

Why Is Initial Bacterial Colonization of the Intestine Important to Infants' and Children's Health?

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ABSTRACT

Microbial colonization of the infant occurs during a critical time window for immune and gastrointestinal development. Infant colonization sets the stage for the adult microbiome. This review is a broad survey of the factors affecting infant colonization and the downstream effects on gastrointestinal health and disease. Major topics affecting colonization include initial inoculation dependent on birth mode, the impact of breast-feeding, and inside-out modulation of the developing microbiome by the immune system. Major outcomes of colonization include the timing-dependent education of the neonatal immune system, which is interconnected with barrier function and metabolism. These all engage in further continuing cross-talk with the microbiome, genetics, and nutrition. This review also briefly examines mechanisms of disease resulting from disrupted colonization as well as nutritional and microbial therapies.

Key Words: breast-feeding, cesarean section, immune programming, infant colonization, microbiome

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Initial colonization of the infant gut by microbes sets the stage for the lifelong, relatively stable adult microbiome (1). Infants rely on colonization to complete development of the immune system and gastrointestinal tract. The first days and weeks of life represent a crucial window of opportunity for shaping the development of the gastrointestinal tract and immune system, as well as the adult microbiome. The nature of infant environmental exposures, acting through the microbiome, affects the likelihood of developing childhood and adult diseases such as obesity, food allergy, and inflammatory bowel disease (IBD).

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NORMAL COLONIZATION AND ITS IMPACT ON HUMAN DEVELOPMENT

In cases of vaginal birth, the infant is inoculated as he or she passes through the birth canal. This inoculum is a mixture of Gram-negative and Gram-positive bacteria, aerobes, and anaerobes (2). At birth, the infant gut is an aerobic environment, which gradually becomes anaerobic during a period of days (3,4). The earliest colonizing bacteria, facultative aerobes, including *Escherichia* and *Enterococcus*, eventually establish an anaerobic environment. This enables the shift to obligate anaerobes, including *Clostridia*, *Bacteroidetes*, and especially *Bifidobacteria* (5). *Bifidobacteria* comprise the largest group within the infant microbiome (4,6). Throughout this succession of organisms, the microbiota increase in diversity (6,7).

These initial colonizing species have come to be recognized as a pioneer microbiome, one that educates the developing immune system and provides favorable conditions for colonization by subsequent microbes, through production of an anaerobic environment, favorable substrates for bacterial growth, and protection from the systemic immune system (4). This pioneer microbiome also has lifelong implications: bacterial strains sampled from adults are often shared between siblings and parents, demonstrating that childhood colonization can persist throughout adulthood (8).

Beneficial Factors That Influence Colonization

Many bacterial sources for the infant derive from the maternal microbiota. Therefore, beneficial infant colonization is dependent upon maternal genetics, environmental exposures, and diet before and during pregnancy as well as during breast-feeding. For example, prenatal maternal exposure to farm animals and consumption of raw milk are associated with decreased atopic disease (9).

Increasing evidence demonstrates that the intrauterine environment is not sterile during pregnancy, contrary to prior dogma (10). Bacteria have been cultured from meconium, umbilical cord blood, fetal membranes, and amniotic fluid. Meconium, the first stool of an infant, is believed to reflect the predelivery intrauterine environment. Indeed, meconium colonization by several gut bacteria has been associated with infants born before 33 weeks of gestation, suggesting a pathologic role of intrauterine bacteria causing inflammation and subsequent preterm delivery (11). Intrauterine colonization has also been associated with worse neonatal outcomes (12). Little is, however, known of the mechanisms that allow for antenatal colonization of the infant. Given this gap in knowledge, as well as an absence of actionable steps to optimize colonization or to treat dysbiosis, this review does not delve deeply into this topic.

Delivery

Ideal infant intestinal colonization begins with oral inoculation by maternal vaginal microbiota. For a vaginally delivered

newborn, the intestinal microbiota closely resembles the microbiota of the mother's vagina (2). During vaginal birth, the infant is also inoculated with maternal intestinal bacteria (13). In addition, maternal breast milk contains viable bacteria, sampled from the maternal gut, which serves as another source of bacterial diversity for the breast-feeding infant (14).

Once the infant has been inoculated, compounds already present in the infant gut as well as from breast milk act as prebiotics and encourage growth of commensals. Prebiotics are compounds that are typically indigestible by human enzymes and instead serve as nutrition for the growth of microbes.

Natural Prebiotics

The vernix caseosa, the waxy skin coating of a fetus, is shed into the amniotic fluid as the fetus approaches term. While still in utero, the near-term fetus swallows amniotic fluid containing pieces of vernix. The vernix is made up of short-chain fatty acids (SCFAs), lipids that are unique to the vernix. Although these vernix SCFAs are indigestible by human enzymes, they provide a rich medium for growth of bacteria (15). When the infant is born, these SCFAs are already present in the gut, and act as the initial prebiotics for the infant. The importance of this role for the SCFAs is underscored in a study on a rat model of necrotizing enterocolitis (NEC), in which SCFAs were found to be protective. The protection was mediated by a change in intestinal microbial ecology, as well as an increase in regulatory cytokine interleukin (IL)-10 (15).

Once the infant begins to breast-feed, breast milk contains additional prebiotics. Colostrum contains especially high concentrations of human milk oligosaccharides (HMOs), which are indigestible by human enzymes alone. Their synthesis requires up to 10% of the total energy expended to produce human milk. These oligosaccharides, like the vernix SCFAs, promote growth of intestinal microbes (16). In vitro, HMOs selectively promote growth of commensals such as *Bifidobacterium longum* subspecies *infantis*, while suppressing growth of pathogens such as *Escherichia coli* and *Clostridium perfringens* (17). The breakdown products of HMOs from *B. infantis*, lactate and SCFAs, also inhibit *E. coli* and *C. perfringens* growth. Furthermore, the growth on HMOs rather than lactose alters the activity of *B. infantis*. *B. infantis* grown on HMOs bound more frequently to intestinal epithelial cells (IECs), and had stronger barrier-promoting and anti-inflammatory effects (18). Prebiotic compounds, such as SCFAs from the vernix caseosa and HMOs, give commensal bacteria an advantage over pathogens in the developing infant gut.

Breast Milk

The myriad of benefits of breast milk in the early postnatal period have been covered in detail in 2 reviews (16,19). We briefly summarize these benefits below and in Table 1.

Breast milk has long been known to contain bacterial DNA. Modern culture-independent techniques identified gut-associated obligate anaerobes in addition to facultative anaerobes in the breast milk as a source of diversity in the infant gut (20). Although viable bacteria are present in low concentrations (20), maternal breast milk contains live, culturable bacteria including bifidobacteria (21). The bacteria were cultured from breast milk collected in a sterile manner, and were not found on the skin. It is believed that during pregnancy, increased intestinal permeability allows increased translocation of gut luminal bacteria to the lamina propria and Peyer patches (14). These bacteria are believed to be engulfed by peripheral blood mononuclear cells and transferred intact to the mammary gland, a process known as the enteromammary pathway. A study comparing

TABLE 1. Breast milk benefits

Probiotic	Bacteria sampled from maternal gut directly inoculate infant gut
Prebiotic	Indigestible oligosaccharides
Innate immunity	Antimicrobial peptides, lactoferrin, and lysozyme sCD14 recognizes LPS Soluble TLR2
Adaptive immunity	Secretory IgA specific to pathogens encountered in maternal gut Glycan decoy cell-adhesion molecules

Maternal breast milk contains both innate and adaptive immune factors, which shape the infant microflora and protect against pathogenic infection. In addition, breast milk contains live bacteria translocated from the maternal gut and prebiotic oligosaccharides to encourage growth of commensals. IgA = immunoglobulin A; LPS = lipopolysaccharide; sCD14 = soluble CD14; TLR2 = Toll-like receptor 2.

pregnant women to nonpregnant women found bacterial DNA in both women's peripheral blood mononuclear cells, but a greater abundance and diversity of bacterial DNA in those of pregnant women (14,22). These studies in humans were complemented by studies of pregnant and lactating mice, which were found to have dramatically increased translocation of viable bacteria to the mesenteric lymph nodes and breast during pregnancy and lactation. The identification of shared species isolated from maternal fecal, peripheral blood, and breast milk samples and infant fecal samples provide further evidence of this pathway as a source of bacterial inoculation for the infant gut.

