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Enteric Immunity
Happy Gut, Healthy Animal

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KEYWORDS

• Bovine • Mucosal • Immunology • Enteric • Microbiome

KEY POINTS

- The largest organ of the immune system is the gastrointestinal (GI) mucosa, making the management of it essential for productivity and health.
- The barrier that consists of mucous, defensins, and immunoglobulin A is a "kill zone" to prevent microbial invasion of the GI epithelium.
- The enterocytes are key cells that maintain the "kill zone" and respond to metabolites and microbial components from the lumen and signals from immune cells to maintain tight junctions and prevent "leaky gut."
- Passive enteric immunity is essential for disease protection of the neonate; anti-inflammatory enteric response is essential disease protection for the growing and adult animal.
- Direct-fed microbials, including nutraceuticals, prebiotics, probiotics, and other dietary supplements, affect commensal "homeostasis" and mucosa immunity to maintain GI health.

INTRODUCTION

In the last decade, there has been an explosion of knowledge on the immune system with substantial implications for enteric health. This increase in knowledge revolves around the realization that the gastrointestinal (GI) tract is the largest immune organ of the body. It is understood that the mucosal immune system begins development in the fetus but does not become functional until epithelial cells of the mucosa in the neonate interact with microorganisms (microbiome) and/or their products in the gut lumen. The interaction between the epithelial cells and the microbiome is necessary for proper immune development, including immune system maturation, regulation, and maintenance of homeostasis. In this article, the interaction of immune system, microbiome, and the ability to maximize immunity are discussed.

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ONTOGENY AND ORGANIZATION OF ENTERIC MUCOSAL SYSTEM

The bovine mucosal immune system prevents bacterial invasion and shapes the gut microbiota, whereas the gut microbiota influences immune system development. The fetal calf is predominately protected by the innate immune system (**Fig. 1**).[1] The innate immune response of phagocytic cells (neutrophils and macrophages) does not fully develop until late gestation and declines before gestation because of fetal cortisol levels.[2] Humoral elements such as complement are present but are at levels below that of the adult. Interferon can be induced in the fetus as early as 60 days of gestation.[3] All of the cellular components of the acquired immune response are present in the fetal calf.[4] The number of peripheral blood T cells dramatically decrease, beginning 1 month before birth of the calf, as they traffic and populate lymphoid tissues of the fetal calf before birth (decrease ~60% to 30% at birth). B cells are much lower in the developing fetus (1%–2%).[4,5] The enteric mucosal lymphoid organ system begins developing at 100 days of gestation when the mesenteric lymph nodes are present (**Fig. 2**).[6–8] The continuous ileal Peyer patch (IPP) (see **Fig. 2**) becomes quite active by day 85 of gestation.[9] The B lymphocytes present are almost exclusively immunoglobulin M $(IgM)^+$ cells, and if the IPP are removed, the animals remain deficient in B cells for at least 1 year because the IPP is the major source of the peripheral B-cell pool.[9] Because the IPP is the site of both proliferation and negative selection, IPP follicles can be inferred as the major site for generation of the preimmune B-cell repertoire in ruminants,[8–10] whereas the discreet Peyer patches (PPs), distributed throughout the jejunum, function as induction sites for the generation of IgA plasma cells (see **Fig. 2**).[10] The role of the rumen in mucosal immunity is unclear because there are few leukocytes in the developing rumen. The first few weeks after birth are essential for long-term enteric immunity as the expression of host microRNAs (miR), and the presence of commensal microorganisms determines long-term gut and host health.[11] By day 21 of age, there is a maximum induction of host miR by high levels of microorganisms of the microbiome.[11] These immune developments include induction of

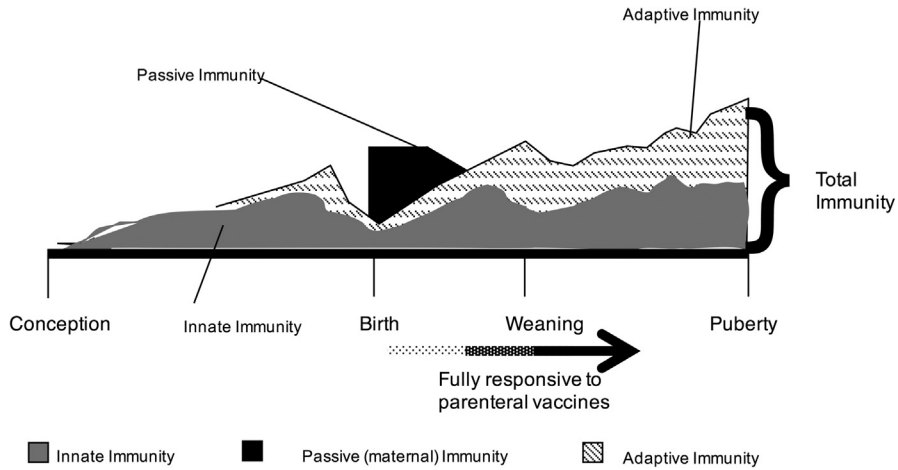

Fig. 1. Development of the immune response in the bovine: from conception to puberty. The calf's passive maternal immunity is only transferred after birth due to its unique placentation. (*Adapted from* Chase, Hurley DJ, Reber AJ, et al. Neonatal immune development in the calf and its impact on vaccine response. Vet Clin North Am Food Anim Pract 2008;24:88; with permission.)

