved through adaptive immune responses. These are expected to target antigenic epitopes encoded by the immunogenic species and, potentially, by related pathogenic species. Recent studies have shown that select commensal bacteria induce polarized $CD4^+$ T-cell responses directed at antigen encoded by those bacteria. Thus, segmented filamentous bacteria (SFB) induces T_H17 cells with specificity for major histocompatibility complex (MHC) class II and SFB antigen (Yang et al. 2014). Other intestinal bacteria can induce T_H1 or Treg cell polarization, and those T cells are likewise specific for the inducing bacterial antigens. For example, colonic Treg cells induced by *Helicobacter hepaticus* recognize peptides generated from the bacterium's proteins (H Xu and DR Littman, unpubl.). In addition, the same bacteria that induce these antigen-specific polarized T-cell responses also induce follicular helper cells that contribute to production of bacteria-specific secretory IgA as well as other immunoglobulins (Fig. 1). It is not yet known whether the T_{FH} cells elicited by different bacteria at diverse sites along the gastrointestinal (GI) tract have unique properties, but with the

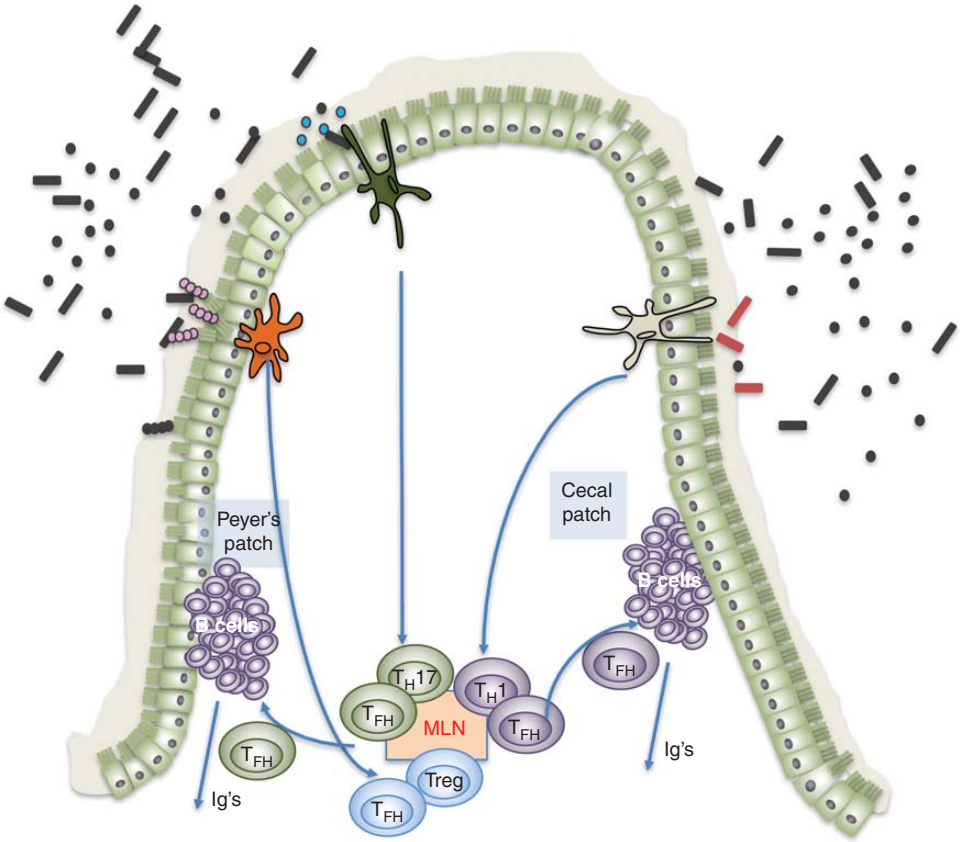

Figure 2. Microbiota-dependent follicular helper cell-mediated antibody diversification. Model for how individual commensal species may induce different types of $CD4^+$ T cells, including follicular helper T cells (T_{FH}) that elicit distinct types of antibody responses. Microbe-specific T cells could potentially be engaged to provide tailored signals to follicular B cells in gut-associated lymphoid tissues, resulting in enhanced diversification and selective isotype switching. This could be effective in conjunction with vaccination to enhance antiviral and antitumor antibody responses. Additionally, bacteria engineered to express tumor neoantigens could be used as vaccines to induce antigen-specific T_H cells with desired cytokine profiles (e.g., T_H1) for tumor immunotherapy. MLN, Mesenteric lymph node.

currently available tools it is now possible to study such cells and determine their influence on B-cell diversification. Optimization of T_{FH} function could have an impact on vaccine design, potentially allowing for enhanced immunoglobulin gene somatic hypermutation and/or directed isotype switching. This could be especially valuable for control of viral infections by eliciting broadly neutralizing antibodies (Doria-Rose and Joyce 2015).

Antibodies that neutralize HIV by targeting conserved epitopes of the viral envelope glycoprotein require numerous rounds of somatic hypermutation. While such antibodies can be detected in infected individuals, they are relatively rare and appear late in the course of disease. Vaccines that incorporate T-cell epitopes encoded by select commensal bacteria that promote T_{FH} differentiation could potentially accelerate affinity maturation, and may thus be efficacious in therapeutic and prophylactic applications (Fig. 2).