During pregnancy, the T helper (Th) cells of the maternal immune system are shifted away from Th1-type immunity and toward Th2-type immunity, to maintain tolerance of the developing fetus (23). Th1 cells protect against intracellular pathogens and protozoa. Th2 cells interact with B cells to fight extracellular helminths. One may imagine that the skew of T helper cells away from Th1-type immunity during pregnancy could be responsible for maternal tolerance of viable bacteria within circulating macrophages. Th1 cytokine IL-12 was observed in a subset of the lactating mothers (22), and caused speculation that in some cases, dysregulation of this permissive bacterial translocation could result in exaggerated Th1 cytokine production, followed by spontaneous abortion or preterm birth.

Through these prebiotic and probiotic mechanisms, breast milk shapes the intestinal microbiome. Exclusively breast-fed infants have overall greater diversity of the microbiome when compared with exclusively formula-fed infants (24). Breast-fed infants also have a higher proportion of phylum Bacteroidetes, and a lower proportion of phylum Firmicutes. Microbiome research often focuses on the relative proportions between these 2 large groups, or phyla, of bacteria in causing disease. These microbial differences cause gene expression changes in the human host. Although breast-fed infants paradoxically have a metagenome enriched in microbial virulence factors, these are in turn associated with a downregulation of host inflammatory genes (24).

Breast milk contains both innate and adaptive immune components that inhibit pathogens from colonizing the infant gut. As part of the innate immune system, breast milk contains antibacterial peptides such as lactoferrin and lysozyme. These antibacterial peptides provide broad-spectrum bacteriostatic and bacteriocidal checks on microbial growth. Breast milk contains glycans that mimic cell surface adhesion molecules on the infant's intestinal epithelium. These glycans act as decoy receptors to specific intestinal pathogens, providing a further level of protection for the infant (25).

Breast milk also contains innate immune receptors, such as soluble CD14 (sCD14) and soluble Toll-like receptor 2 (sTLR2) (19). These soluble receptors act as part of a tightly controlled immune response to colonizing microbes. sCD14 is a co-receptor that facilitates the response of TLR4 to lipopolysaccharide (LPS). sCD14 acts as a sentinel in the proximal gut lumen, and is necessary for inflammatory epithelial cytokine secretion (IL-8, tumor necrosis factor [TNF]- α) in response to Gram-negative bacteria (26). sCD14 is only partially resistant to degradation. It passes intact through the stomach, but is degraded by pancreatic enzymes before it reaches the bacteria-rich distal gut (27). TLR4 is briefly upregulated in the early neonatal period, followed by downregulation (19), which likely reflects the process of developing tolerance to commensals. sCD14 in the breast milk is similarly expressed at high levels in the first days after birth, after which it decreases gradually (26).

A variety of innate immune receptors in the milk further modulate the immune response. sTLR2 interacts with sCD14 in breast milk to suppress the inflammatory response (28). LPS-binding protein (LBP), present at low concentrations in the breast milk, attenuates the sCD14-mediated TLR4 inflammatory response (26). Another 80-kDa molecule present in breast milk, at present unidentified, increases TLR4 and TLR5 responsiveness, while decreasing TLR2 and TLR3 responses (29). These receptors demonstrate the importance of breast milk in interacting with microbial colonization to effect immune homeostasis during the first days of the infant's life.

In addition to the innate immune peptides and receptors, breast milk contains adaptive immune components. Specifically, maternal milk contains high levels of secretory immunoglobulin A (sIgA). These sIgA are specific to those pathogens encountered by the mother's own intestinal tract, targeting those pathogens the infant is most likely to encounter. Maternal gut exposure results in plasma cell production of sIgA in the mammary gland (30). These sIgA pass intact through the distal gut (27), where they affect microbial colonization in a lasting manner. In a mouse knockout model for the polymeric immunoglobulin receptor, IgA was unable to be translocated from mammary gland into the breast milk (31). The pups that did not receive passive transfer of sIgA in maternal milk had microbiota that were significantly different from controls. Furthermore, the microbial distance between groups magnified as the pups matured into adulthood, even as the pups began to produce endogenous IgA. Pups of polymeric immunoglobulin receptor knockout dams had decreased expression of epithelial maintenance genes, and were more susceptible to intestinal invasion of aerobic bacteria, and more susceptible to chemically induced colitis. These downstream effects underscore the importance of breast-feeding on the microbiota, as well as the influence of the microbiota on gene expression and disease susceptibility.

Diet in Later Childhood

Much of what we know about the impact of diet on colonization comes from observing and comparing 2 populations. One study compared the microbiota of young European children from Italy with those of children from a rural African farming community in Burkina Faso. European children breast-fed for a shorter time, ate more calories, more fat and animal protein, and less fiber when compared with those from Burkina Faso (32). This resulted in a microbiome that was dramatically less diverse, with nearly opposite ratios of the bacterial phyla Bacteroidetes and Firmicutes (Italy: 22.4% Bacteroidetes, 63.7% Firmicutes; Burkina Faso: 57.7% Bacteroidetes, 27.3% Firmicutes). Microbiota with higher proportions of phylum Firmicutes, which includes *Clostridia* and *Enterococcus*, are associated with diseases including obesity (33). Although these phylum-level differences are unlikely solely

owing to duration of breast-feeding, this suggests a benefit to increased duration of breast-feeding.

In another study of infants and adults from the United States, Amerindians from Venezuela, and individuals from rural Malawi, the differences in gene expression in the microbiome reflect a striking adaptation of the microbiota to the different diets in these populations. Among infants, the presence of urease distinguished non-US (Venezuelan and Malawian) infants from infants in the United States (34). The presence of urease signifies nitrogen scavenging in a low-protein diet. Indeed, the United States has a comparatively high-protein diet compared with the Amerindian Venezuelan diet or rural Malawian diet. Similar differences were found among the microbiota of adults, with the United States adults' microbiota enriched in genes for amino acid degradation and simple sugar breakdown, whereas those from Venezuelan and Malawian adults had microbiota enriched in genes for amino acid synthesis and starch breakdown, again reflecting the diets of the different cultures (34).

Normal Colonization Maintains Homeostasis of the Immune System

The microbiota play a role in shaping the architecture of the immune system (eg, Peyer patches), the development of specific immune cell populations (eg, regulatory T cells [T_{reg}]), and the balance between immune cell types. Two features of the gastrointestinal immune architecture affected by microbiota are the mucus layer and gut-associated lymphoid tissue (GALT), which includes Peyer patches.