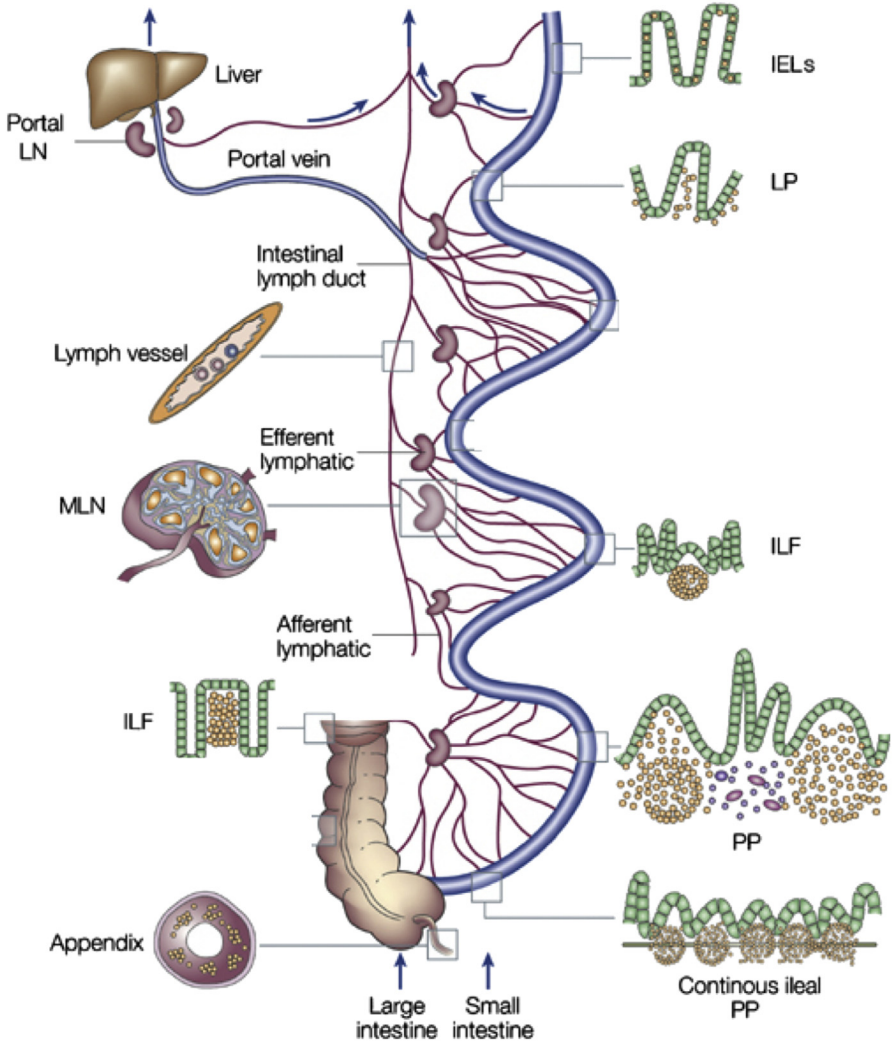

Fig. 2. Organization of the gut lymphoid tissue. Lymphocytes can leave the surface epithelium (intraepithelial lymphocytes [IEL]) or Lamina propria (LP) via draining afferent lymphatics to mesenteric lymph nodes (MLNs), or via portal blood reaching the liver where induction of tolerance occurs. The M cells in the follicle-associated epithelium of PPs transport antigen to prime B cells in the isolated lymphoid follicles (ILF) of the PPs of the jejunum, ileum, and the large intestine. The continuous IPPs are a primary lymphoid organ responsible for B-cell development. The IPP can be up to 2 m long and constitute 80% to 90% of the intestinal lymphoid tissue. LN, lymph node. (*Adapted from* Brandtzaeg P, Kiyono H, Pabst R, et al. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol 2008;1(1):35; with permission.)

tolerance to dietary components, reduction of mast cells that causes increased gut permeability, and decreased pathogen responses. Changes in diet in the early period of 7 to 21 days of age greatly influence microbiome and miR and therefore the level and longevity of the enteric immune response.[11,12]

COLOSTRUM AND ENTERIC IMMUNE DEVELOPMENT

Colostrum is composed of antibodies, cytokines, and cells. Antibody is the most important component of colostrum and provides an immediate source of antibody for the intestinal tract. Bovine colostrum contains ~55 mg/mL of total IgG (48 mg/mL IgG1, 3 mg/mL IgG2, and 4 mg/mL IgA).[13] Preparturient vaccination of the cow for enteric diseases, such as colibacillosis, *Clostridium perfringens*, cryptosporidiosis,[14] and rotaviruses,[15] results in production of pathogen-specific antibodies that provide protection for the neonate against severe disease. A second component of colostrum is cytokines.[16,17] These immunologic hormones help in the development of the fetal immune response. These cytokines are produced by the immune cells that traffic to the mammary gland. Interleukin 1-beta (IL-1β), IL-6, tumor necrosis factor alpha (TNF-α), and interferon-gamma are present in bovine colostrum and associated with a proinflammatory response and may help in the recruitment and development of neonatal lymphocytes into the gut to aid in normal immune development. Colostrum rapidly improves the ability of neutrophils to phagocytize bacteria, which is accomplished by absorption of proinflammatory cytokines.[18] Colostrum also contains high levels of the anti-inflammatory cytokines IL-10[19] and transforming growth factor beta (TGF-β)[20] that suppress local secretion of proinflammatory cytokines in the intestine to maintain tight junctions and also allow gut microbial colonization. The third component of colostrum is cells. Colostrum contains viable leukocytes in percentages similar to peripheral blood with more macrophages (40%–50%) and less lymphocytes (22%–25%) and neutrophils (25%–37%).[21,22] The vast majority of lymphocytes are T lymphocytes with less than 5% being B lymphocytes. Some of these maternal cells enter the circulation and reach peak levels 24 hours after birth.[23] Animals that receive colostrum containing maternal leukocytes develop gut antigen-presenting cells (APC; macrophages and dendritic cells [DC]) faster,[22] which is important because APCs are the keystone cell for the development of an acquired immune response to pathogens or vaccines. Additional pathogen-specific maternal T lymphocytes from vaccinated cows have been isolated from the neonatal calf with maximum proliferation at 1 day following birth.[24] The exact role of these cells in the long-term development of pathogen-specific mucosal-acquired immunity is not clear, because they are no longer detectable at 7 days of age.

