What is all the fuss over the microbiome and immunity? It is more than bugs, guts and tears

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Abstract

The interaction of the gastrointestinal tract (GIT) and the microbiome (all the microbes in the lumen of the GIT) has become an increasing area of interest in terms of the effect on the immune system and overall health of cattle. Feed changes (including feed restriction) and dehydration (including water restriction), let alone the use antimicrobials, plus the normal stressors (weather, density, weaning, etc.) all effect both the mucosal epithelial (ME) cells of the GIT and the microbiome. The ME maintain a kill zone barrier to keep out pathogens in concert with the commensal microorganisms (microbiome) and other cells of the immune system. The microbiome functions best when it is in a stable condition resulting in immune homeostasis. Immunoregulation by the ME and microbiome results in the establishment of a mucosal firewall. Disruptions in the microbiome result in dysbiosis, which decreases the kill zone, allows leaky gut, and increases inflammation. This increased inflammation is seen as an important part of pathogenesis of infectious diseases of the GIT, respiratory, and reproductive tract. This inflammation often results in a "cytokine storm"- a perfect storm that involves a physiological component of the GIT along with microbiome changes in the gut (diet change), triggering systemic changes which result in enhanced disease. By improving mucosal barriers and the decreasing stressors, overall health and productivity of the cattle can be enhanced.

Key words: bovine, microbiome, immunity

Immunity

The gut immune system consists of 3 lines of defense systems: barriers, innate immunity, and adaptive or acquired immunity (Figure 1) that work together to give ruminant gastrointestinal tract (GIT) protection from disease. In this brief overview, I will provide some basic principles. For a more complete review see Chase 2018,[2] Chase & Kaushik 2019,[3] and Gomez 2019.[6]

The intestinal barrier system is probably the most overlooked defense mechanism, but it eliminates 99.9% of all infections. This system is very susceptible to dehydration and changes in microbial populations. The gut mucosal immune system alone contains more than a trillion (10^{12}) lymphocytes and has a greater concentration of antibodies than other tissues in the body. It protects against harmful pathogens but also tolerizes (induces tolerance) the immune system to dietary antigens and normal microbial flora. The 3 defense components of the gut mucosal immune system are integrated together.

Intestinal epithelium - the largest immune organ

The first line of defense is the epithelium in the GI tract. The epithelial cells (enterocytes) function for secretion and absorption. When it comes to their immune function, they are essential. First, these cells knit themselves together with special proteins that form "tight junctions" (Figure 2). A tight junction is the physical barrier that keeps pathogens out. Healthy enterocytes will maintain that tight junction. Unhealthy epithelium becomes leaky. On the surface of the mucosa epithelium there is a physical barrier (Figure 2). There are 3 components of this barrier, also known as the "Kill Zone" levels: mucus and mucins, antimicrobial proteins, and secretory IgA. The goblet cells secrete mucus and mucins (the enterocytes also secrete mucins) that provide the initial mucous barrier (Figure 2).[14] The mucosal barrier contains defensins (aka as antimicrobial peptides [AMP] and host defense proteins [(HDP]) produced by the enterocytes.[15] In the lamina propria, B cells produce antibody. This antibody production is driven by what happens in mucosa epithelium. The key is that IgA, to become secretory IgA, has to go through a healthy enterocyte. Enterocytes are involved in the export of IgA from the lamina propria to the lumen (Figure 2) and avoid inflammation. The enterocytes cannot do all their functions with inflammation going on. Enterocytes need to be hypo-responsive (anti-inflammatory) because of the constant contact with the microbial environment (Figure 3).[13] If they responded to the microbes in the lumen all the time, they would be constantly producing inflammatory signals and turning on inflammation.[16] That's what happens in Crohn's disease. The enterocytes get anti-inflammatory signals from the microbiota and/or their products, either directly affecting the enterocytes and/or by turning on peripheral T regulatory (pTreg) cells in the lamina propria (Figure 3). These Treg cells inhibit T helper inflammatory cells (Th1 and Th17) and induce IL-10, an anti-inflammatory cytokine that increases sIgA production.