Microbiota Maintain a Sterile Mucus Layer

The mucus layer maintains spatial segregation between the bacteria-rich gut lumen and the intestinal epithelium. This bacteria-free zone, approximately 50 μ m thick, protects against otherwise continuous immune stimulation and inflammation. This serves to augment the barrier function of the epithelial layer, which is a single cell thick.

Intestinal microbes provide the stimuli for maintenance of the mucus layer. Germ-free (GF) animals have a thinner mucus layer than conventional animals (35). *Akkermansia muciniphila*, which actually degrades mucin, is believed to increase the mucus layer's thickness through increased turnover (36). Similarly, probiotics have been shown to increase mucus production. In an IEC line, lactobacillus increased mucin production (37) by binding to the epithelial cells (38). In doing so, these probiotics inhibited the adherence of enteropathogens enterohemorrhagic *E coli* O157:H7 and enteropathogenic *E coli* (37).

The innate immune system maintains the sterility of the mucus layer. IECs produce antibacterial RegIII γ in a MyD88-dependent manner (39). MyD88 is an adaptor protein in the TLR pathway. RegIII γ is an antibacterial C-type lectin that targets gram-positive bacteria. MyD88 is both necessary and sufficient to main separation and prevent bacteria from interacting with epithelial cells. RegIII γ knockout mice exhibited increased adaptive immune activation, as evidenced by increased fecal IgA and increased Th1 cells in the lamina propria; these increases were dependent on the presence of the intestinal microbiota. This physical segregation of the microbiota from the intestinal epithelium serves as an example by which the innate immune system maintains tolerance of the intestinal microbiota by limiting contact with the adaptive immune system.

Resistance to Pathogens

A healthy microbiome further protects the host by forming its own protection against colonization. Commensals, like those in

probiotics, can also protect against pathogens by preventing the pathogen from attaching to the colonic epithelium, a phenomenon known as colonization resistance (40). As mentioned above, microbiota stimulate the mucus layer and also stimulate the epithelium to secrete antimicrobial peptides into this mucus layer, providing a barrier against pathogens (37). Finally, commensals themselves can produce substances to block pathogenic infection. Commensal *Bifidobacteria* protect against enterohemorrhagic *E. coli* O157:H7 infection in a mouse model by production of acetate (41).

Stimulation of GALT by Microbiota

The microbiota stimulate the formation of GALT. As evidence of the role microbiota play in forming these immunologic structures, GF animals have dramatically reduced germinal centers in the GALT and reduced secretory IgA (42,43).

IgA produced by the GALT acts in an immunomodulatory manner (44). Intestinal dendritic cells (DCs) sample the intestinal lumen. DCs that encounter bacterial polysaccharide A, a component of the capsular polysaccharide of commensal *Bacteroides fragilis*, stimulate the local adaptive immune system to produce secretory IgA. The locally produced IgA coats the antigen, resulting in decreased activation of the innate immune response. This allows commensals to persist without detection by the systemic immune system (Fig. 1) (45).

The GALT comprises a self-contained immune system, where antigens are recognized and generate an immune response, but the response (both the cells and the immunoglobulins they produce) is contained within the mucosal compartment (46,47). The segregated circulation of cells within the GALT concentrates the

immune response in the gut, the site of the bacteria, at the same time avoiding a systemic inflammatory response.

These data find further support in epidemiologic studies. In a cohort of Swedish infants, increased diversity of *Bifidobacterium* species in early fecal samples is associated with increased salivary secretory IgA at 6 months of age (48). This reflects the increased IgA production, distributed along the gastrointestinal tract. Increased salivary secretory IgA has been linked to protection against allergy (49), as further evidence of local immunity protecting against systemic reactions.

Microbiota Balance Immune Responses

Permissive Colonization

In the perinatal period, the infant immune system shifts from one with a propensity to hyperstimulation (50) to one of tolerance. The rapid colonization of the infant's gut and other surfaces requires this shift. Elahi et al (51) recently demonstrated 1 mechanism by which the neonatal gut allows colonization by microbes: a specific population of neonatal CD71⁺ erythroid cells dampens the innate immune response. This permissive microbial colonization (ideally by commensals) comes at the cost of an increased susceptibility to systemic infection in the neonatal period. The obvious magnitude of this cost underscores the evolutionary benefit of intestinal colonization in the neonatal period.

Anti-Inflammatory Stimuli

In addition to the immunomodulatory signals from the host during infancy, anti-inflammatory stimuli come from the

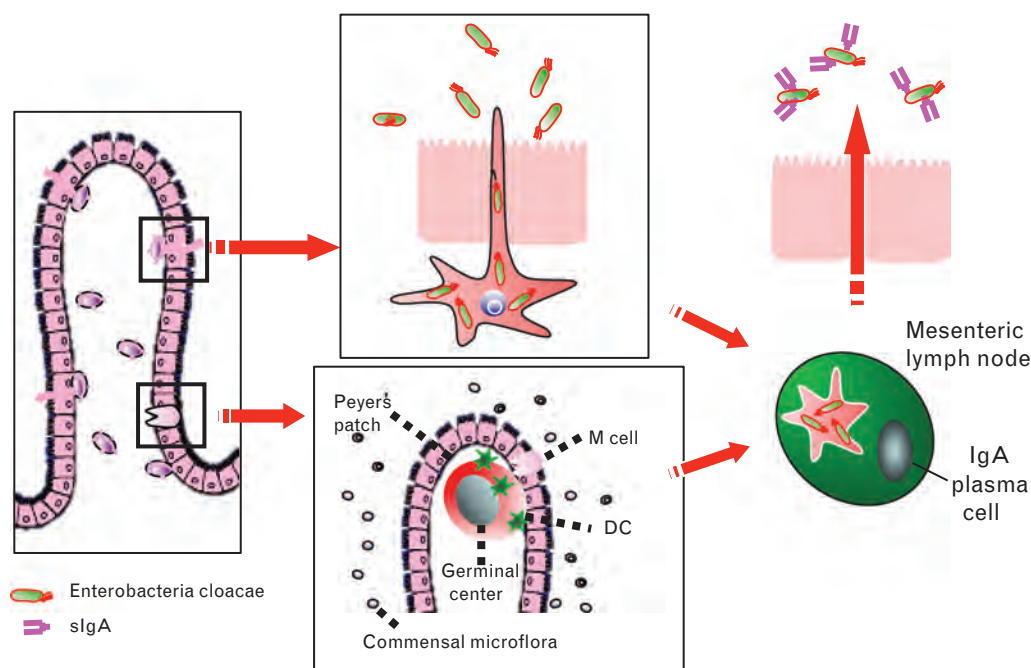


FIGURE 1. Symbiotic bacterial induction of sIgA via DCs. DCs sample gut bacteria, which stimulates the formation of local sIgA by plasma cells. This sIgA is distributed along the length of the gut but not systemically. This allows the immune system to block antigenic stimulation at the epithelial surface and preserve systemic reactivity should the bacterium pass the epithelial barrier. Adapted from (45). DC = dendritic cell; sIgA = secretory immunoglobulin A.

microbiota as well. SCFAs such as propionate and butyrate affect T_{reg} populations. High levels of SCFAs, produced when fermenting bacteria break down dietary fiber, also have immunomodulatory effects. Butyrate, in the presence of bacteria, increases the differentiation of progenitor cells to become T_{reg} cells (52). SCFAs specifically expanded the population of colonic T_{reg} cells (53). Both of these studies found an increase in IL-10 production with increased T_{reg} cells (52,53). SCFAs stimulated peripheral T_{reg} generation (butyrate and propionate) and colonic accumulation (propionate and acetate) in microbiome-depleted mice (54). Increased T_{reg} differentiation was mediated by epigenetic changes to the *FoxP3* gene locus (54).