FUNDAMENTALS OF ENTERIC IMMUNITY

The enteric mucosal immune system provides the first immune defense barrier for more than 90% of potential pathogens (**Figs. 3** and **4**). The gut mucosal immune system alone contains more than a trillion (10^{12}) lymphocytes and has a greater concentration of antibodies than other tissue in the body. It protects against harmful pathogens but also tolerize (induces tolerance) the immune system to dietary antigens and normal microbial flora. The components of the gut mucosal immune system are integrated together (see **Figs. 3** and **4**).[25] The health of the enterocytes, which are the epithelial cells that line the GI tract, is important not only for the growth and development of cattle, through secretion and absorption in the gut, but also to provide a first immune response to microorganisms (see **Fig. 4**). The goblet cells secrete mucous and mucins (the enterocytes also secrete mucins) that provide the initial mucous barrier (see **Fig. 4**).[26–29] The mucosal barrier contains defensins (also known as antimicrobial peptides [AMP] and host defense proteins [HDP]) produced by the enterocytes (see **Fig. 4**). Secretory immunoglobulin A (sIgA) is produced when dimeric IgA is secreted by the plasma cells in the lamina propria (LP) and is transported to the mucosal surface of the epithelial cell. The inner mucous layer along with the AMP

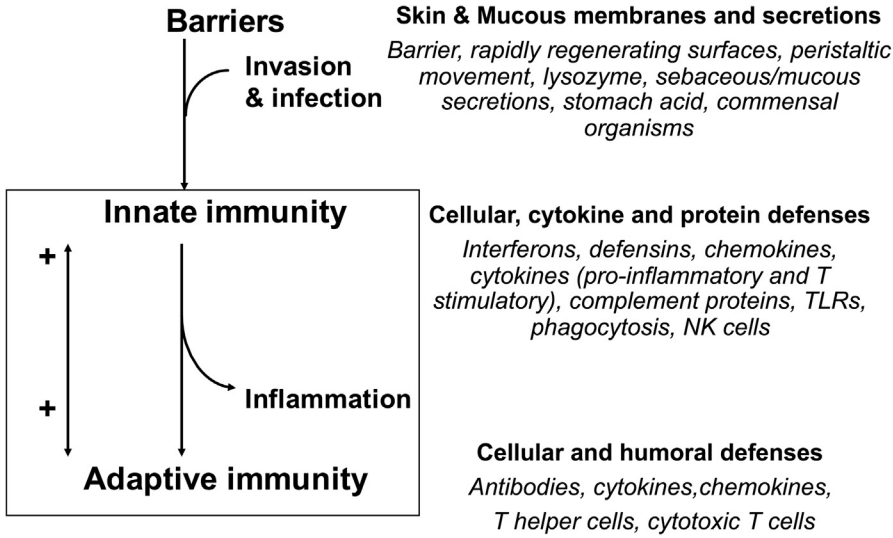

Fig. 3. Gut immune responses: the barrier, innate, and adaptive immune components. (*Adapted with permission from* D. Topham, PhD, Rochester, NY.)

and sIgA forms a "kill zone" that few pathogens or commensals have evolved strategies to penetrate (see **Fig. 4**).[26] The "kill zone" along with the tight junctions that knit the enterocytes together forms a "barrier" against pathogens.

Once microorganisms breech the barriers, the innate immune system is the first responder to pathogen invasion. The system consists of white blood cells (macrophages, monocytes, DC, basophils, neutrophils, eosinophils, mast cells, and natural killer [NK] cells) (**Fig. 5**), complement, and the secreted immune system mediators, including chemokines and cytokines. These innate immune mediators include interferon, the proinflammatory mediators TNF-α, IL-1β, IL-6, macrophage inflammatory protein 1-alpha, and the anti-inflammatory mediator IL-10.[30] The innate response occurs in 2 waves. The first wave that occurs in the first few hours following damage or infection features the activation of macrophages, the major producer of proinflammatory cytokines that recruit other white blood cells and activate neutrophils, nonspecific killers of bacteria to increase killing of pathogens. If the proinflammatory response in the gut mucosa is excessive, "leaky gut" will occur (**Fig. 6**A).[31,32] The proinflammatory cytokines, particularly TNF-α, stimulate the myosin II regulatory light chain kinase (MLCK), which causes the tight junctions to break down so the epithelium becomes leaky (see **Fig. 6**A). Mucosa epithelium needs to be hyporesponsive under the influence of the anti-inflammatory cytokines[31] so healthy mucosa enterocytes will maintain tight junctions. A local increase of the anti-inflammatory cytokine IL-10 results in inhibition of the local proinflammatory response and increases eosinophils in the tissue. Cattle that are resistant to GI parasites like *Cooperia* and *Ostertagia* have an increase of both proinflammatory and anti-inflammatory mediators in the mucosa with a large influx of eosinophils into the tissue and lumen.[33] With only a proinflammatory response, there is little resolution of disease and enhanced collateral damage and immunopathology.[34] Immunopathology is seen in protozoal diseases like cryptosporidia, where localized neutrophilia is enhanced in young animals[34–36] and also has been hypothesized as the major contributor to the lesions of *C perfringens* alpha toxin.[37] The proinflammatory anti-inflammatory mucosal response increases with

