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See ARTICLE page 148

Host immune response to antibiotic perturbation of the microbiota

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Mammals are superorganisms, being a composite of mammalian and microbial cells existing in symbiosis. Although the microbiota is not essential for life, commensal and intestinal epithelial cell interactions are critical for the maturation of the immune system. Antibiotic treatment alters this delicate balance by causing compositional changes in the intestinal microbiota, and may lead to a homeostatic imbalance through alterations in expression of IEC tight junction proteins, mucin, antimicrobial peptides, and cytokines. Dysregulation of the homeostasis between mammals and their intestinal symbionts has been shown to predispose the host to enteric infection, and may lead to development of inflammatory bowel diseases.

As western societies have progressed, advances in health and hygiene have altered human–microbe interactions through increased sanitation, antibiotic usage, and vaccination. Concordantly, epidemiological studies have shown an alarming increase in the occurrence of immune-mediated disorders, including inflammatory bowel disease (IBD). Susceptibility and severity of IBD, including Crohn's disease and ulcerative colitis, has been linked to alteration of the microbiota composition. However, it is unknown if such changes precede and contribute to the onset of IBD or are simply a result of IBD. The composition of the microbiota is significantly affected by the use of antibiotics, which are often used extensively, and can lead to antibiotic-associated diarrhea and development of secondary infections such as urinary tract infections. The alteration in microbiota composition is believed to reduce carbohydrate fermentation and impair metabolism of bile acids, as well as creating niches for pathogens to proliferate. A major example of this is *Clostridium difficile*-associated disease. Hence, it is of importance to understand the mechanisms by which antibiotic-induced fluctuations of the microbiota perturb the homeostatic state of the intestinal immune system.

A recent investigation by Sekirov *et al.*[1] has shown that antibiotic-mediated disturbance in the composition, but not total numbers of the intestinal microbiota predisposes mice to higher colonization by *Salmonella typhimurium* and more severe pathology. This shows that altering the microbiota composition, without creating vacant niches in the microbial community, predisposes the host to enteric infection. Two factors could be responsible for the increased susceptibility to enteric infection. Antibiotic treatment could result in selective removal of a group of commensal organisms that serve as a barrier to *S. typhimurium* colonization and/or persistence. Disturbance of the microbiota could also result in alterations in the mucosal immune response, thereby indirectly affecting Salmonella's ability to cause disease. Studies using germ-free (GF) mice and probiotics suggest that changes in microbiota composition are sufficient to perturb the homeostatic state of the intestine and result in mucosal immune defects, which would impact host susceptibility to enteric infections. The importance of a healthy microbiota in the maintenance of intestinal homeostasis and defense against enteric infections and, perhaps, even other gastrointestinal diseases such as IBD is a concept that is receiving increased attention.

MICROBIOTA AND INNATE IMMUNE RESPONSES

Changes in the microbiota composition induced by antibiotic treatment, and those seen in IBD, may lead to variable concentrations of microbe-associated molecular patterns (MAMPs) present in the gut. MAMPs are evolutionarily conserved molecules expressed by both pathogens and commensals that include cell surface markers such as lipopolysaccharide, polysaccharide A, lipoteichoic acid, and peptidoglycan. MAMP concentrations are detected by pattern-recognition receptors (PRRs) of dendritic cells, M cells,