The coevolution of host and microbiome raises the possibility that some commensals are selected for their propensity to elicit tolerance mechanisms and/or specialized immune

responses that protect the host from diverse pathogens. Such a protective immune response may indeed be carried out by cells designated as T_H1^*, which have been described as having properties of both T_H1 and T_H17 cells (Sallusto 2016). These cells produce IFN-γ but, unlike T_H1 cells that express the chemokine receptor CXCR3, they additionally express CCR6, which is regulated by the T_H17-cell transcription factor RORγt. Most tellingly, patients with homozygous null mutations in *RORC* are highly susceptible to *Mycobacterium tuberculosis*, and cannot control bacterial growth after immunization with bacillus Calmette–Guérin (BCG), an attenuated strain of *Mycobacterium bovis*. Such individuals appear to have normal T_H1 responses to viruses, but are also susceptible to opportunistic *Candida albicans*, which is controlled by T_H17 cells (Okada et al. 2015). T_H17 cells induced by bacteria such as SFB do not produce IFN-γ and are thought to protect epithelial barriers but to be generally noninflammatory. In contrast, IL-23R/RORγt-dependent "pathogenic" T_H17 cells, potentially the mouse equivalent of human T_H1^* cells, produce various combinations of IL-17, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and have been implicated in multiple autoimmune diseases (Hirota et al. 2011). It is not yet known whether there are distinct constituents of the microbiota that induce such cells under homeostatic conditions. However, in the context of proinflammatory conditions, as observed following a blockade of IL-10 signaling, distinct bacterial species guide the differentiation of "pathogenic" T_H17 cells that contribute to inflammatory bowel disease (Ahern et al. 2010). It has also been shown that T_H17 cells induced by gut bacteria can function systemically in promoting autoimmune diseases, including a spontaneous model of arthritis and myelin protein–induced experimental autoimmune encephalomyelitis (Wu et al. 2010; Lee et al. 2011). Treg cells induced in the large intestine by colonization with a consortium of *Clostridial* species have likewise been shown to restrain systemic inflammatory processes (Atarashi et al. 2013). These findings suggest that it may be possible to engineer specific bacterial strains endowed with desired inductive functions to serve as vaccine vectors. The T cells would be primed in the intestinal mucosa–associated lymphoid tissues, most likely the mesenteric lymph nodes, and could then exert their protective functions either in the gut or elsewhere. SFB-specific T cells induced by the colonizing bacteria can be found disseminated in lymphoid organs distal to the intestine, which is consistent with the notion that priming of microbiota-specific T cells in the gut mucosa can contribute to systemic immunity. Whether such primed T cells can also take up residence in tissues distal to the gut, where they could exert long-term protective functions, has not been determined.