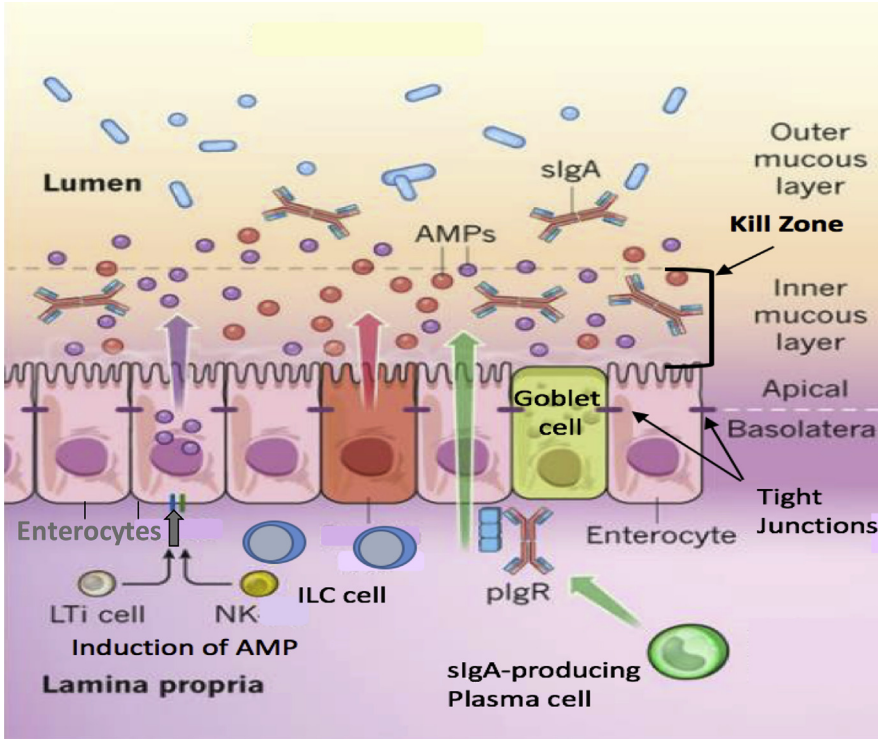

Fig. 4. The mucosal defenses of the GI tract. Distinct subpopulations of intestinal epithelial cells are integrated into a continuous, single-cell layer that is divided into apical and basolateral regions by tight junctions. Enterocytes sense the microbiota and their metabolites to induce the production of AMPs. Goblet cells produce mucin and mucous that is organized into a dense, more highly cross-linked inner proteoglycan gel that forms an adherent inner mucous layer, and a less densely cross-linked outer mucous layer. The outer layer is highly colonized by constituents of the microbiota. The inner mucous layer is largely impervious to bacterial colonization or penetration due to its high concentration of bactericidal AMPs, as well as commensals sIgA, which is moved from their basolateral surface, where it is bound by the polymeric Immunoglobulin receptor (pIgR), to the inner mucous layer. Responding to the microbiotal components, innate lymphoid cells (ILC), lymphoid tissue inducer cells (LTi), and NK produce cytokines, which stimulate AMP production and maintain the epithelial barrier. (*Adapted from* Maynard CL, Elson CO, Hatton RD, et al. Reciprocal interactions of the intestinal microbiota and immune system. Nature 2012;489:235; with permission.)

age and results in less disease. Neutrophils (see **Fig. 5**) also known as polymorphonuclear cells die after a short time at sites of inflammation. The hydrolytic enzymes are released and contribute to the inflammatory response and tissue destruction, which contributes to collateral damage and enhanced disease. Neutrophil granule proteins induce adhesion and emigration of inflammatory monocytes to the site of inflammation. Neutrophils also create extracellular defenses by the formation of neutrophil extracellular traps (NETs) (**Fig. 6**B).[38–40] The NET formation is induced by agents like bacterial aggregates and biofilms, fungal hyphae, and protozoan parasites (cryptosporidia, *Neospora*, and coccidiosis) that cannot be phagocytized.[35,36,41,42] Neutrophils use the potent oxidative metabolism system to kill bacteria. The NET reaction is one of the most potent bactericidal mechanisms of neutrophils and is

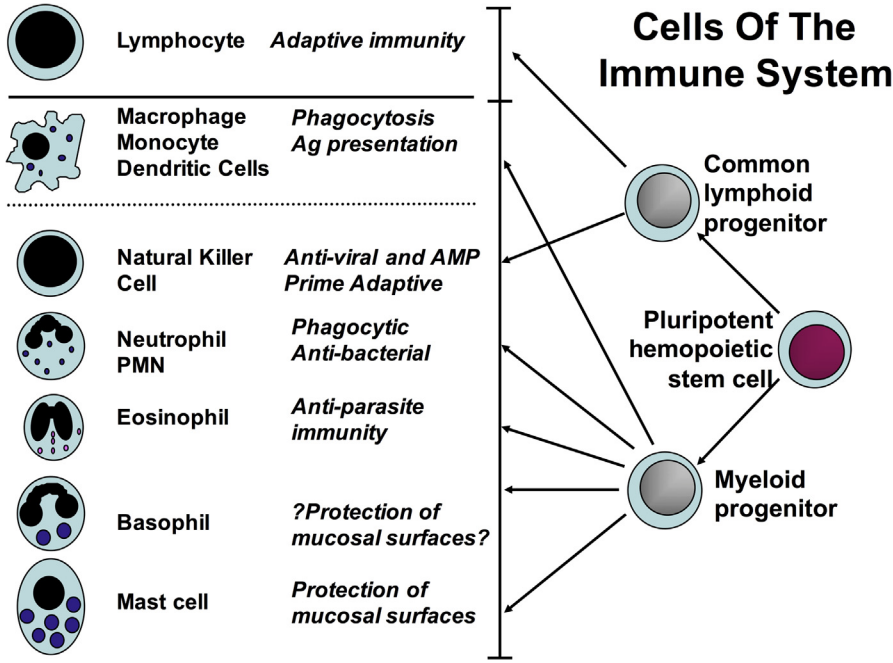

Fig. 5. The cells of the immune system. The innate and acquired immune cell lines have overlap with the macrophages and NK cells having important innate and acquired responses. Ag, antigen; PMN, polymorphonuclear cells. (*Adapted with permission from* D. Topham, PhD, Rochester, NY.)

potentially fungicidal, parasiticidal, and virucidal. The eosinophil is capable of the same phagocytic and metabolic functions as the neutrophils but focuses the host's defense against the tissue phase of parasitic infections (see **Fig. 5**). Eosinophils are more capable of exocytosis than phagocytosis; that is, rather than ingesting and killing small particles, they efficiently attach to and kill migrating parasites that are too large to be ingested. Eosinophils are also important in helping to control certain types of allergic responses. Basophils and mast cells (see **Fig. 5**) have been associated primarily with allergic reactions because of their binding of IgE. These cells have an important regulatory role. They release inflammatory mediators necessary for the activation of the acquired immune response.[43,44] Interferon, the last component of the innate response, sets up an immediate wall against virus infections. The second wave that occurs a day or 2 later is the NK cells (see **Fig. 5**) that enhance defensin production,[25,26] kill parasites,[35,36] and virally infect cells[45] but also produce cytokines to help the adaptive immune response.[45]

The adaptive phase occurs in the organized gut-associated lymphoid tissues (GALT) described above.[8] GALT is the initial induction site for mucosal immunity for antigens that are sampled from mucosal surfaces. The number and maturity of DCs and T cells in the GALT in the jejunum and ileum are very similar in the newborn and the weaned calf, indicating that the mucosal adaptive response is functional at birth.[46] The DCs are important because they are APCs that help in discriminating between dietary antigens, commensal microflora, and pathogens, and in providing a proper adaptive immune response with T cells.