Microbiota

On the other side of the epithelial cell in the lumen is where the microbiota (microbiome) is located. It is a collection of organisms found in the animal's GIT and is dependent

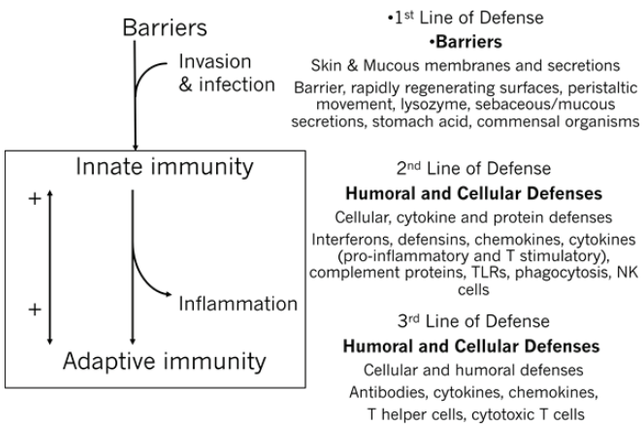


Figure 1. The 3 lines of gut immune defense: the barrier, innate, and adaptive immune components.

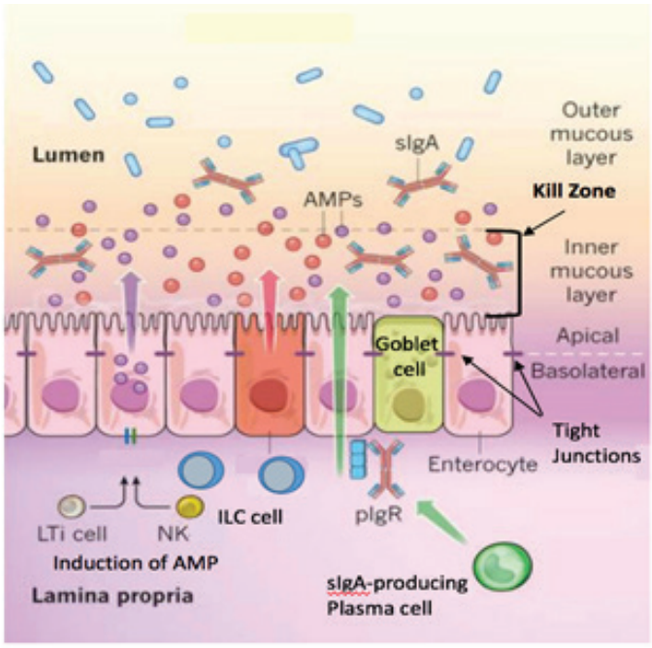


Figure 2. Mucosa Epithelium and the Kill Zone. The Kill Zone is in close apposition to the gastrointestinal enterocytes and contains mucus, mucins, antimicrobial peptides (AMPs) and secretory immunoglobulin A (sIgA).[11] The IgA is producing by B cells in the lamina propria that have matured to become plasma cells and the IgA has to be bound by polymeric Ig receptor (pIgR) and exported by the enterocytes to the lumen to be sIgA. The lamina propria also contains difference types of lymphocytes including innate lymphoid cells (ILC), natural killer (NK) cells and lymphoid tissue inducer (LTi) cells that also produce cytokines that induce AMPs. Adapted from Maynard et al 2012[11].