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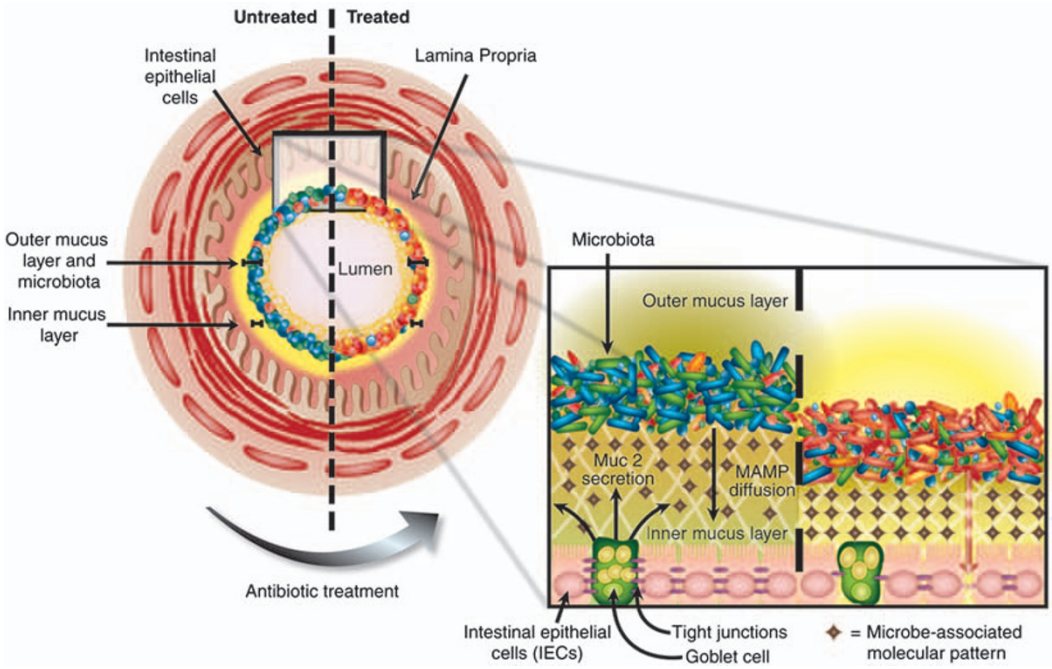

Figure 1 Antibiotic-induced perturbation of the microbiota composition affects intestinal homeostasis. Microbiota-specific fluctuations are detected by intestinal epithelial cells (IECs) and may result in a defective mucus and IEC barrier. Decreased Muc2 secretion by goblet cells could lead to increased stimulation of IECs through increased MAMP diffusion and commensal contact. Altered IEC–commensal interactions could result in decreased tight junction protein expression, increasing the permeability of the IEC barrier.

and intestinal epithelial cells (IECs).[2] A significant alteration in MAMP concentrations could disturb homeostasis of the gut-associated lymphoid tissue through weakening of the IEC barrier and changes in mucin, cytokine, and antimicrobial peptide production by IECs (see **Figure 1**). For example, administration of a commensal surface molecule, polysaccharide A, to mice resulted in suppression of IL-17 and promotion of IL-10 production by CD4+ cells, effectively protecting the host from experimentally induced colitis by *Helicobacter hepaticus*.[3] It is likely that a large portion of the microbiota have similar MAMPs, functioning as symbiosis factors,[3] to promote protective intestinal immune responses. Probiotics have also shown promise in improving colonic health, and much focus has been given to possible therapeutic strategies for IBD using these agents.[4] The beneficial effect of administering symbiosis factors and probiotics likely involves restoration of altered MAMP concentrations detected by PRRs by the mucosal immune system. Effectively modulating intestinal inflammation through changes in IEC tight junction proteins, mucins, antimicrobial peptides, and cytokines as discussed below (see **Table 1**).

Microbiota composition and mucus function

The utilization of mucin knockout mice, germ-free mice, and probiotics suggest that the intestinal mucus layer is a major mediator of IEC–commensal interactions, and that its function is largely affected by the microbiota. The mucus layer consists of two stratified layers, mainly composed of the secreted mucin Muc2.[5] The inner layer is of dense composition and devoid of commensal bacteria.[5] The outer layer is built as a loose matrix housing commensal bacteria, and may serve to sequester antimicrobial proteins.[5] Muc2 knockout mice spontaneously develop colitis, suggesting that defects in mucin production lead to altered commensal–IEC interactions.