Finally, secreted products from *Bifidobacteria* have been shown to downregulate proinflammatory cytokines IL-8 and interferon- γ , while upregulating anti-inflammatory cytokine IL-10 in a human fetal enterocyte line and fetal intestinal xenograft (55). Early colonization with high levels of *B. fragilis* is associated with decreased expression of TLR4 messenger RNA, as well as with decreased cytokine responses to LPS (48).

One of the persistent paradoxes in understanding the relation between the host immune system and the microbiota remains how the host differentiates between commensals and pathogens. One such mechanism for differentiation involves recognition of bacterial DNA. Bacterial DNA from probiotics attenuates responses to inflammatory stimuli, whereas that from pathogens such as *Salmonella* augments the inflammatory response (56). Pretreatment of human IECs with probiotic DNA before stimulation with proinflammatory TNF- α reduces IL-8 secretion, in a mechanism that involves decreased degradation of the inhibitory molecule I κ B resulting in delayed activation of transcription factor NF- κ B. These observations were supported in a Crohn colitis model, the IL-10 knockout mouse. Oral administration of probiotic DNA to IL-10 knockout mice reduced TNF- α and interferon- γ messenger RNA as well as histologic injury scores (56). This improvement in an in vivo Crohn colitis mouse model is encouraging for understanding therapeutic targets for IBD, especially given limited successes in treated IBD with probiotics (57). These DNA-dependent effects are believed to be mediated by TLR9 recognition of unmethylated CpG motifs in DNA of pathogens (56,58), yet more research is needed to identify the precise mechanism of this differentiation.

Microbes Maintain Balance Between T Helper Cell Subsets

The early gut microbiota also play a key role in regulating the balance between populations of CD4⁺ helper T cells, $Th1$ and $Th2$ cells. As mentioned before, the maternal immune system is shifted toward $Th2$ -type immunity during pregnancy (23). This $Th2$ -skewed cytokine milieu causes the infant immune system to also be shifted toward $Th2$ -type immunity. During the first weeks and months of life, infants gradually increase $Th1$ activity and restore balance of helper T cells. Without this shift, $Th2$ persistence is associated with atopic diseases including asthma (59). A bacterially derived carbohydrate is sufficient to cause this shift: gut DCs protruding through the intestinal epithelium sample commensal *B. fragilis* from the gut lumen. The sampled capsular polysaccharide A from *B. fragilis* is transported to the systemic immune system, where it restores the balance between $Th1$ and $Th2$ cells in GF mice (60). Infants born by cesarean section exhibit a delayed activation of $Th1$ -type immunity, owing to their altered colonization (6).

Similarly, $Th17$ cells, inflammatory cells that protect against infection at mucosal surfaces, are also regulated by the microbiota. Specifically, $Th17$ cells are induced by segmented filamentous bacteria (SFB) (61). In mice, SFB act via major histocompatibility

complex class II (MHCII) on intestinal DCs to increase differentiation of CD4⁺ T cells into $Th17$ cells in the lamina propria (62). In addition, an unknown stimulus acts in an SFB-independent manner through MHCII located on innate lymphoid cells to inhibit $Th17$ differentiation; lineage-specific knockout of this MHCII also results in spontaneous $Th17$ activation (62).

Appropriate Colonization and Immune Response Allows for Optimal Gut Function

Through appropriate colonization and the resulting “education” of the gastrointestinal immune system, infants develop more optimal gut function. These gut functions include vascular supply (63), epithelial healing (64), and nutrient absorption (65).

The microbiota affect gene expression in host epithelial cells. In a landmark article in 2001, GF mice were monocolonized with *Bacteroides thetaiotaomicron*, which caused changes in gene expression of lipid metabolism (66).

In another example, appropriate adaptive immune responses (IgA) to the gut microbiota are required to allow for lipid absorption and metabolism: Shulzhenko et al (65) identified interconnected regulatory signaling networks, which balance innate defense mechanisms and metabolism in gut epithelial cells. In circumstances of decreased IgA, these regulatory networks become unbalanced. The lack of an adaptive immune response causes the gut epithelium to upregulate innate immune pathways, with a coincident downregulation of genes responsible for fat uptake and metabolism. This discovery helps to explain the lipid malabsorption and cachexia seen in patients with human immunodeficiency virus and combined variable immunodeficiency. We hypothesize that the runaway immune activation seen in NEC and IBD could have similar effects on lipid absorption.

DYSBIOSIS AND ITS CONSEQUENCES: DISEASE

Dysbiosis is abnormal colonization, or the imbalance of microbes inhabiting a certain part of the body. The circumstances known to promote or to be associated with intestinal dysbiosis can be divided into the following categories based on their origin: those that stem from abnormal microbial exposures, disruptions in diet, antibiotic usage and other medications, and the influence of host genetics. It is likely that there are other as-of-yet unknown causes as well.

Abnormal Microbial Exposures

Consequences of Cesarean Delivery

In 2010, a landmark study by Dominguez-Bello et al (2) demonstrated that the intestinal microbiota of infants born by vaginal delivery closely resembled their mother's vaginal microbiota, whereas those of infants born by cesarean section instead reflected the microbes present in the infants' environment (including *Staphylococcus*). Although the majority of mothers are presumed to have normal colonization, if the infant is born vaginally and the mother is in a state of dysbiosis, she will pass this on to her infant. In a mouse model, dysbiosis has been shown to transmit phenotypic traits such as allergy (67) and obesity (68) to the recipient mouse.

If the infant is born by cesarean section, the infant gut is colonized later by environmental rather than intestinal bacteria, and those bacteria are less diverse (69,70). Infants born by cesarean section are specifically lacking in presence (69) of and diversity (70) within the *Bacteroidetes* phylum. Although further research is needed to prove causality, cesarean birth has been associated with increased rates of later celiac disease (71,72), obesity (73), and

asthma (74), presumably through the microbiota. This association is particularly strong in cases of elective rather than emergent cesarean birth (71); in a study of birth mode, microbial diversity was highest in emergent cesarean birth, followed by vaginal birth, with elective cesarean section having the least diversity (69). Although many attribute this to the possibility of retrograde colonization with prolonged ruptured membranes in emergent cesarean, differences in hormones cannot be ruled out.

Decreased Diversity

Twenty-five years ago Strachan (75) published the “hygiene hypothesis,” now a familiar concept more accurately known as the “microbial hypothesis.” He hypothesized that allergic diseases may be “prevented by infection in early childhood, transmitted by unhygienic contact with older siblings.” Further investigation has supported this concept, with new data providing support for an interaction among the high-fat/high-sugar Western diet, hygiene, and the microbiota (32,33,76).

Altered microbiota, such as that resulting from cesarean delivery, have been associated with numerous disease states. Although the strength of these assertions varies, decreased diversity has been seen in diseases such as NEC (77), colic (78), IBD (79), obesity (1), and *Clostridium difficile* colitis (80).

There are several proposed mechanisms by which decreased diversity may cause disease. As described above, early intestinal bacteria act to modulate the immune system, resulting in maintenance of the sterile internal mucus layer (39), increased secretory IgA (44,48), colonization resistance (40), immunomodulatory cytokines (55), and decreased permeability (81). Immune stimulation of the GALT actually results in decreased activation of the systemic immune system (47). Insufficient diversity may weaken these beneficial immunomodulatory signals.