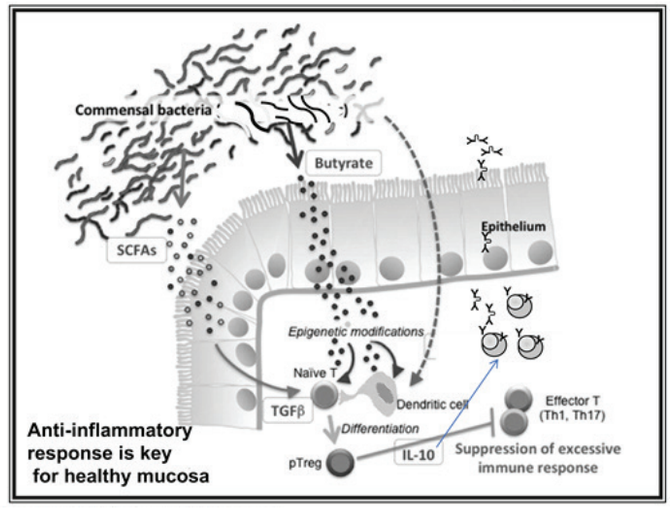
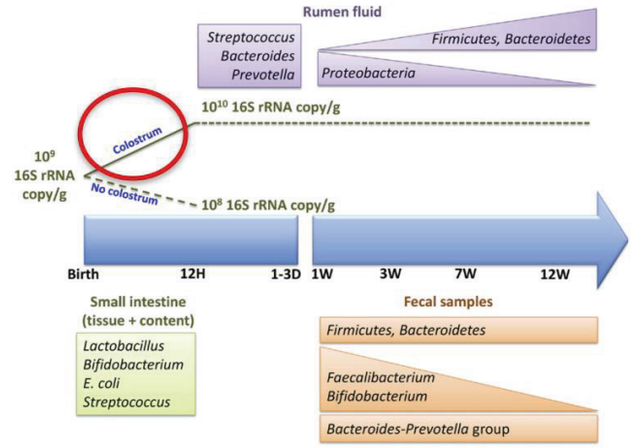


Figure 3. Microbiome and Anti-inflammatory Response. Commensal organisms (probiotics) can produce short chain fatty acids (SCFAs i.e. butyrate), other metabolites (prebiotics) and/or microbial components (flagella etc) that induce the anti-inflammatory response.[13] Transforming growth factor-beta (TGF-β) is the cytokine that is induced that has the biggest anti-inflammatory effect. It converts naïve T cells into Treg cells that then block inflammatory cells (T helper 1, Th1 and T helper 17, Th17) and produce IL-10 that turns on sIgA production. Other lymphocytes like natural killer cells and innate lymphoid cells (ILC) also produce signals to make enterocytes produce more antibacterial peptides; again, helping the "kill zone" defense mechanism (Figure 2). Adapted from Monteiro J, 2014.[12]



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The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract

Figure 4. The changes in the commensal bacteria in the GIT of a calf from birth to 12 weeks of age. Adapted from Malmuthuge et al, 2015.[9]

on the region of the GIT and the age of the animal (Figure 4).[9] Colostrum is important for microbiome development, as it increases microbiome density (Figure 4). In the rumen microbiome alone, there are 10^{16} bacteria (quadrillion), 10^{13} protozoa and 10^{9} fungi. In the rest of the GIT based on a monogastric animal, there are about 10^{14} microorganisms of which 10^{12} are commensals to help the host. The microbiome

is essential for immune development and the composition will influence the host immune status. When it comes to the immune system, the lower intestinal tract is thought to be the most important part of the immune development and the microbiome interaction that occurs. The large intestine is less important. The wild card is the rumen. There is not much understanding on the interaction of the microbiota of the rumen and the immune system. There is not a lot of immune tissue in the rumen and rumen epithelium is stratified squamous cell, which is not as active as GIT epithelium, but that doesn't mean it couldn't influence the immune response.