These mucosal aggregates or follicles of B cells, T cells, and DCs are covered by epithelium that contains specialized epithelial cells called dome or M cells that are found

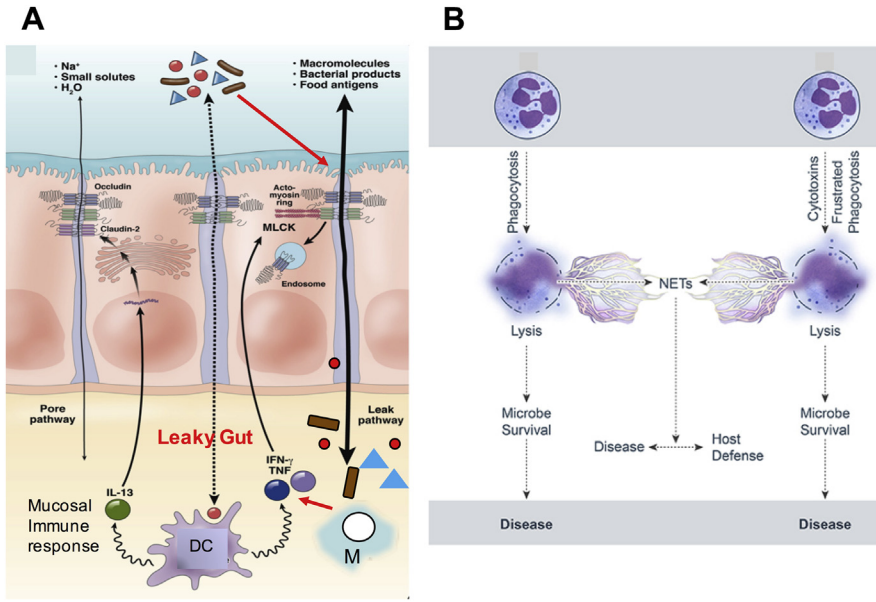

Fig. 6. Innate immunity and the mucosa. (*A*) Pathogenesis of leaky gut. The epithelial barrier normally restricts passage of luminal contents, including microbes and their products, but a small fraction of these materials do cross the tight junction. This diagram shows how DCs, and macrophages (M) react to these materials. These innate immune cells release cytokines that exert proinflammatory (TNF and interferon-gamma [IFN-γ]) and anti-inflammatory (IL-13) effects. If proinflammatory signals dominate and signal to the epithelium, MLCK can be activated to cause barrier dysfunction through the "leak pathway," allowing an increase in the amount of luminal material presented to immune cells. In the absence of appropriate immune regulation, immune activation may cause further proinflammatory immune activation, cytokine release, and barrier loss, resulting in a self-amplifying cycle that can result in disease. (*B*) Neutrophil collateral damage from NET formation. Neutrophil lysis after phagocytosis. Cytolysis can be programmed, for example, necroptosis, or caused by direct damage. Neutrophil lysis is caused by cytolytic toxins, pore-forming agents, physical injury, or frustrated phagocytosis. This can result in the formation of NETs during neutrophil lysis. Hydrolytic enzymes–DNA complexes are released in the NETs, enhancing the proinflammatory response and tissue destruction, contributing to collateral damage and disease. ([A] *Adapted from* Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol 2013;11(9):1078, with permission; and [B] Kobayashi SD, Malachowa N, DeLeo FR. Influence of microbes on neutrophil life and death. Front Cell Infect Microbiol 2017;7(4):159, with permission.)

in the GALT. These dome cells pinocytose antigen and transport it across the epithelial layer (**Fig. 7**).[47] The antigen may then be processed by APCs and presented to T and B lymphocytes; indeed, intestinal APCs play a central role in the induction and maintenance of mucosal immunity.[47] These follicles are organized like lymph nodes with T-cell areas and B-cell germinal centers.[7,46] The lymphocytes that emigrate from these organized areas into the surrounding LP are referred to as diffuse lymphocytes.[48] The hallmark of the mucosal immune system is that local stimulation will result in memory T and B cells in the nearby mucosal tissue but also in other mucosal tissues.

In the mucosal lymphoid tissues, mature T cells and B cells that have been stimulated by antigen and induced to switch to produce IgA will leave the submucosal lymphoid tissue and reenter the bloodstream.[49] These lymphocytes will exit the bloodstream through