Delayed Colonization

The timing of colonization matters because the immune system receives its microbial programming during the early neonatal period. Cesarean delivery results in delayed colonization, that is, a longer period after birth with fewer microbes and less diversity. There is a longer lag until the establishment of those intestinal

anaerobes most commonly associated with the adult gut, such as *Bacteroidetes* (6).

In mouse models, timing of colonization of GF mice affects the ability of the microbiota to affect immune development. In a 2012 study, 1 week or more of GF development (followed by conventionalization by cohousing) was sufficient to cause altered mononuclear cell populations and increased levels of regulatory cytokines (82). One group of mice was inoculated with populations different from the animal facility’s typical microbiome. Those inoculated after 3 weeks showed persistently altered microbiota compared with the facility, whereas those inoculated at 1 week assimilated to the facility microbiome. Mice that remained GF for 3 weeks demonstrated greater inflammatory responses in cells harvested from the mesenteric lymph node, but also increased T_{regs} and increased tolerogenic DCs (82).

In a mouse model using oral antigen (OVA) exposure, intestinal colonization was required to induce systemic tolerance (83). Monocolonization of GF mice with *B. infantis* was sufficient to produce immune responsiveness, and therefore to induce tolerance to OVA. This effect was, however, dependent upon colonization during infancy. When *B. infantis* was introduced in adulthood, the GF phenotype persisted, and oral OVA did not induce systemic tolerance.

In another study, GF mice were more susceptible to both ulcerative colitis (UC) and OVA-induced asthma than specific-pathogen-free mice (84). This susceptibility was mediated by increased invariant natural killer T cells and increased chemokine ligand CXCL16. The susceptibility was diminished when the GF pups and their mothers were colonized on the day of birth, but not when the mice were colonized at 5 weeks of age. In further experiments, this immunologic programming was found to be at least partly mediated by time-sensitive epigenetic changes. Specifically, the absence of colonization during the newborn period resulted in increased hypermethylation of the *Cxcl16* gene, causing increased invariant natural killer T cell accumulation in the colon and lungs (Fig. 2).

Taken together, these studies demonstrate the concept of a window of opportunity for microbial education of the developing immune system, which results in persistent alterations in systemic gene expression and, potentially, persistent changes in microbial populations. These data underscore the importance of the microbial health in the neonatal period. They also stimulate questions

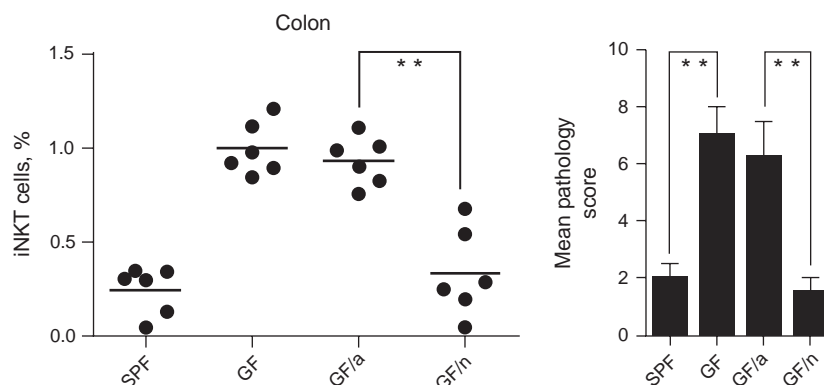


FIGURE 2. Early colonization, iNKT cells in the colon and disease. Left, Colonization of GF mice during the newborn period (GF/n) is sufficient to program the immune system to prevent iNKT cell accumulation in the colon. When GF mice are colonized as adults (GF/a), their immune system persists with the same phenotype as a GF mouse. Right, GF mice and GF/a mice are susceptible to UC. GF/n mice are protected from colitis by colonization in infancy. GF = germ free; iNKT = invariant natural killer T; SPF = specific-pathogen free; UC = ulcerative colitis. From Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 2012;336:489–93. Reprinted with permission from AAAS.

regarding probiotic therapy and microbial transplants. The circumstances of both donor and recipient could produce unforeseen effects on colonization, as well as on the resulting immunologic changes in the recipient. For example, the ages and environmental circumstances (eg, diet, microbial exposure) of both donor and recipient and the timing in relation to other microbial and immunologic events (antibiotic therapy, pro- or anti-inflammatory milieu, immunosenescence) may play a role in the success and safety of microbial transplants.

Nutritional Contributions to Dysbiosis

When to Introduce Antigens

The infant microbiome undergoes dramatic shifts upon introduction of solid foods (7). This may represent a second window of development for the intestine and immune system, especially regarding oral tolerance (85). The data on the timing of antigen introduction to reduce likelihood of an autoimmune or allergic reaction are not uniform (86). Some circumstances suggest delaying antigen introduction: in a small randomized controlled trial of infants at risk for celiac disease, the delayed introduction of gluten from 6 to 12 months resulted in a decrease in the incidence of celiac disease at 2 years, as well as decreased development of anti-gliadin immunoglobulin G antibodies (87). The authors hypothesize that the genetic predisposition may have resulted in an immature, altered microbiome and provided a ripe environment for the development of gluten sensitization during infancy.

Conversely, recent observational studies suggest that earlier introduction of antigens may decrease food allergy. One study found that Israeli Jewish children, who have a lower prevalence of peanut allergy, have significantly increased rates of early peanut consumption, whereas Jewish children in the United Kingdom, who have a higher prevalence of peanut allergy, rarely consume peanuts during the first year of life (88). Another study associated early introduction of complementary food (anything other than breast milk or formula before 4 months) with decreased rates of peanut-specific immunoglobulin E in children with a parental history of allergy or asthma (89). A third study of Finnish children at risk for type 1 diabetes mellitus found that earlier and more diverse introduction of complementary foods decreased risk of sensitization to several food and inhaled antigens (90). These studies are in contrast to earlier recommendations of delaying introduction to allergenic foods. The role of the microbiome in the interaction between genetics and food antigens remains obscure and is a possible avenue for future investigation.

Nutrition and the Mucus Layer

Alterations in nutrition play an important role in moderating host–biome interactions. As described above, the intestinal mucus layer limits the stimulation of the host immune system by the microbiota. Evidence is, however, mounting that dietary emulsifiers can degrade the mucus layer. Emulsifiers serve to improve food texture, for example, in ice cream. When added to mouse chow or water, emulsifiers decrease the mucus layer thickness and result in bacterial colonization of the inner mucus layer (91). The changes in the mucus layer result in changes in the microbiota, as well as in effects of the microbiota on the host. Mice treated with emulsifiers have increased serum levels of flagellin and systemic inflammatory markers. Although these mice do not exhibit histologic evidence of inflammation, mice treated with emulsifiers have shorter colons, which is a phenotypic marker of intestinal inflammation. Furthermore, these mice eat more and gain more weight than controls (91).

Given this groundbreaking evidence of the effects of emulsifiers on the mouse mucus layer, one can imagine the effects of

dietary emulsifiers in humans. Excessive emulsifiers in processed foods may break down the barrier between intestinal biota and host defenses, resulting in increased immune stimulation, altered microbial populations, and altered metabolism.