The Good Response

Having a "healthy microbiome" results in optimal GIT mucosa function. For example, certain clostridial species do a good job of producing butyrate. Butyrate and other small chain fatty acids have a calming effect and cause the GIT epithelium to be much calmer and inhibit the inflammatory response (Figure 3). These anti-inflammatory signals are not coming from the host side of the GIT, but from the bacteria along with metabolites. That's why commensals are so important. They are affecting the host response. The host response is not to just the bacteria and other microorganisms, but also on microbial cell components and metabolites of bacteria. Very few of the metabolites and microbial components have been characterized, but the ones that have been the most characterized are the fatty acids, and particularly butyrate. Transforming growth factor-beta (TGF-β) is the cytokine that is induced that has the biggest anti-inflammatory effect, It converts naïve T cells into Treg cells that then block inflammatory cells (T helper 1, Th1 and T helper 17, Th17) and produce IL-10 that turns on sIgA production. Other lymphocytes like natural killer cells and innate lymphoid cells (ILC) also produce signals to make enterocytes produce more antibacterial peptides, again, helping the defense mechanism (Figure 2). This achieves homeostasis in the GIT mucosa by inducing the protective responses to pathogens, maintaining the regulatory pathways for tolerance to innocuous antigens and preventing inflammation, making for a happy gut and an animal that can more closely achieve its maximum genetic potential.[1,8]

The Bad Response

When homeostasis is achieved between the GIT mucosa and microbiome, there is a solid "kill zone" and healthy microbiome (Figure 5, left half). When the microbiome is disrupted due to stress (weaning, transportation, parturition, surgical procedures, etc.), changes in feed intake (after weaning, feed changes, etc.), dehydration, or use of oral antibiotics, the microbiome becomes depleted and undergoes dysbiosis, and the kill zone decreases and the mucosa becomes inflamed[1,5] (Figure 5, right half). The innate immune system, particularly macrophages, becomes activated and begins to produce interleukin-1 beta (IL-1b), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF) and begins to recruit in inflammatory cells (Figure 6).[10] TNF then stimulates a kinase pathway MLCK that results in the breakdown of the tight junctions and the development of "leaky gut", which can be a vicious circle– more bacteria and antigens leak through and a more severe inflammatory response develops. The inflammatory mediators, TNF, IL-1b and IL-6, enter the portal blood stream and affect the liver, and cause it to switch from being an efficient metabolism machine to becoming an inefficient "immune organ". This results in poorer growth and performance of the animal.[7] If the "leak" and inflammation are controlled, the animal recovers but will expend energy for tissue repair and the activation of the liver, which will decrease performance (average daily gain, feed efficiency, etc.).[7] This inflammatory response has systemic effects and can affect the respiratory system and enhance bovine respiratory disease.[3]

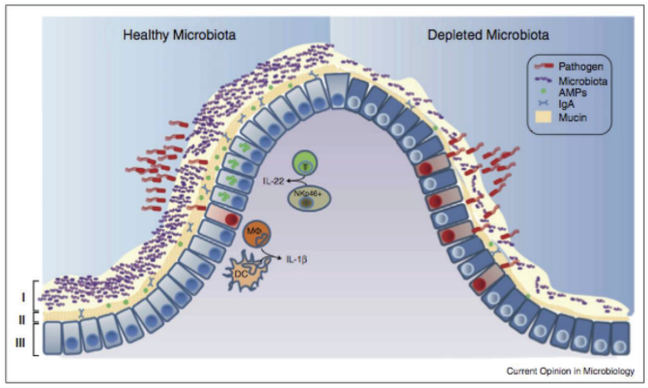

Figure 5. Healthy mucosal defenses and mucosal dysbiosis. The intestinal microbiota promotes 3 levels of protection against enteric infection. (I) Saturation of colonization sites and competition for nutrients by the microbiota limit pathogen association with host tissue. (II) Kill Zone- Commensal microbes prime barrier immunity by driving expression of mucin, immunoglobulin A (IgA) and antimicrobial peptides (AMPs) that further prevents pathogen contact with host mucosa. (III) Finally, the microbiota enhance immune responses to invading pathogens. This is achieved by promoting IL-22 expression by T cells and NK cells, which increases epithelial resistance against infection, as well as priming secretion of IL-1b by intestinal macrophages (MΦ) and dendritic cells (DCs), which promotes recruitment of inflammatory cells into the site of infection. In conditions in which the microbiota is absent, there is reduced competition, barrier resistance and immune defense against pathogen invasion. Adapted from Khosravi and Mazmanian 2013.[8]