CONCLUDING REMARKS

The commensal microbiota's influence on quality and quantity of innate and adaptive immune responses and its role in protection from invasive pathogens suggests that it will have a place in design of future therapeutic and prophylactic vaccines. However, the success of such approaches will require a much deeper mechanistic understanding of how individual microbes or defined communities achieve their effect. The key parameters for the establishment of durable antigen-specific responses will need to be characterized in animal models, potentially with human microbiota constituents. As there is growing evidence that the microbiota contributes to immune control of tumors, select microbes could potentially also be used to deliver neoantigens in vaccination strategies aimed at targeting tumor cells or the tumor microenvironment (Fig. 2) (Perez-Chanona and Trinchieri 2016). Although the value of microbiota-based vaccination remains highly speculative, what is certain is that many new insights will be gained from studying immune responses to commensals in the next several years.

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Do the Microbiota Influence Vaccines and Protective Immunity to Pathogens?

Issues of Sovereignty, Federalism, and Points-Testing in the Prokaryotic and Eukaryotic Spaces of the Host-Microbial Superorganism

Andrew J. Macpherson

Maurice Müller Laboratories (DKF), Universitätsklinik für Viszerale Chirurgie und Medizin (UVCM), Inselspital, University of Bern, 3010 Bern, Switzerland

Correspondence: andrew.macpherson@insel.ch

In contrast to live attenuated vaccines, which are designed to induce immunity through a time-limited bloom in systemic tissues, the microbiota is a persistent feature of body surfaces, especially the intestine. The immune responses to the microbiota are idiosyncratic depending on the niche intimacy of different taxa and generally adapt the host to avoid overgrowth and maintain mutualism rather than to eliminate the organisms of that taxon. Both the microbiota and the host have so much molecular cross talk controlling each other, that the prokaryotic and the eukaryotic spaces of the host-microbial superorganism are federal rather than sovereign. This molecular cross talk is vital for the immune system to develop its mature form. Nevertheless, the microbiota/host biomass spaces are rather well separated: The microbiota also limits colonization and penetration of pathogens through intense metabolic competition. Immune responses to those members of the microbiota mutually adapted to intimate association at mucosal surfaces have attractive potential durability, but for clinical use as persistent vehicles they would require personalization and engineered reversibility to manage the immune context and complications in individual human subjects.

OUTLINE

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The principles of vaccination are to manipulate the immune system to combat disease. This is widely and successfully applied to prevent infections, and is in development as an approach to treat tumors. Live attenuated vaccines—mostly viruses—have been massively successful in altering the epidemiology and complications of infectious disease, although there is a sting in the tail from potential revertant strains or direct complications of vaccination itself (such as oral vaccine–induced paralytic poliomyelitis). As the complications of the diseases themselves fade from the public memory in developed countries, the small risks of vaccination complications make uptake and herd immunity of the human population a challenge. Yet, this is in a setting in which we have good information that the benefits vastly outweigh the risks.

In this essay, I shall take the microbiota as meaning the stable consortia of microbes at body surfaces, and not transitory or undefined changes associated with the consumption of preparations with limited evidence for biomedical effectiveness. I shall also focus on the intestine, although the considerations are different for different surfaces. Taking the yardstick of live organisms, the permanence of the microbiota has huge durable potential for delivering health benefits. Deliberate alterations in permanent microbial colonization also need to address the potentially pervasive complications that might develop with a long lead time, with or without unforeseen genetic recombination. Whether the risks are reasonable will depend on the clinical situation: An adult patient requiring salvage treatment from tumor in relapse or treatment of intractable inflammatory bowel disease contrasts considerably with manipulating the microbiota of a healthy baby.