The inner mucus layer functions as a barrier, which serves to minimize microbial translocation and prevent excessive immune activation. However, MAMPs are thought to diffuse through this layer to stimulate the underlying IECs through PRRs.[2] This was shown in germ-free mice, as they respond to microbiota colonization by increasing Muc2 sulfate incorporation.[6] Sulfate incorporation of Muc2 occurs within goblet cells before secretion and is thought to confer resistance to enzymatic degradation. In addition, *in vitro* treatment of mucin-secreting IECs with a probiotic strain, *Lactobacillus plantarum* 299v, increased *MUC2* expression and inhibited enteric pathogen adherence.[7] Current findings suggest that probiotic strains may protect the host from intestinal inflammation by induction of mucus-associated genes, which strengthens the mucus barrier and protects against colonization by enteric pathogens.

A defective mucus barrier could lead to increased stimulation of IECs by the microbiota through increased MAMP diffusion (see **Figure 1**), commensal contact with IECs, and commensal translocation to the underlying lamina propria (LP). Hyper-stimulation of IECs and commensal translocation would lead to disruption of intestinal homeostasis and induction of an inflammatory response, leading to increased host pathology and inflammation upon *S. Typhimurium*

Table 1 Examples of the impact of microbiota composition on mucosal immune responses

Change in microbiota composition via:	Effect on mucosal immunity:	Ref.
Colonization of germ-free mice		
Colonization by normal microbiota	Increase in RegIIIγ expression	Cash *et al.*[10]
Colonization by normal microbiota	Increase in Muc2 sulfate incorporation	Schwerbrock *et al.*[6]
Antibiotic treatment		
Metronidazole, neomycin, and vancomycin combination treatment of wild-type mice	Decrease in RegIIIγ expression	Brandl *et al.*[11]
Vancomycin treatment of C57Bl/6 mice	Decrease in IL-17 secretion by CD4+ cells	Ivanov *et al.*[12]
Streptomycin or vancomycin treatment of C57Bl/6 mice	Increase in *S. Typhimurium* susceptibility	Sekirov *et al.*[1]
Colonization with probiotic strains		
Lactobacillus plantarum 299v and *Lactobacillus rhamnosus* GG colonization of HT-29 intestinal epithelial cell line	Increase in MUC2 expression	Mack *et al.*[7]
VSL#3 (4 strains of *Lactobacilli*, 3 strains of *Bifidobacteria* and *Streptococcus thermophilus*) colonization of BALB/c mice	Increase in tight junction protein expression during chemically-induced colitis	Mennigen *et al.*[8]
MAMP stimulation		
Polysaccharide A (PSA) administered to C57Bl/6, *Rag*$^{-/-}$ and *IL10*$^{-/-}$ mice	Decrease in IL-17 and increase in IL-10 secretion by CD4+ cells after induction of colitis	Mazmanian *et al.*[3]
Lipopolysaccharide (LPS) administered to wild-type mice	Increase in RegIIIγ expression	Brandl *et al.*[11]

infection as seen by Sekirov *et al.*[1] Similarly, an aberrant inflammatory response to commensals is thought to be a major component in the etiology of IBD, and defects in mucin production, induced by intestinal microbiota shifts, could be a mechanism by which this occurs.

Microbiota composition and IEC barrier function

The intestinal epithelium and its protective mucus layer cover are the primary defenses against pathogen permeation and commensal leakage into the underlying LP. Colonization of the gut by probiotics results in protection of the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis upon chemically induced colitis.[8] Therefore, changing the composition of the microbiota, through antibiotic administration, could change the strength of the IEC barrier through alterations in tight junction protein expression. Decreased expression of tight junction proteins would increase the permeability of the IEC barrier allowing commensal leakage into the underlying LP, leading to inflammation that is indicative of IBD.