The Ugly Response

The immune system is a major consumer of energy, and in times of negative energy like seen in the newly weaned calf or a transition cow, it can be difficult times for the immune system to respond. In addition, the mobilization of energy from adipose tissue (fat) results in infiltration of

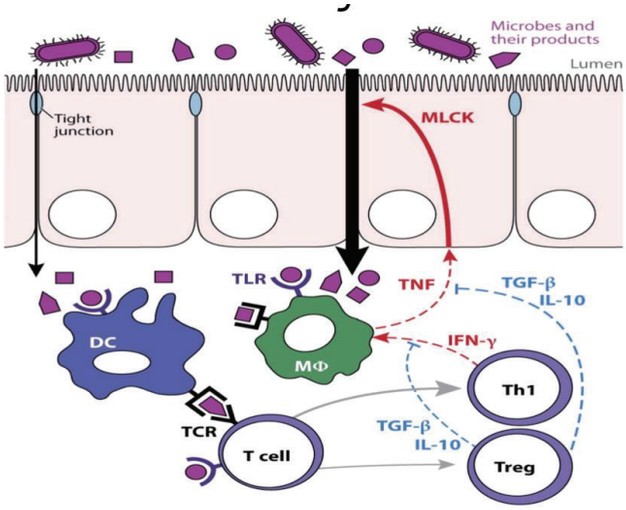

Figure 6. Pathogenesis of Leaky gut. The epithelial barrier normally restricts passage of luminal contents, including microbes and their products, but a small fraction of these materials do cross the tight junction. This diagram shows how dendritic cells (DC), macrophages (M), and T cells react to these materials. The naive T lymphocyte (T cell) responds to antigenic and other stimuli within the lamina propria, becoming a Th1-polarized cell (Th1), a T regulatory cell (Treg), or other differentiated T cell types. These innate and adaptive immune cells release cytokines that exert pro-inflammatory (TNF and IFN-γ) and anti-inflammatory (IL-10, TGF-β) effects. If pro-inflammatory signals dominate and signal to the epithelium, MLCK can be activated to cause barrier dysfunction, which would allow an increase in the amount of luminal material ("leaky gut") presented to immune cells. In the absence of appropriate immune regulation, this activation may cause further pro-inflammatory immune activation, cytokine release, and barrier loss, resulting in a self-amplifying vicious cycle that can result in disease. Abbreviations: IL, interleukin; MLCK, myosin II regulatory light chain kinase; TGF, transforming growth factor; TNF, tumor necrosis factor. From Marchiando et al 2010.[10]

macrophages as activity of adipocytes (fat cells) results in inflammation.[18] These macrophages are particularly sensitive to signals from gut bacteria, including endotoxin from gram-negative bacteria.[4] Animals with "leaky gut", along with other metabolic or major stressors, are at higher risk. With diet changes that occur at weaning or at parturition for the dairy cow, the microbiome has major changing populations. This combination of adipose remodeling, macrophage activation, and "revamped" microflora can result in a cytokine storm, which is the "bad inflammatory" described above going crazy (Figure 7).[17] A cytokine storm (hypercytokinemia) is the systemic expression of a healthy and vigorous immune system resulting in the release of more than 150 known inflammatory mediators (cytokines, oxygen free radicals, and coagulation factors). It is an overreaction of the immune system. Both pro-inflammatory cytokines (TNF, IL-1, and IL-6) and anti-inflammatory cytokines (such as interleukin 10 and interleukin 1 receptor antagonist) are elevated in the serum of people or animals experiencing a cytokine storm. This results in systemic spillover affecting other systems. An animal with a systemic inflammatory response (cytokine storm) will have increased bovine respiratory disease, more severe mastitis and metritis. The development of cytokine storms in people are believed to be responsible for many of the human deaths during the 1918 influenza pandemic, which killed a disproportionate number of young adults. In this case, a healthy immune system may have been a liability rather than an asset. Preliminary research results also indicated this as the probable reason for many deaths during the sudden acute respiratory syndrome (SARS) epidemic in 2003 in China and with current COVID19 pandemic.