FEDERALISM RATHER THAN SOVEREIGNTY

In these days of Brexit (British exit) and the political suggestions of building walls along borders, let us start with biological sovereignty. I would argue that true sovereignty hardly exists in the host-microbial superorganism. Of course, at first sight, there is a fair distinction between the living spaces of the predominantly prokaryotic microbiota and those of the eukaryotic cells of the host. Yet the mutual exchange of metabolites between these eukaryotic and prokaryotic spaces (Holmes et al. 2011), which themselves have extensive mutual signaling properties, effectively provide cross-governance between the microbiota and its host. This is generally true of the consortial arrangement in higher organisms, although I focus here on mammals. The metabolic exchange is much more than a free-trade agreement: It is the promiscuous sharing of chemical directives that determine behavior of cells in the other living space (Fig. 1). The extent of this cross-governance means that almost all organ systems in the mammalian body, especially the immune system, are profoundly shaped by the presence of the microbiota (Smith et al. 2007). Compare germ-free and colonized animals: The presence of a microbiota matures the hypoplastic germ-free secondary lymphoid structures, increases myeloid output from the bone marrow, normalizes B-lymphocyte numbers and immunoglobulin (Ig) levels, and primes innate and adaptive components of mucosal immunity (Hooper et al. 2012). In animals that are born and bred in a colonized environment (and microbes colonize body surfaces in early life), there is very little ingress of live microbiota organisms to central tissues. However, there is pervasive exchange of metabolites helping the immune system to mature.

A caveat is necessary here. Following colonization of germ-free mice, development of most maturity in organ systems, including immunity, can be recapitulated at any age. There are important exceptions, such as regulation of invariant natural killer (NK) T-cell content and (i)T_{reg} induction, which are most effective when colonization starts during early postnatal development (Gensollen et al. 2016). This is also a time when the lymphocyte immune repertoires are very sensitive to microbial exposure. For example, the "natural" antibody repertoire in mice can be shaped by early life exposure to killed microbes. This has a later functional benefit on later protective anti-glycan responses, for example, to *Streptococcus pyogenes* (Kearney et al. 2015). Given that both systemic antibody isotype preference (Cahenzli et al. 2013) and the