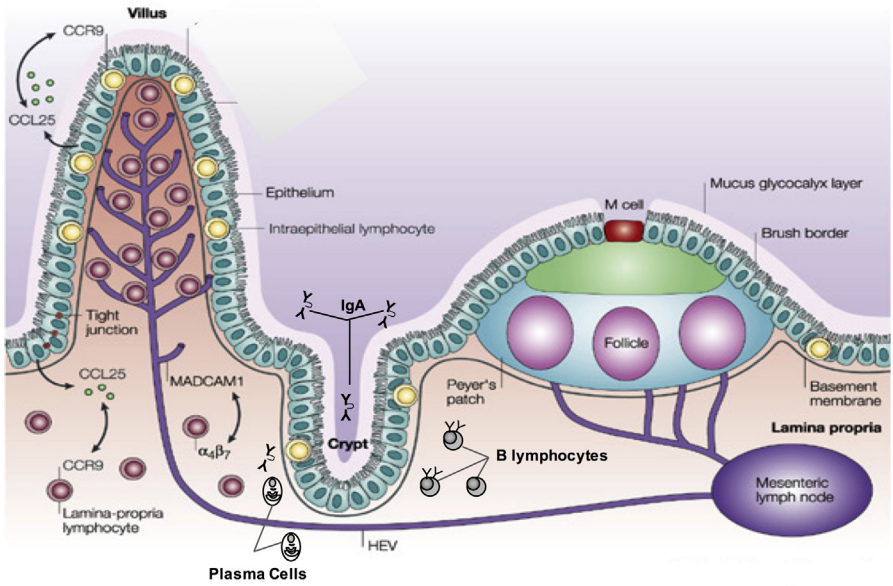

Fig. 7. Mucosal immune system of the gut epithelium. The LP contains scattered T cells and lies beneath the epithelium, which contains intraepithelial lymphocytes (IEL). B cells are scattered in the LP but are more frequent in the crypt regions along with plasma cells that produce IgA that is transported and secreted into the lumen. M cells facilitate antigen uptake and delivery to the organized lymphoid tissues. T cells activated in the PP and mesenteric lymph node express mucosa specific receptors, which interact with cell-adhesion molecules on the HEVs, assisting in homing these T cells to the mucosal LP. The chemokine CCL25 produced by epithelial cells recruits lymphocytes expressing CCR9 receptors to the LP. (*Adapted from* Cheroutre H, Madakamutil L. Acquired and natural memory T cells join forces at the mucosal front line. Nat Rev Immunol 2004;4(4):291; with permission.)

high endothelial venules (HEV) as described above and locate in the LP (see **Fig. 7**). B cells will differentiate into plasma cells that will secrete dimeric IgA. Many of these cells will return to the same mucosal surface from which they originated,[49] but others will be found at different mucosal surfaces throughout the body. The homing of lymphocytes to other mucosa-associated lymphoid tissue sites throughout the body is referred to as the "common mucosal immune system" (**Fig. 8**). Therefore, oral immunization can result in the migration of IgA precursor cells to the bronchi in the respiratory tract and subsequent secretion of IgA onto the bronchial mucosa.

MICROBIOME AND ENTERIC IMMUNITY

The microbiome is essential for immune development in the neonatal calf; then the microbiome-gut-immune-brain axis maintains the health of the calf.[50–53] As the calf develops, there is a "succession" of microbes that finally culminates in what is called a "climax" community that occurs as the gut transitions to an anaerobic environment.[50,54] Microbiome succession is influenced by nutrition, stress, and environment. This microbial community of commensals and their metabolites controls the health of the gut mucosa and the underlying immune cells in the LP (**Fig. 9**).[51,55] These

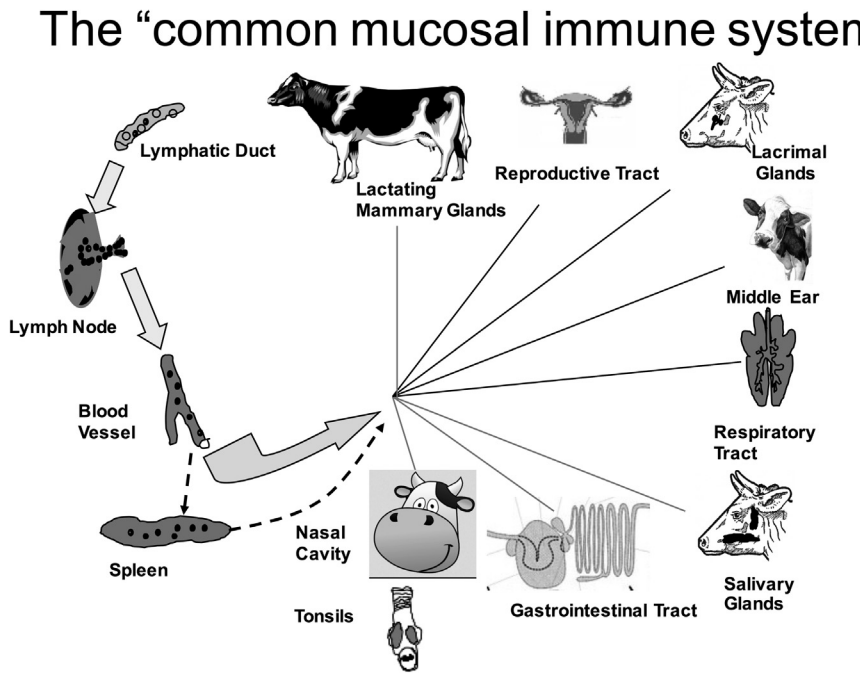

Fig. 8. Lymphocyte circulation and common mucosal immune system of the bovine. As illustrated on the left side of the figure, lymphocyte circulation with lymphocytes entering the lymph nodes by afferent lymphatics and exiting by efferent lymphatics. The common mucosal system involves the circulation of B and T cells between lymphoid tissues on mucosal surfaces.

commensal metabolites stimulate enterocytes to produce TGF-β, which is essential for the development of T-regulatory (Treg) lymphocytes that produce anti-inflammatory IL-10 (see **Fig. 9**). The microbial components in the microbiome also stimulate the enterocytes to produce serum amyloid A that stimulates DCs to activate another important mucosa regulatory T cell, T_H17 cells (see **Fig. 9**). These microbial metabolites also directly stimulate an NK-like cell, type 3 innate lymphoid cells to produce IL-22 to induce the enterocytes to produce more defensins (eg, REGIIIγ and REGIIIβ) (see **Fig. 9**). The composition of the microbiome varies by gut location with the numbers and diversity of populations being high in the rumen and increasing dramatically from the abomasum to the colon with the ileum being a key organ for microbial-immune development. These microbial communities (the microbiome) have evolved to help protect the animal by improving barrier and immune function; understanding the complexity of the gut microbial ecosystem is essential.[51,56]

