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Invited Review: Ruminal microbes, microbial products, and systemic inflammation[1,2]

M. Garcia,* B. J. Bradford,*[3] and T. G. Nagaraja†[3]
*Departments of Animal Sciences and Industry, and †Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan 66506

ABSTRACT

The ruminal ecosystem is inhabited by complex communities of microbes that include bacteria, protozoa, archaea, fungi, and viruses. The immune system of the animal has evolved to maintain tolerance to innocuous gut commensals and allow the induction of protective responses to pathogens. However, ruminal microbes can also promote local and systemic inflammation. The ruminal epithelium–vascular interface allows absorption of fermentation products and also serves as a selective barrier to prevent translocation and systemic dissemination of bacteria, bacterial toxins, and immunogenic factors. Ruminal dysbiosis that increases ruminal acidity and osmolarity may increase permeability and even induce a breach in the integrity of the epithelial and vascular endothelial barriers, thus facilitating entry of bacteria or bacterial antigens into the portal vein. Upon reaching the liver, bacteria and their products can cause local inflammation and alter function of the organ; if they manage to bypass the liver, they can cause systemic inflammation and affect other organs. Shifts in microbial populations associated with dysbiosis result in increases in concentrations of potentially toxic and inflammatory substances that include lipopolysaccharides, lipoteichoic acids, and leukotoxins, among others. Lipopolysaccharides are constituents of all gram-negative bacteria, which are the dominant ruminal microbes. The entry of lipopolysaccharides into the systemic circulation, either from the rumen or lower gut, could trigger the release of proinflammatory cytokines, reactive oxygen and nitrogen intermediates, and bioactive lipids. An activated immune system drastically increases its demand for nutrients; however, the nutritional requirements of an activated immune system in the context of systemic physiology are still unknown. In conclusion, ruminal microbes and their products generate many complex interactions with the host immune system, and dysbiosis has the potential to induce systemic inflammation. Although inflammation is generally a protective reaction, the persistence of inflammatory mediators could have negative consequences for the host.

Key words: cattle, microbial product, ruminal microbe, systemic inflammation

INTRODUCTION

The reticulo-rumen is a vast microbial ecosystem, dominated by bacteria, but also populated with protozoa, archaea, fungi, and viruses (Puniya et al., 2015). The microbial community is influenced by the diet, which is primarily composed of plant polysaccharides containing a variety of sugars and glycosidic linkages. Ruminal microbes, in homeostatic conditions, work in a coordinated manner to optimize nutrient utilization, exemplifying a symbiotic or mutualistic relationship with the host. The establishment of a stable microbiota–host relationship also necessitates avoidance of potentially deleterious immune and inflammatory responses in the rumen. Ruminal bacteria with a vast array of carbohydrate-metabolizing enzymes are evolutionarily adapted to extract nutrients from the diet. Unlike gut pathogens, such as *Salmonella*, ruminal bacteria are not armed with virulence factors that allow them to invade and exploit epithelial tissue for nutritional benefit and subvert the host immune system (Rasmussen et al., 2005; McCuddin et al., 2006). Several studies have reported that dysbiosis and rumen disruption promote the proliferation of opportunistic microbes and their products, leading to pathogenic outcomes and subsequent inflammatory responses (Haskins et al., 1969; Vance et al., 1972; Nagaraja et al., 1978a,b,c; Liu et al., 2013; Devant et al., 2016). The detrimental effects of inflammatory responses and metabolic disorders in dairy cattle have received considerable attention. This review focuses on ruminal microbes and their effects on ruminal function and immunity. We will highlight recent findings that suggest a surprisingly direct role of the ruminal epithelium in mediating inflammation. The critical role of immune cells in recognition of microbial and endogenous products, the latter released after the damage of the ruminal epithelium, will be discussed in the context of in vitro and in vivo research. Last, we will discuss the pathogenic effects of *Fu-*

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[3] Corresponding authors: tnagaraj@vet.k-state.edu and bbradfor@ksu.edu

sobacterium necrophorum and lipopolysaccharide (**LPS**), which can escape from the rumen and enter the portal and systemic circulation.

RUMINAL MICROBES AND RUMINAL FUNCTION

Ruminal Microbes and Their Products

Microbes, including the commensals, produce an array of products that include metabolites and parts of their cell membrane known as microorganism-associated molecular patterns (**MAMP**), which are recognized as nonself-molecules by the host (Janeway, 1992; Medzhitov and Janeway, 2002; Neish, 2009). Although the terms are sometimes used interchangeably, "MAMP" and "virulence factors" are not equivalent. Microbial virulence factors have evolved, via mutation or acquisition of mobile genetic elements, to adapt to specific environments within the host with the purpose of enhancing their proliferation and avoiding host immune recognition, whereas MAMP are essential products for microbial survival and are highly conserved among microbial classes (Medzhitov, 2001).