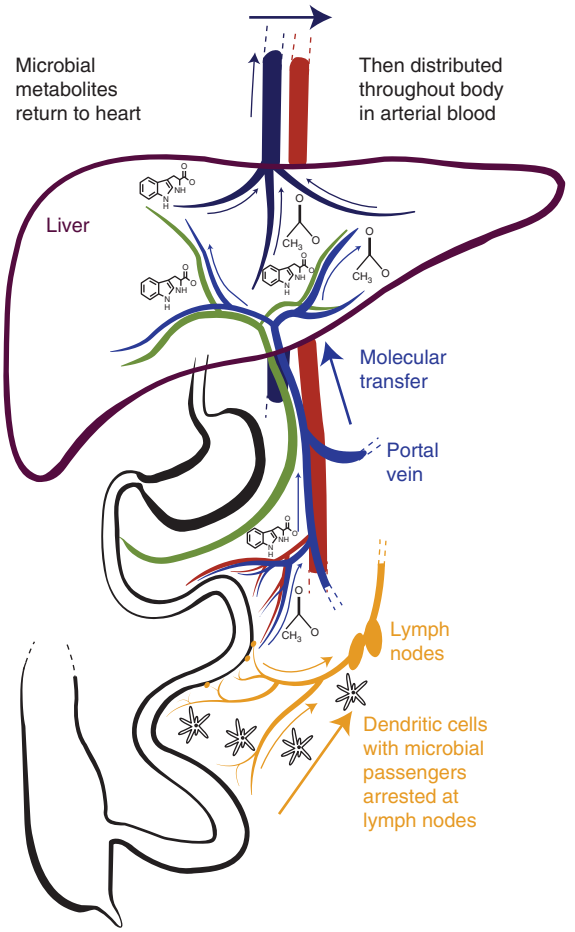

Figure 1. Sampling of live organisms and microbial molecular products from the intestinal microbiota. Live microbes are sampled at the epithelial surface by dendritic cells that can induce responses locally in the mucosal lymphoid structures or following transit to the draining lymph nodes. Microbial molecular products are absorbed into the venous blood draining the intestine, which is a tributary of the hepatic portal vein, delivering these compounds to the liver. They are then subject to hepatic metabolism and/or are released into the hepatic vein, which returns blood to the heart for recirculation over the entire body. Pervasive molecular penetration throughout body tissues contrasts with the live lymphatic microbial sampling mechanism, because the draining lymph nodes contain most microbial mutualists within intestinal tissues.

timing of mucosal antibody induction (Harris et al. 2006) are dependent on early microbial exposure, it seems likely that the early life window is a time when the developing repertoires can be shaped by the succession of postnatal colonization taxa to benefit later protective immunity to pathogens. It is also a time when the microbiota is unstable, with both immune responses and the microbiota composition being tuned by transferred maternal antibodies (Rogier et al. 2014; Koch et al. 2016). Understanding the mechanisms underlying the microbial and immunological dynamics of this process remains an important challenge, but it has massive potential to be manipulated to benefit human health.

IMMIGRATION CONTROL

Coming back to the idea of a wall, for the hypothetic eukaryotic politician promoting barriers

as a means of sovereignty, one must admit that this means of separating prokaryotic and eukaryotic living spaces is rather effective. Yet the barriers are themselves a eukaryotic response to prokaryotic chemical directives. In the intestine, one example of this is Toll-like receptor (TLR)-triggered secretion of antimicrobial peptides such as RegIIIγ (Vaishnava et al. 2011). Another example is the way in which microbial metabolic exchange between a mother and her offspring matures the barrier in neonates. In experiments in which the mother is transiently colonized during pregnancy and delivers her pups germ free, even though the germ-free neonates have never been directly exposed to live microbes, the molecules of the maternal microbiota extensively reprogram neonatal epithelial gene expression (including upregulation of RegIIIγ and the mucus synthetic pathways). This means that the young animal is conditioned to avoid penetration of challenge doses of intestinal bacteria into host tissues (Gomez de Aguero et al. 2016). In other words, the presence of the microbiota is truly mutualistic, because its chemical directives include separation of the living spaces. These chemical directives also mature the immune system in terms of homeostatic lymphocyte proliferation, increased bone marrow output, and development of secondary lymphoid structures: All of these are establishing conditions for an effective immune response (Kieper et al. 2005; Balmer et al. 2014).

There is rather effective immigration control from the cumulative effect of bilateral chemical directives, high population densities of the microbiota, and metabolic specialization/competition in the prokaryotic biomass. In the early days of germ-free husbandry, it was found that axenic animal colonies were exquisitely sensitive to pathogens. Indeed, avoiding catastrophic infections in vivaria was the original motivation to create standardized microbiotas, such as that proposed by Russell Schaedler (Macpherson and McCoy 2015). This illustrates the importance of colonization resistance as a body-surface effect of protective immunity, which sets a higher threshold for the doses of infective enteric pathogens such as *Salmonella* and *Shigella*, and probably explains the clinical effectiveness of stool transplantation in patients with recurrent infections of *Clostridium difficile* in which repeated doses of antibiotics have failed (van Nood et al. 2013). The concept of colonization resistance is not restricted to preventing a pathogen from initially establishing itself and penetrating host tissues, as postinfective restoration of microbiota diversity is necessary to clear pathogenic bacteria (Endt et al. 2010; McDonald et al. 2015; Imhann et al. 2016).