The stress of weaning, co-mingling, and abrupt diet changes results in major microbial population shifts in the luminal microbial ecosystem, the microbiome. Stress lowers the defenses against pathogen entry, leading to increased risk of disease. Stress also leads to dysbiosis, the loss of good bacteria with an overgrowth of harmful organisms (**Fig. 10**).[57,58] However, dysbiosis is not just the loss of microbiome, it results in depletion of the “kill zone”(see **Fig. 4**); the mucous layer becomes thinner, and the amount of sIgA and defensins declines precipitously to allow the barrier to become weakened, allowing pathogens to interact with the mucosa and cause

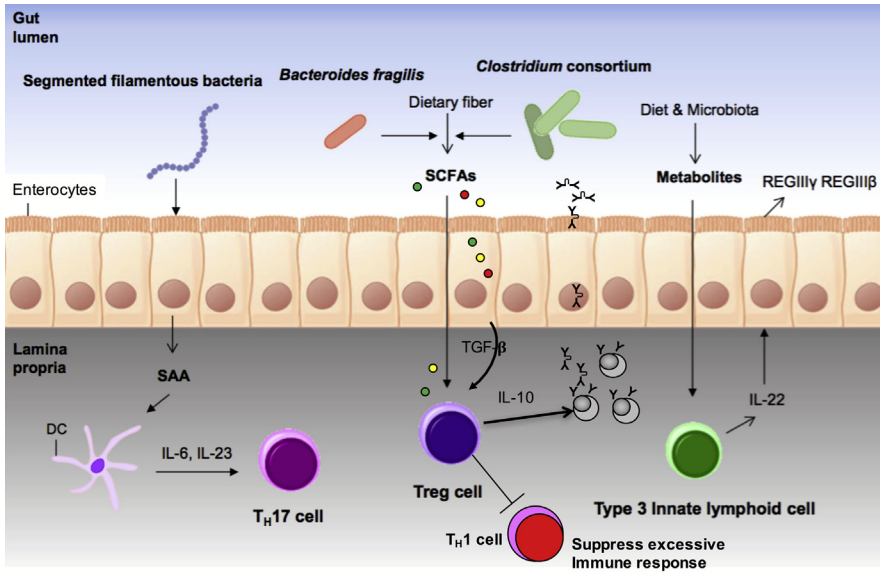

Fig. 9. Gut microbiota and their products shape the development of epithelial cells and immunity. Segmented filamentous bacteria (related to Clostridium) promote the production of serum amyloid A (SAA) protein from epithelial cells, which activates DCs to produce IL-6 and IL-23, resulting in the generation of Th17 cells that are important for T-cell development. *Clostridium consortium* and *Bacteroides fragilis* produce short chain fatty acids (SCFAs) from dietary carbohydrates that induce directly or indirectly by the production of TGF-β by the enterocytes the differentiation of Treg cells to enhance IgA production and to help minimize inflammatory response. Diet- or microbiota-derived metabolites upregulate the number of IL-22-secreting type 3 innate lymphoid cells (ILC3s) that induce the production of defensins (AMP/HDP-REGIIIβ and REGIIIγ) from epithelial cells. (*Adapted from* Kim, Yoo SA, Kim WU. Gut microbiota in autoimmunity: potential for clinical applications. Arch Pharm Res 2016;39:1568; with permission.)

disease. In addition, commensal organisms that help stimulate the mucosa to be anti-inflammatory are no longer available so tight junctions become weakened; "leaky gut" occurs, and pro-inflammatory responses occur that further weaken the gut epithelium (see **Fig. 6**A). One major factor leading to the dysbiosis and diarrhea that we can learn from pigs is low feed and water intake.[59] Dysbiosis is also associated with susceptibility to Johne disease.[60]

Homeostasis, "maintaining" a stable microbiome, is essential for good health and production. Oral antibiotics affect the microbiome homeostasis and therefore effect gut immunity and the incidence of disease. For example, the use of the antimicrobial bacitracin methylene disalicylate[61] altered the fecal microbial composition of calves by increasing the number of opportunistic pathogens such as *Escherichia*, *Enterococcus*, and *Shigella*, and decreasing beneficial bacteria. In another study, the microbiome population of *Lactobacillus* decreased with all antibiotic treatments, but the greatest reduction in *Lactobacillus* was observed with oxytetracycline, a broad-spectrum antibiotic.[61] To make things worse, the reduction of lactic acid–producing bacteria (*Lactobacillus*) during weaning raises intestinal pH, increasing disease susceptibility because low gut pH is bactericidal to *Escherichia coli*.[59] It takes weeks to months to return the microbiome populations back to normal following antibiotic treatment.

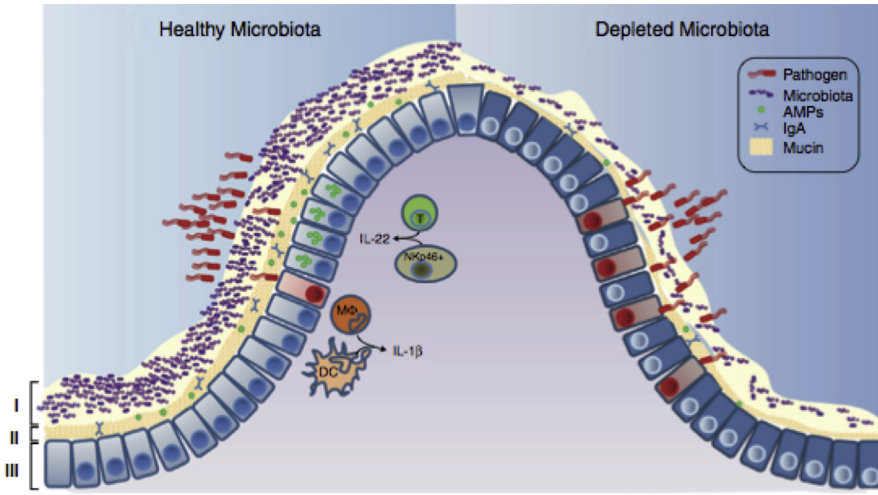

Fig. 10. Healthy mucosal defenses and mucosal dysbiosis. The intestinal microbiota promotes 3 levels of protection against enteric infection. (I) Saturation of colonization sites and competition for nutrients by the microbiota limit pathogen association with host tissue. (II) Kill zone: Commensal microbes prime barrier immunity by driving expression of mucin, IgA, and AMPs that further prevents pathogen contact with host mucosa. (III) Finally, the microbiota enhances immune responses to invading pathogens. Enhanced immune protection 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 monocytes (MΦ) and 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. (*From* Khosravi A, Mazmanian SK. Disruption of the gut microbiome as a risk factor for microbial infections. Curr Opin Microbiol 2013;16(2):222; with permission.)