Thin mucus layers are found in sites of inflammation in adolescent patients with IBD when compared with noninflamed sites and with controls with functional abdominal pain (92). In patients with UC, sites of more severe inflammation have been observed to have thinner mucus layers (93). When examining colonoscopic biopsies from adults (94) and children (95), patients with IBD were found to have significant bacterial colonization of the intestinal mucosal surface, whereas in controls the mucosa was nearly sterile. Furthermore, increasing mucosal bacteria were associated with increased severity of inflammation (94). In an animal model of Crohn disease, a high-fat/high-sugar diet resulted in dysbiosis, decreased mucus layer thickness, increased permeability, and increased susceptibility to colonization with pathogenic *E coli* (96). Changes in the mucus layer may allow for increased systemic inflammation, with dramatic effects on metabolism.

Nutrition, Inflammation, and Obesity

A landmark article associated chronic systemic inflammation with metabolic syndrome (97). In a mouse model, levels of LPS in the blood (endotoxemia) were increased in mice fed a high-fat diet, and this caused inflammation. The authors hypothesized that this endotoxemia was responsible for the metabolic syndrome seen in both humans and mice consuming high-fat diets. The endotoxemia and systemic inflammation were recapitulated by direct subcutaneous infusion of LPS in mice. This resulted in increased fasting glucose, insulinemia, weight gain, adipose inflammation, hepatic insulin resistance, and increased liver triglycerides—all markers of metabolic syndrome.

In a follow-up study, researchers determined that the microbiota of high-fat diet mice provided the inflammatory stimulus for these metabolic changes (98). Broad-spectrum antibiotics given to high-fat diet mice reduced the intestinal bacterial load, which resulted in improved barrier function (less permeability), reduced endotoxemia, and reduced inflammatory markers. Specifically, antibiotic treatment normalized levels of TNF- α , IL-1, and plasminogen activator inhibitor-1. Plasminogen activator inhibitor-1 is elevated in patients with metabolic syndrome and is believed to be at least partially responsible for the increased cardiovascular disease seen in metabolic syndrome (99). Antibiotic treatment also normalized markers of oxidative stress and macrophage infiltration in the visceral adipose tissue, with a subset of these also reduced in subcutaneous adipose tissue. These were associated with improved glucose tolerance, reduced insulin resistance, and decreased visceral adiposity. Results in an *ob/ob* mouse model were similar to those in the high-fat diet mouse model (98).

A retrospective case-control study (sampled from a prospective study) compared the infantile microbiota of overweight/obese and normal-weight children. Children were matched for mode of delivery, duration of breast-feeding, use of antibiotics and probiotics, and body mass index of the infant at birth. Children who became overweight/obese at 7 years of age had a trend toward lower proportions of *Bifidobacteria* and significantly higher concentrations of *Staphylococcus aureus* in their stool at 6 and 12 months of age (100). As discussed later, *Bifidobacteria* are associated with improved barrier function and an improved metabolic profile (101).

In patients with obesity (as well as in mice), there is a decrease in the ratio between the bacterial groups *Bacteroidetes* and *Firmicutes* (B:F ratio) as compared with lean patients who have

a higher ratio (33). Through dieting, patients with obesity gradually increased their B:F ratio. After a year of dieting and weight loss, the B:F ratio of patients with obesity was similar to that of lean patients.

This increased B:F ratio was also seen in African children from Burkina Faso when compared with European children from Italy (32). Both the African children and patients with obesity on dietary therapy had increased fiber intake, decreased animal fats, and decreased total caloric intake, resulting in microbial change. As mentioned earlier, breast-fed infants have an increased B:F ratio compared with non-breast-fed infants (24). This increased B:F ratio seen in infancy may initiate a cycle of microbiome affecting diet and metabolism, which in turn alters the microbiome, and help explain the protective effect of breast-feeding against developing obesity (102,103).

Reinhardt et al (104) outline how psychological stress affects obesity through the microbiome. Animal studies of newborn mouse pups and pregnant macaques demonstrate that stress results in dysbiosis. In the mouse studies, this dysbiosis resulted in increased intestinal permeability, akin to that seen in obesity. This dysbiosis and permeability was reversed by neonatal administration of probiotics.

Taken together, these nutritional studies demonstrate the critical importance of diet in influencing not only the mucus layer and intestinal health but also metabolism. Childhood dietary choices interact with and have the potential to shift the microbiota established in infancy by breast milk and environmental exposures, in turn shaping the child's metabolic and immune profile (105).

Antibiotic Usage

The effects of antibiotics on the intestinal microbiome provide further evidence for the importance of a diverse microbiome in maintaining homeostasis, particularly in the perinatal period. In a population-based cohort study of children in the United Kingdom ($N = 1,072,426$), increased antibiotic use was associated with an increased risk of developing IBD (106). The younger the age of exposure, the stronger the relation was with development of IBD. The study identified anti-anaerobic antibiotic use as their primary analysis because of the observed specific lack of diversity in anaerobes seen in patients with active IBD (79). The relation persisted when examining any antibiotic use. Finally, a dose-response relation was observed: those with greater numbers of courses of anti-anaerobic antibiotics had increased hazard ratios of IBD. Of note, the study excluded antibiotics prescribed in the period immediately before the diagnosis of IBD that may have been prescribed to treat symptoms of undiagnosed IBD. Without this exclusion, patients who received antibiotics leading up to their diagnosis of IBD may have falsely skewed the results toward an even greater association between antibiotics and IBD.

Similarly, a large, multicenter cohort analysis of extremely low birth weight infants ($N = 5693$) found that prolonged empiric antibiotics increased the risks of NEC, death, and the composite outcome of NEC or death in a linear fashion (107). For example, there was approximately 7% increase in the odds of developing NEC for each additional day of empiric antibiotics. The association remained even after adjusting for sicker infants receiving longer courses of antibiotics.

Finally, a study in mice demonstrated that early antibiotic usage had lasting effects on immunity and metabolism because of transient changes in the microbiome (108). Early exposure to low-dose penicillin (LDP) contributed to obesity and decreased expression of several genes related to immunity. The timing of LDP exposure was significant, with earlier penicillin exposure resulting in greater metabolic changes. The effects of LDP were additive with diet, such that LDP-exposed mice fed a high-fat diet had greater fat

mass accumulation than those either fed a high-fat diet or exposed to LDP alone. The metabolic changes were caused by alterations in the microbiota: when the microbiota from LDP-exposed mice were transplanted into GF mice, the metabolic and immune changes were recapitulated. Perhaps most important, short periods of LDP exposure resulted in “durable phenotypes.” Despite normalization of the microbiota after cessation of antibiotics, the phenotypes of elevated fat mass and decreased expression of immune-related genes persisted. This study incorporates several important concepts related to infant colonization including synergy of microbiota and diet, transmissibility of phenotypes through the microbiome, and most important the window of opportunity for regulation of the immune system and metabolism.

Genetics and Dysbiosis

Although research into the effects of dysbiosis on the host abounds, the effects of the host on the microbiome are more limited. In a mouse model of genetic susceptibility to food allergy, the baseline microbiome of genetically susceptible mice differed significantly compared with wild-type mice (67). Upon sensitization with allergen ovalbumin (OVA), the microbiome of allergy-prone mice shifted further from baseline.

Dysbiosis resulting from genetic mutations have also been shown to be transmissible. Genetically, obese mice had microbiota with an increased capacity for energy harvest compared with lean littermate mice and were not attributable to differences in food consumption in this study (68). This increased capacity for energy harvest was demonstrated by the decreased energy remaining in cecal contents of obese mice. That this microbial population was responsible for the obese phenotype was confirmed by microbial transplant. GF mice that received microbiota from obese mice increased their body fat by 47%, significantly greater than those that received lean microbiota (27% increase). There was no difference in diet between the 2 groups.