AGITATION ACROSS FEDERAL BORDERS: THE POINTS TEST FOR DIFFERENT MICROBIAL TAXA

The very reason for the existence of an immune system is that barriers are imperfect. It is clear that the immune system has immense flexibility to generate responses to a wide range of taxa, for example, the diverse and rather distinct T-helper subset differentiation responses to different microbial taxa (Sallusto 2016). The microbiota has a correspondingly broad composition, embracing bacteria, archaea, protists, viruses, helminths, and fungi. Even those taxa that are part of the rare biosphere may have functional effects outstripping their numerical frequency, although the discussion here will be on intestinal bacteria. To appreciate the impact of the microbiota on immunity, we need to consider individual intestinal niches and how the host adapts to accept different taxa in these different physical situations.

The essential microbiological difference between a bacterial pathogen compared with a mutualist of the same species is in terms of pathogenicity islands, those relatively short stretches in the bacterial genome that encode a facility for invading or damaging host tissues and surviving host phagocytic biocidal mechanisms. The evidence comes from genetic manipulation of pathogens (abrogating pathogenicity by deleting pathogenicity island components) or genetic manipulation of the host (increasing host susceptibility to nonpathogens by deleting host genes encoding biocidal mechanisms). There is no question that when some of the pathogenic effect in bacteria is attenuated, the manipulated organism can be exploited to manipulate B- and T-lymphocyte repertoires with

protective effect, making such microbes very effective immunogenic vehicles (Chatfield et al. 1989). The capacity for systemic pathogenicity (and immunogenicity) carries a metabolic cost that is rather unsuitable for persistence in the microbiota (Diard et al. 2013). So we are left with mutualists that are well adapted to existence in the prokaryotic spaces and pathogens that have adapted to bloom in the eukaryotic spaces. It is relatively easy to attenuate a pathogen to transiently immunize against the acute bloom of a pathogen, exploiting the fact that it will itself be eliminated; but this is rather different from persistently maintaining or stably controlling an immunogen in the microbiota.

There is a wide spectrum from benign behavior to proinflammatory potential in the different taxa that colonize the intestine. In contrast to the blooms of pathogens that are eliminated by pulsed systemic immune responses, there is evidence that the mucosal innate and adaptive immunity is acting more continuously to preserve mutualism with the microbiota. This is even true of the noninflammatory mutualist *Bacteroides thetaiotaomicron*, whose niche is restricted to the intestinal lumen or outer layers of mucus, which shows transcriptional signals in the bacteria of oxidative stress and of inflammatory networks in the mucosa in the absence of secreted immunoglobulin A (IgA) (Peterson et al. 2007). IgA also generally limits intestinal bacterial overgrowth (Wei et al. 2011). Generally, those taxa that are the most likely to generate intestinal inflammation are also the more strongly targeted by secreted Ig (Lecuyer et al. 2014; Palm et al. 2014; Bunker et al. 2015). Nevertheless, these responses to benign microbes are mainly focused on the intestinal mucosa, unless the intestinal permeability barrier is breached and the live organism reaches systemic secondary lymphoid structures (Macpherson et al. 2000; Konrad et al. 2006). Weak systemic responses may be enough to induce polyclonal expansions (Zeng et al. 2016), making pre-induced vaccine responses more durable (Pinna et al. 2009), but are likely less ideal for de novo induction of systemic protective immunity unless one were to compromise by making the organism more invasive.

At the other end of the spectrum are microbiota organisms that are certainly potentially proinflammatory, and so depend on more idiosyncratic mucosal immune responses to be mutualists. These often have rather intimate niches at or close to the epithelial surface. The particular idiosyncratic feature(s) (such as innate lymphoid cell cytokine secretion, regulatory T-cell induction, T_H17 induction, interleukin [IL]-10 expression) varies according to taxon and the niche it occupies (Kullberg et al. 2006; Round et al. 2011; Sonnenberg et al. 2012; Atarashi et al. 2015). It is likely that these known adaptations for mutualism in models represent examples of a wider range of microbial taxa in the context of different host species. As with the benign microbes, mutualism depends on the immune responses that are induced, although these may now show a clear extension into systemic immunity.