Modulation of the response–what can we learn from human medicine

Although we have been using prebiotics, probiotics, essential oils and/or organic acids in animal production for years, the approaches have often been empirical and based on 1 or 2 components with little understanding of the mechanism of action. In looking at human medicine and the prevention and treatment of inflammatory bowel disease, a more holistic multi-pronged approach has been developed (Figure 8).[16] Like veterinary medicine , the initial approaches for prevention and/or treatment of GIT disease were pharmaceutical-based with antibiotics being a major tool. Using a multi-pronged approach in humans has been aimed at reducing the use of exogenous corticosteroids and/or antibiotics (Figure 8, circle lower left). There are several GIT health goals from these multi-pronged approaches. First, maintain a healthy "kill zone" and mucosa and block specific pathogen attachment (Figure 8, center green box). Second, correct dysbiosis and restore normal microbial function (Figure 8, upper left blue box), and normalize the immune dysfunction and repair barrier defects (Figure 8, upper right

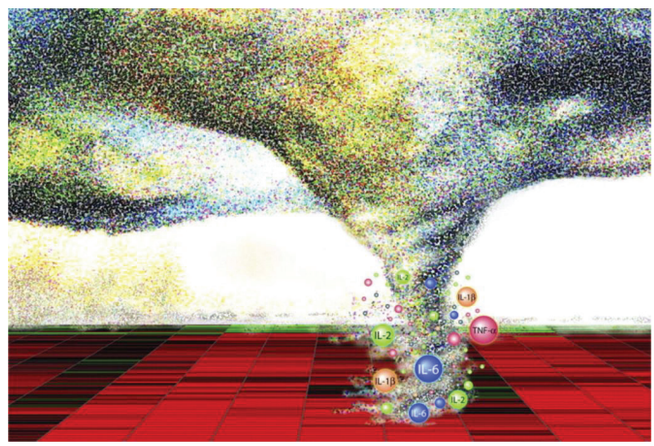

Figure 7. Imagery of the "Cytokine Storm". Many different cytokines are activated and create "Cytokine Storm". From Tisoncik et al 2012.[17]