Similar studies in mouse models of Crohn disease (109) and UC (110) provide further evidence of the effects of the host on the microbiome. Each study demonstrates that genetic mutations cause dysbiosis, which can then be transplanted into GF wild-type mice, and recapitulate the phenotype seen in mice with that genetic mutation.

These genetic studies contribute to the concept described by Hooper et al (46), that the host exerts inside-out control over the microbiota, whereas the microbiota also exert outside-in programming of host immunity and metabolism. This cycle of crosstalk among the microbiota, host genetics, nutrition, immunity, and metabolism is initiated in infancy (Fig. 3). The window of opportunity to establish host immunity, and therefore inside-out control of the microbiome, whose effects will persist throughout life, depends on appropriate infant colonization through prenatal maternal exposures (9), delivery mode (2), breast-feeding (14,16,19), and judicious use of antibiotics (106).

POTENTIAL SOLUTIONS AND RECOMMENDATIONS

Treatments for dysbiosis are in their infancy. Ideally, in the future, treatments will be tailored to the cause of dysbiosis and will reflect knowledge of microbial-gut homeostasis. Patients are aware of microbial therapies such as probiotics and fecal microbiota transplant (FMT) (111). Physicians have a responsibility to inform themselves about these therapies and, in turn, educate their patients as to the risks and benefits of microbial therapy.

When dysbiosis has already occurred, there are 2 major categories of treatment: nutritional changes to encourage the growth

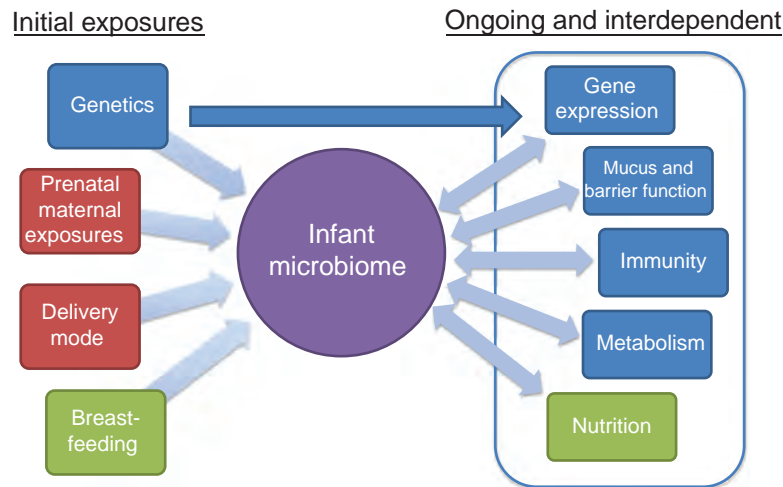


FIGURE 3. Cross-talk between microbiome and intestinal homeostasis. Initial infant colonization results from genetics, microbial exposures such as delivery mode and antibiotic usage, and breast-feeding. This, in turn, sets in motion the cross-talk among the microbiome, nutrition, immunity, barrier function, metabolism, and gene expression. The initial colonization of the infant and microbiome-directed therapies represent a major avenue for the prevention and treatment of immune and metabolic disease.

of normal endogenous microbes and direct administration of live microorganisms.

Nutritional Measures That Directly Affect the Microbiome

Breast-feeding remains one of the most important factors in determining initial infant colonization and future health (22). In cases in which breast milk is not available, donor human breast milk represents a preferred alternative to infant formula, especially in cases of prematurity (112). Donor breast milk and maternal breast milk, however, exhibit important differences. The immunologic composition of breast milk changes significantly depending on time since delivery and gestational age at delivery (113). For example, levels of sIgA, IgG, and many growth factors are increased in breast milk from mothers of preterm infants, and are highest in colostrum, tapering with increasing time since delivery. Conversely, lactoferrin is decreased in milk from mothers who deliver prematurely compared with that from mothers who deliver at term, and also tapers over time. Cytokine profiles also vary with gestational age and time since delivery. In addition, the bacterial inoculum in mother's milk, which includes bacteria sampled from the maternal gut, is killed during donor milk pasteurization (114). Given the differences in adaptive and innate immune components of milk depending on gestational age at delivery and time since birth, compounded by pasteurization, donor milk remains an imperfect substitute for maternal breast milk, particularly in the preterm infant (113).

The effects of diet as an independent driver of the microbiome are well established. In a simplified mouse model of microbial populations, changes in diet predicted more than half of the observed changes in the microbiome (115). Mice were colonized with 10 species common to the healthy human gut, with varying metabolic functions. Mice were fed combinations of 4 purified macronutrients: casein, corn oil, cornstarch, and sucrose. The observed changes were sufficient data to produce a linear model of microbial population change, which was then used to predict changes in the mouse microbiome in response to different macronutrient components of the diet. The experiment was repeated using common pureed baby foods in place of purified macronutrients (115).

Levels of fiber, fat, and animal protein are often cited as the major differences and drivers of microbial change in human diets. Low levels of fiber in the Western diet are believed to decrease substrates for the production of SCFAs and therefore lead to decreased SCFAs and decreased T_{reg} cells (53). In another example showing the impact of diet, in a study of older adults, those who lived in the community had much more diverse microbiota than those who lived in long-term care facilities (76). Upon closer examination, the location of dwelling was not the primary driver of microbial change. Rather, the change in diet from one of high fiber with low or moderate fat to one of low fiber with moderate or high fat resulted in a dramatic shift away from the community-associated microbiome to one associated with long-term care facilities.

Initial observations of children undergoing nutritional therapy for Crohn disease suggest that changes to the microbiome are associated with clinical improvement. Nutritional therapy results in significant changes in the microbiome (116), with increased *Bacteroidetes* and increased overall diversity (117). A larger study is examining the effects of elemental diet therapy for pediatric IBD on the microbiome (118).

Prebiotics

Prebiotics alter the ratios of endogenous bacteria in the intestine, in turn affecting gut function and metabolism. As described in the beginning of this review, prebiotics in the vernix caseosa and breast milk promote growth of commensals such as *B. longum* (17). Prebiotics can correct bacterial imbalances, with far-reaching effects. For example, the prebiotic oligofructose increases the proportion of colonic *Bifidobacteria*, improves gut barrier function, decreases endotoxemia, and improves glucose tolerance (101,104,119). These benefits of oligofructose and *Bifidobacteria* are mediated through glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). GLP-1 and GLP-2 are gastrointestinal hormones that decrease serum glucose and promote satiety. The effects on glucose and satiety in turn alter host metabolism and diet. One may speculate that the anti-inflammatory properties of the secreted products of *Bifidobacteria* seen in the immature gut (55) also play a role in mediating the benefits of prebiotics.

Prebiotics have been shown to increase absorption of minerals, especially calcium (120). Prebiotics, in combination with probiotics (so-called synbiotics), have also demonstrated efficacy in treatment of UC (121). Prebiotics generally have mild adverse effects, such as flatulence and increased stool output when taken in larger amounts (120). Despite this, and given the unknown risks associated with administration of microbial communities, prebiotics represent an appealing and safe therapeutic option for treatment of dysbiosis.