The best-worked-out example of this more aggressive class within the microbiota is segmented filamentous bacteria ([SFB] *Candidatus arthromitus*). This organism has evolved with an exquisitely intimate niche attached to the epithelial cells of the lower small intestine. The position is ideal for SFB to harvest the many amino acids it needs from the host or diet (because its genome has dispensed with the necessary synthetic pathways). It uses special structures named "holdfasts" to anchor itself onto the epithelial layer to avoid being expelled into the large intestine where its amino acid auxotrophy would be much harder to satiate (Kuwahara et al. 2011; Sczesnak et al. 2011). In addition to provoking extremely effective IgA responses (which appear to limit overgrowth during colonization), SFB induces a remarkably specific and numerous population of mucosal T_H17 cells with differentiation of the RORγt subset promoted by serum amyloid A secreted from the epithelial cells to which it is tethered, and a feedback circuit in which epithelial protective IL-22 is secreted by class 3 innate lymphoid cells (Fig. 2) (Lecuyer et al. 2014; Yang et al. 2014; Atarashi et al. 2015; Sano et al. 2015). Although this T_H17 population is not directly proinflammatory, under the right conditions of major histocompatibility complex (MHC)

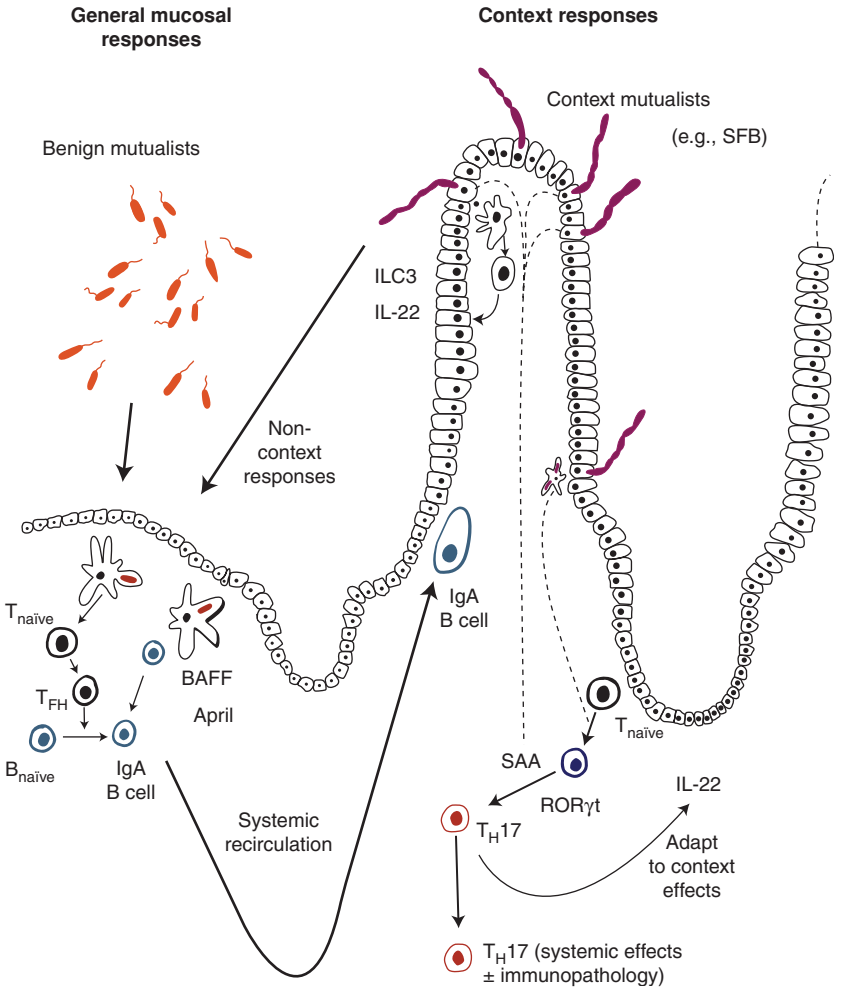

Figure 2. Induction of immune responses by members of the intestinal microbiota. The extent and character of mucosal immune response induction by the microbiota varies according to the organism concerned. Benign noninflammatory mutualists residing in the lumen or confined to outer layers of mucus induce B- and T-cell responses within the lymphoid structures of the intestine and its draining lymph nodes. These are largely focused on the mucosa itself, because the induced lymphocytes mainly home back to the intestine after systemic recirculation through the lymphatics and the blood stream. B-cell responses may be induced with T-cell help or following direct stimulation of B cells by B-cell-activating factor of the tumor-necrosis factor family (BAFF) and a proliferation-inducing ligand (APRIL) secreted by mononuclear cells. At the other end of the spectrum, organisms with especially intimate niches induce a range of additional idiosyncratic immune responses. An example is segmented filamentous bacteria (SFB), which is responsible for generating a substantial population of specific mucosal T_H17 cells, with cues from serum amyloid protein (SAA) secreted by the epithelial cells that tether the microbe in the lower small intestine. SFB also very effectively induces specific IgA, which limits overgrowth during colonization. The epithelial layer to which SFB is attached is sustained by interleukin (IL)-22 secreted by class 3 innate lymphoid cells (ILC3) through a feedback loop. Although the T_H17 cells induced by SFB are noninflammatory, in the right major histocompatibility complex (MHC) background of autoimmune predisposition, they can provide help for B-cell-dependent autoimmune arthritis.