Intestinal intraepithelial immune cells, including natural killer cells and γδT cells, have also been shown to have a role in maintenance of IEC barrier function. During intestinal homeostasis, natural killer cells contribute to secretion of IL-22 that binds to the IL-22 receptor expressed specifically on IECs.[9] IL-22 is believed to mediate epithelial innate immunity by promoting maintenance of IEC-barrier integrity through induction of the C-type lectins RegIIIβ and RegIIIγ.[9] It is plausible that these innate immune cells respond to changes in MAMP concentrations through increased secretion of pro-inflammatory cytokines and decreased secretion of protective cytokines like IL-22. This shift in the intestinal cytokine profile would promote inflammation and increase susceptibility to intestinal diseases.

Microbiota composition and IEC-mediated antimicrobial peptide secretion

Impaired antimicrobial defense results in enhanced bacterial penetration into the LP resulting in an inflammatory response and tissue damage. Antimicrobial proteins secreted by IECs (enterocytes and paneth cells) include defensins, cathelicidins, and C-type lectins (RegIIIβ and RegIIIγ).[2] They function by disrupting bacterial surface structures and contribute to the maintenance of microbiota composition. A recent study showed that administration of a combination of the broad-spectrum antibiotics metronidazole, neomycin, and vancomycin led to significant depletion of the microbiota and decreased expression of RegIIIγ by IECs. RegIIIγ expression has been shown to rely on IEC stimulation by microbes and their products.[10] This decrease in both microbiota and RegIIIγ resulted in increased intestinal colonization by vancomycin-resistant *Enterococcus*.[11] Importantly, defective RegIIIγ expression could be corrected by administering specific MAMPs post-antibiotic treatment to selectively stimulate IEC PRRs. The addition of MAMPs mimicked the lost commensal–IEC interactions after metronidazole, neomycin, and vancomycin treatment.[11] The authors found that lipopolysaccharide, but not lipoteichoic acid, stimulation induced expression of RegIIIγ and decreased vancomycin-resistant *Enterococcus* colonization.[11] The ability of MAMP administration to regulate RegIIIγ expression provides evidence that microbiota composition has a role in regulating epithelial innate immunity.

MICROBIOTA COMPOSITION AND DISRUPTION OF INTESTINAL HOMEOSTASIS

Microbiota-specific fluctuations are likely detected by IECs and other components of the mucosal immune system, and

may alter the expression of IEC tight junction proteins, mucin, antimicrobial peptides, and cytokines (see **Table 1**). These changes in innate immunity could result in or be exacerbated by differential regulation of the Th17/Treg balance in the LP of the intestine.

Ivanov *et al.*[12,13] showed that C57BL/6 mice purchased from commercial vendors, Jackson and Taconic, have significantly different numbers of IL-17-producing cells in the LP, which correlates to the presence or absence of segmented filamentous bacteria (SFB). Taconic mice are abundant with SFB and show a higher frequency of IL-17 producing cells than Jackson mice, which lack SFB.[13] Transfer of Taconic mouse intestinal microbiota to Jackson mice results in intestinal colonization by SFB, effectively increasing the frequency of IL-17-producing cells in Jackson mice.[12,13] Also, they showed that using antibiotics to shift the microbiota composition in adult mice served to alter the frequency of IL-17-producing cells in the LP.[12] Treatment with clinical levels of vancomycin resulted in decreased levels of IL-17-producing cells, but treatment with metronidazole plus neomycin did not.[12] This suggests that microbiota composition can regulate the Th17/Treg balance in the LP, a critical process of the host immune response.

CONCLUSION

Antibiotics are often used in the clinic to treat bacterial infections, but the effects of these drugs on microbiota composition and, on intestinal immunity are poorly understood. Changes in the intestinal microbiota composition, consequential of antibiotic usage, could induce weakening of the IEC barrier through changes in mucin, cytokine, and antimicrobial peptide production by IECs. These resulting disturbances in mucosal innate immunity may lead to differential regulation of the Th17/Treg balance affecting intestinal immune responses. Alterations in microbiota composition would render a host in a state of intestinal homeostatic imbalance and predisposed to enteric infection and potentially other inflammatory bowel diseases.

DISCLOSURE

The authors declared no conflict of interest.

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