MAXIMIZING ENTERIC IMMUNITY: PASSIVE IMMUNITY, VACCINES, AND DIRECT-FED MICROBIALS

Passive immune therapy has been used for more than 50 years in calf enteric disease. Polyclonal antisera has been administered orally and/or subcutaneously to prevent/treat bacterial diarrheal diseases colibacillosis and *C perfringens* type A and C with variable results.[62] One of the first successful uses of passive treatment was the oral administration of K-99 monoclonal antibody for the prevention/treatment of colibacillosis in calves.[63] Antirotavirus chicken egg yolk immunoglobulins fed in milk replacer decreased rotavirus diarrhea and enhanced rotavirus antibody-secreting cells.[64] Major success has been obtained by vaccinating cows before calving to enhance passive colostral antibodies for the protection of the calf against colibacillosis, *C perfringens* type C enterotoxemia, rotavirus, and coronavirus diarrhea.[62,65,66]

Both parenteral and mucosal vaccinations have been used to prevent enteric disease. Immune protection has been done indirectly by vaccinating the cow to obtain high levels of colostral antibodies for passive transfer to the calf against the neonatal diseases discussed above.[62,65,66] In the neonate, acquired immunity with parenteral vaccination of the neonatal calf has been used for *C perfringens* type C in herds wherein the disease occurs in calves older than 14 days of age.[62] Although oral and intranasal vaccines for rotavirus and coronavirus have the potential for active mucosal immunity in the neonatal calf (less than a week of age),[67] early onset of these diseases

(<14 days of age) along with the induction time for the immune response (7–10 days) makes efficacy poor in young calves (<14 days of age). Interestingly, oral coronavirus infections result in infection of both the enteric and the respiratory system[68] so coronavirus vaccines administered intranasal stimulated the common mucosal system and provided respiratory protection (see **Fig. 8**).[69] Several *Salmonella* vaccines: inactivated (whole cell; Rough mutants lacking oligosaccharide side chains (Re) mutant common core), MLV (genetically altered), and subunit (siderophores) vaccines have been licensed for parenteral administered but efficacy has been less then optimal.[70,71] A major problem with *Salmonella* vaccines has been adverse reactions. The off-label oral administration of MLV *Salmonella* vaccines also has variable efficacy.[70,72] There is a single *Mycobacterium avium subsp paratuberculosis* (MAP; paratuberculosis; Johne disease) vaccine available. The vaccine is efficacious and used in sheep.[73] The parenterally administered whole inactivated bacterin with Freund's adjuvant also produces cross-reactivity to both paratuberculosis and bovine tuberculosis tests.[74,75] These false positives interfere with national bovine tuberculosis eradication testing programs, which limits the use of the vaccine to approval by regulatory officials. Cross-reactivity is also a major deterrent for animal health companies to design new MAP vaccines for cattle.

Mucosal delivered vaccines have the advantage of not being affected by maternal antibody interference, being able to induce a response in neonates less than 7 days of age, and priming the common mucosal immune system (for example, oral coronavirus vaccination would provide specific coronavirus immunity to the respiratory mucosa).[67] The use of novel adjuvants with parenteral vaccines[76] that induce mucosal responses in addition to novel mucosal delivered adjuvants will also enhance enteric immunity.[77]

The area with the most opportunity that is also the least characterized is the use of direct-fed microbials to enhance enteric immunity and animal health while reducing antimicrobial usage.[78] Direct-fed microbials includes nutraceuticals, prebiotics, probiotics, and other dietary supplements. The effect of these direct-fed microbials on gut mucosal immunity and health has generated much interest.[50,51,54,56,61] Prebiotics (oligosaccharides, beta-glucan, and fiber), fiber metabolites (butyric acid and other short chain fatty acids), organic acids (ie, formic acid, citric acid), and botanicals (ie, vanilla, oregano, pepper oil) enhance the tight junctions in mucosal barrier and have an anti-inflammatory effect on mucosa (see **Fig. 9**).[79–82] Probiotics (ie, yeast, *Lactobacillus*, *Bifidobacteria*, and their metabolites) maintain microbiome homeostasis, increase secretory IgA, and decrease local inflammatory and APC responses to improve mucosal immunity (see **Fig. 9**).[83–88] This anti-inflammatory activity could have an impact on protozoal (eg, coccidia and cryptosporidia) and bacterial diseases (eg, *Salmonella* and Johne disease) where a proinflammatory response is part of the pathogenesis mechanism. Additional research needs to be done to further understand mechanisms and develop formulations that contain combinations of direct-fed microbials for different applications and age groups.

SUMMARY

The enteric mucosal immune system provides the first immune defense barrier for more than 90% of potential pathogens. The gut mucosal immune system alone contains more than a trillion (10^{12}) lymphocytes and has a greater concentration of antibodies than other tissue in the body. It protects against harmful pathogens but also induces immune system tolerance to dietary antigens and normal microbial flora. The health of the enterocytes, which are the epithelial cells that line the GI tract, is important not only for the growth and development of cattle, through secretion and

absorption in the gut, but also to provide a first immune response to enteric microorganisms. The enterocytes 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, "homeostasis." Disruptions in the microbiome's homeostasis result in dysbiosis, which decreases the "kill zone," allows "leaky gut," and increases inflammation. Increased inflammation is seen as an important part of pathogenesis of infectious diseases, including coccidia, cryptosporidia, *C perfringens* type A, *Salmonella*, and Johne disease. Maintaining microbiome homeostasis, the "kill zone," and the mucosa anti-inflammatory response are the keys to maintaining good gut and animal health and reducing antimicrobial usage.

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