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susceptibility, the SFB T_H17 subset can provide the necessary germinal center help to generate arthritis via autoantibodies (Maloy et al. 2003; Wu et al. 2010; Teng et al. 2016).

Given the local focus of most mucosal immune responses induced by the microbiota, the most promising opportunities are to use these responses to manipulate the system to dampen proinflammatory responses from its more aggressive members. In inflammatory bowel disease, one is seeking to identify the immunopathogenic microbes in specific host contexts (Maloy and Powrie 2011). For immunization, one would need to identify host contexts in which a particular microbe can be accepted or substituted with a feasible mutualistic response in that individual that is engineered to be more durable than current anti-inflammatory or immunosuppressive treatments. Provided the specificity of re-adaptation of the systemic repertoires and subsets can avoid host immunopathology or dysbiosis, context mutualists could be effective vehicles to induce systemic protective immune responses. An example of the principle is the protective immunity to malaria, which can be produced in mice by the induction of anti-gal antibodies following colonization by the pathobiont *Escherichia coli* O86:B7 (Yilmaz et al. 2014). The challenges of design and personalization are that we still need better understanding of some key features of the responses (such as the initiation of proinflammatory rather than homeostatic T_H subsets). We also need accurate personalized a priori models of how a particular microbe can be confined to a required niche and how it will be received immunologically depending on genetics, epigenetics, and background metabolic signaling in the recipient. For safety, especially in view of the inevitability of longer-term genetic recombination, either the systems will have to be transitory or there will need to be excellent switches to eliminate aberrant responses.

PROSPECTS

I have advanced the view that there are important differences between manipulating the immune system to eliminate the bloom of an infectious pathogen (in which the immune system is trying to secure sovereignty of central tissues) and using the federalism of persistent host-microbial interactions for the same ends (when mucosal immune responses are to a large extent gardening the microbiota to maintain mutualism).

Manipulating the microbiota is in itself a multidimensional problem of community structures and dietary conditions. It seems to me that there are reasonable prospects of microbiota design to benefit general metabolism or exploiting context mutualists as transitory immunogenic vehicles. We are currently mainly following empirical approaches, which may turn out to be enough. Nevertheless, in an age of rapidly declining empiricism in biomedicine, the real challenge is to understand the microbial and host parameters well enough to have good predictive models of the outcomes of manipulations, bringing with them the opportunity to safely bioengineer a personalized microbiota for the health of the individual human host.

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