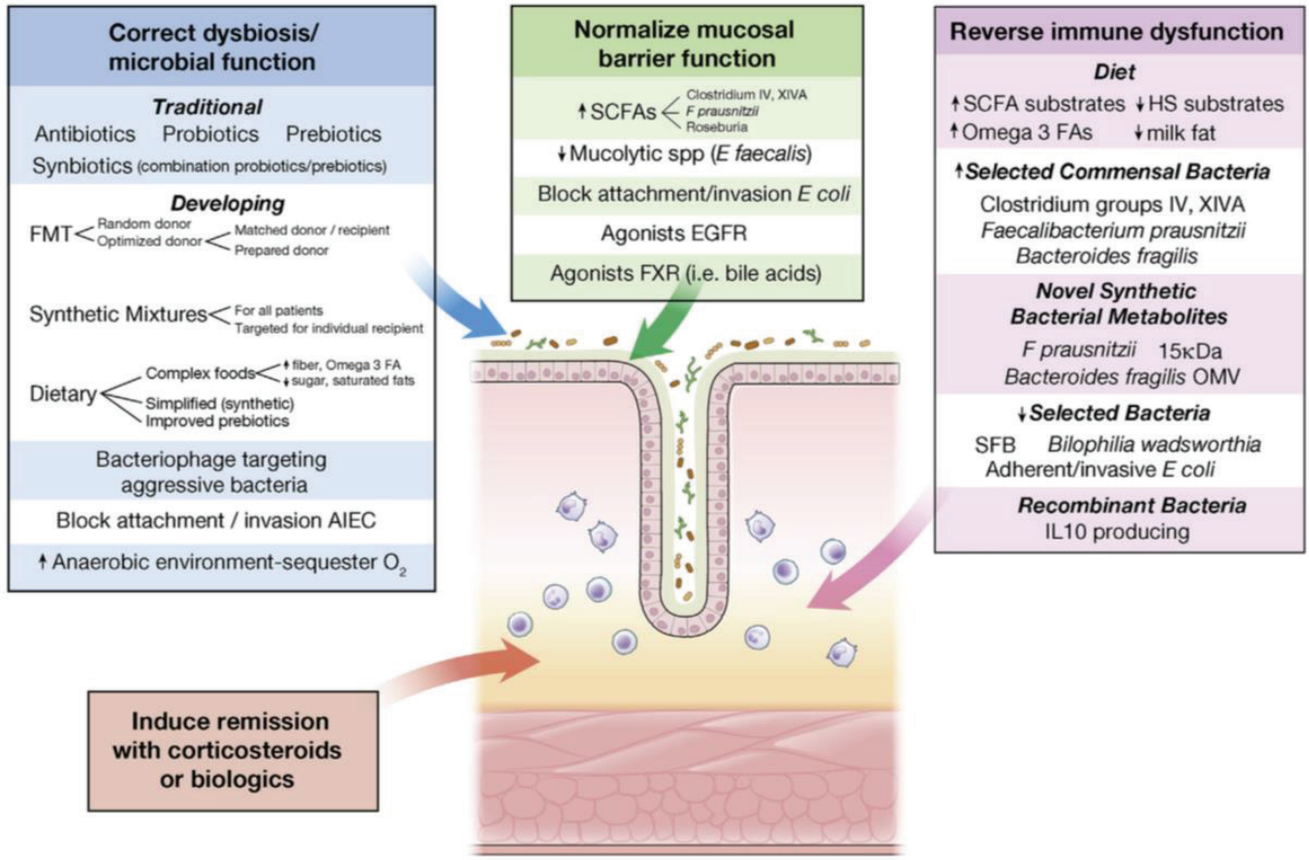

Figure 8. Targeting the mucosa with nutraceuticals that specifically enhance the microbiota and improve barrier and immune function. AIEC, attaching and effacing *E. coli*; EGFR, epidermal growth factor receptor; FA, fatty acid; FXR, farnesoid X receptor; FMT, fecal microbial transplant; HS, hydrogen sulfide; IL-10, interleukin 10; OMV, outer membrane vesicles; SCFA, short chain fatty acids; SFB, segmented filamentous bacteria. From Sartor and Wu, 2017.[16]

lavender box). These approaches may be accomplished by using traditional approaches (probiotics, organic oils, high-fiber diets, or combinations of these); cutting-edge methods (fecal microbial transplants; synthetic mixtures of defined microbes, personalized for an individual's specific microbiota profile) and personalized diets. Then there are novel experimental approaches (bacteriophages targeting key aggressive bacteria, using synthetic microbial metabolites or recombinant bacterial species) that also have promise.

In livestock, we have several other unique approaches to improving GIT health in addition to the traditional approaches (probiotics, organic oils, high fiber diets, or combinations of these). These approaches include prebiotics {refined functional carbohydrates (RFC); inhibiting bacterial attachment, promoting a more anaerobic environment; blocking bacterial receptors; stimulating protective mammalian pathways}; mixtures of defined microbes based on culture and sensitivity testing that are herd and/or region-specific and hen egg IgY antibodies against specific organisms. With ruminant housing and pasture management exposure to feces (and rumen content transplants), there is an on-farm "microbial transplant" opportunity.

Conclusions

The gastrointestinal tract is the largest immune organ of the body. The mucosal barrier- the tight junctions and the "kill zone", along with the gut mucosa and maintaining an "anti-inflammatory" state, are essential for "good gut health". The microbiome- the microorganisms in the GIT, which has more cells then the entire animal's body, is essential for immune development, immune response, and maximizing ruminant productivity. Management of the bovine GIT immune system is not a simple process. It begins with consumption of colostrum. Stressors, along with the intake of feed and hydration, affect the microbiome and the intestinal epithelial cells, resulting in important immune interactions. Nutraceuticals (i.e., probiotics, prebiotics, hen yolk IgY, essential oils, organic acids) aid in both microbiome stability "homeostasis" and immune function.

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