Microbial Therapies

Maternal Microbiota

The state of the maternal microbiota affects the colonization of the infant from birth. As such, the prenatal and even prepregnancy maternal microbiota represent a target in the prevention of infant dysbiosis. As mentioned before, maternal farming exposures and consumption of raw milk modulate infant immunity, as demonstrated by decreased risk of atopic disease (9). Further research is, however, needed before widespread adoption of maternal interventions such as probiotic use.

A study is ongoing to evaluate the exogenous administration of vaginal secretions to infants delivered by cesarean section (122). This study could lead to an exciting change in practice. In certain circumstances, the cause of cesarean delivery, such as macrosomia or preterm delivery, could, however, be the result of maternal dysbiosis, such as diabetes (98) or infection of the birth canal (123). Further research will be needed before changes can be implemented widely.

Probiotics

In theory, probiotics represent a tempting fix to complex dysbiosis: identify the missing bacteria and replace them. In practice, this has proved more difficult. Probiotics have been found to be effective in some diseases, such as irritable bowel syndrome and pouchitis. The results in other diseases such as Crohn disease have, however, been disappointing (40), likely owing to the variability in pathogenesis and in the course of Crohn disease. The treatment of NEC with probiotics or probiotic-secreted factors remains a tantalizing possibility (55). The mechanisms of action of probiotics are diverse and include nearly every effect on the host as by the endogenous microbiome. These include stimulation of mucus production (37), inhibition of pathogen adherence (38), and improved barrier function (18). Probiotics downregulate expression of innate immune receptors (40), balance subsets of T helper cells (60), and alter gene expression (66).

Despite more than a decade of basic research and clinical trials on a range of single-organism and cocktail probiotics (40), the efficacy of a probiotic for a given condition remains hard to predict. The adult microbiome is stable and relatively difficult to perturb (8). The evidence for treatment of conditions such as Crohn disease is equivocal (57). In a notable trial, probiotics in the setting of severe pancreatitis has been shown to be harmful (40). Given these mixed results, probiotic research remains in a guess-and-check stage. Those probiotics that have been shown to be helpful, such as combination probiotic VSL#3 for pouchitis, or *Lactobacillus GG* and *B. lactis* for allergy, may, however, be used with confidence (124).

Helminths

The use of helminths as an anti-inflammatory therapy has caught the attention of both physicians and the public. Research into the anti-inflammatory properties of helminth infection is inspired

by the hygiene hypothesis (75), and the eradication of helminths in the industrialized world, where autoimmune diseases are most prevalent. Indeed, a case-control study in South Africa found childhood helminth infection to be protective against IBD (adjusted odds ratio 0.2) (125). The majority of studies use the porcine whipworm, *Trichuris suis*, because of its accessibility in large numbers, its ability to temporarily and safely colonize humans, and the lack of transmission between close human contacts (126). Present data are, however, extremely limited. A randomized controlled trial of 54 patients with UC demonstrated improvement in 13/30 (43.3%) patients who received *T. suis* ova (TSO), versus improvement in 4/24 (16.7%) patients in the placebo arm (127). An open-label trial of TSO administration in 29 patients with Crohn disease showed a 79.3% response rate (23/29 patients) (128). These and further studies (129) have found *T. suis* therapy to be safe, even with patients concurrently taking immunosuppressants.

The mechanisms of helminth therapy are just beginning to be elucidated. Another worm, *Heligmosomoides polygyrus bakeri*, was found to induce activation of Foxp3⁺ T_{reg}s in a mouse model (130). These T_{reg} cells were transplanted into Rag mice, where they prevented piroxicam-induced colitis. T_{reg} cells from uninfected mice were not protective.

Further studies suggest that administration of secreted/excreted worm products may be sufficient to produce anti-inflammatory effects. Secreted/excreted products from *T. suis* were applied to mouse IECs. This reduced the inflammatory reaction to LPS, although surprisingly it increased the permeability of the epithelial layer (131).

Despite these early promising studies, study cohorts for helminth therapy have so far been small and study durations short. There is limited understanding of mechanisms of action of helminth therapies. Furthermore, nearly all of these investigations have originated from the same group of scientists. Further studies are needed at multiple sites, with more patients and longer trials.

FMT

FMT has demonstrated efficacy in adults for recurrent *C. difficile* colitis (132). Public interest in FMT has grown (111) and FMT is proposed for an increasingly diverse range of pediatric conditions (133). The evidence in pediatric patients is, however, limited to a few cases. Given the interest in FMT, coupled with the limited data in pediatric FMT, we cover this topic in detail.

In 2010, Russell et al (134) reported a case of a 2-year-old girl with relapsing *C. difficile* infection, refractory to 8 months of traditional and experimental antibiotics and probiotics. After appropriate donor screening, a single FMT from the patient's father via nasogastric tube was effective.

An open-label pilot study of 10 children, ages 7 to 21 years, evaluated FMT in mild-to-moderate UC (135). Five consecutive daily treatments were administered by enema from screened donors. One patient could not retain the enema and was excluded. A total of 7 of 9 patients responded to FMT at 1 week, of whom 6 maintained the response at 1 month. Three of 9 patients reported remission at 1 week. Adverse effects included fever in 2 patients, which were controlled with antipyretics.

Most recently, a 1-year-old patient with early-onset colitis was treated with FMT (136). The patient presented with bloody diarrhea at 10 months, refractory to mesalamine, azathioprine, prednisolone, infliximab, probiotics, antibiotics, and amino acid-based formula. Genetic conditions and infectious causes were ruled out. At age 18 months, the patient received 4 courses of FMT from an age-matched cousin with temporary response. The first course resulted in a marked reaction, with profuse sweating, vomiting, paleness, tachycardia, and fever. This reaction recurred but

diminished with the following 2 treatments. After a total of 7 treatments, the patient was able to taper off medications. The patient was asymptomatic with normal histology 6 months after the last FMT, 3 months after stopping all medications.

These limited data demonstrate the variable response to FMT, as well as a potential for inflammatory reactions to treatment. Although no major adverse events such as sepsis have been reported, the UC study was careful to exclude patients receiving anti-TNF therapy or patients with severe disease (135). All of the studies performed careful screening of donors (134).

The mechanism of improvement in FMT is still unknown. Longer-term efficacy and safety profiles of FMT are also unknown. As demonstrated in this review, dysbiosis can occur secondary to altered immunity, diet, or genetics, with insidious effects on host metabolism and immunity. Related microbial donors may share a genetic mutation with the patient, resulting in a similar dysbiosis. The microbiome has been implicated in diseases with insidious onset, such as cardiovascular disease (137), making adverse effects of FMT difficult to track. Finally, it is unknown which bacteria may be commensals in one patient, but pathogens in another.

Given the enthusiasm for and perception of FMT as “natural” demonstrated by patients (111), we recommend open discussion of FMT with patients, but reservation of FMT for cases where traditional treatments have been proven futile.

CONCLUSIONS

The mechanisms of relations among host, microbiota, and environment are gradually being revealed. Infant exposures set into motion simultaneous and reciprocal development of the immune system, gastrointestinal tract, and metabolism. The exposures affecting the infant microbiota, including maternal exposures, birth mode, breast-feeding, and antibiotics, continue to affect the individual and his or her physiology throughout childhood and into adulthood. Treatment options for dysbiosis have gained greater awareness and acceptance in the general population. The evidence from human trials of therapeutic helminths and FMT are, however, based on small numbers of patients. These therapies should be exercised with caution in patients for whom traditional therapies have been exhausted.

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