Bacteria account for most of the microbial population colonizing the rumen and include more than 200 species with a total population of up to 10^{11} bacteria/mL of ruminal contents (Hungate, 1975; Mackie et al., 2000). Lipopolysaccharides and lipoteichoic acids are the most studied MAMP for gram-negative and gram-positive bacteria, respectively (Medzhitov and Janeway, 2002). Other MAMP, generally constitutive parts of the bacterial outer membrane required for bacterial survival, are peptidoglycans, lipopeptides, porins, flagellin, and bacterial DNA (Anas et al., 2010). Other bacterial products, considered virulence factors that suppress host recognition and promote bacterial proliferation, are exotoxins (leukotoxins, hemolysins, platelet aggregation factors, and so on), hemagglutinins, adhesins, and extracellular enzymes (Law, 2000; Nagaraja et al., 2005).

Ciliated protozoa are the second most abundant microbial population in the rumen, and populations range from 10^4 to 10^6/mL of ruminal contents, representing over 25 genera (Hungate, 1975; Mackie et al., 2000). Based on defaunation studies, protozoa are not essential for normal ruminal function, but their presence or absence has been associated with the structure and pathogenicity of different bacterial and archaeal communities, as well as modification of fermentation patterns (Yáñez-Ruiz et al., 2015). Potential virulence factors associated with 2 protozoa genera, *Cryptosporidium* and *Giardia*, are those associated with motility, attachment, invasion, and maintenance, primarily (Certad et al., 2017).

Fungi represent the third most abundant ruminal microbes, but the population is difficult to quantify because of the 2-stage life cycle (Mackie et al., 2000). Although ruminal fungi have a major beneficial role in fiber degradation (Ribeiro et al., 2016), they can also damage the mucosal epithelium via their less known virulence factors, some of them quite similar to those from bacteria (Brunke et al., 2016). Fungal infection of the gastrointestinal tract (**GIT**) of cattle has been reported worldwide, with predominance of species from the genus *Aspergillus* and class Zygomycetes, and minor occurrence of the genus *Candida* (Jensen et al., 1994). A recent study has reported that *Candida albicans*, a fungal species found in the rumen, can synthesize a pore-forming enzyme capable of damaging epithelial cells and penetrate the mucosal epithelium (Moyes et al., 2016).

Anaerobic methanogens constitute the ruminal community of Archaea, primarily of the order of Methanobacteriales. It is unknown whether archaea have virulence factors, although the paracrystalline cell surface S-layer of many archaea, and its release by membrane vesicles, may play a role evading the host immune response (Eckburg et al., 2003; Deatherage and Cookson, 2012). Ruminal viruses are primarily phages infecting bacteria and archaea (Gilbert and Klieve, 2015). In spite of being quite prevalent in the rumen, they are the least studied population (Ross et al., 2013). Enteric viruses invade gut microbes to carry out replication and transmission (Kuss et al., 2011), and viruses have been isolated from several well-known ruminal bacteria, such as *Prevotella ruminicola* and *Streptococcus bovis* (Gilbert and Klieve, 2015).

Microbial Population and Ruminal Homeostasis

The GIT of ruminants has the same general function as in nonruminant species, i.e., feed prehension, digestion, and absorption. The difference resides in the complex stomach of ruminants, with the reticulo-rumen inhabited by an array of resident anaerobic microbes. The stratified squamous structure of the ruminal epithelium appears to have evolved to deal with the abrasive feed and large microbial population colonizing the rumen. Certainly, as ruminants have evolved to adapt to dietary grain, their immune system has probably evolved in parallel or may continue evolving to become more tolerant and selective to a new array of microbes responsible for digesting highly fermentable carbohydrates (Ley et al., 2008). Under steady-state conditions, the normal response of the GIT to commensal microbes and food antigens was described by Medawar (1961) as "a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response."

Regardless of an apparent evolution of immune defense, environmental stressors still negatively affect ruminal microbe/immune cell homeostasis. Adoption of dietary strategies has been the major means to regulate ruminal fermentation, with the objective to alter microbial communities to maximize the efficiency of feed utilization. This can be achieved in part by minimizing or eliminating inefficient (e.g., methanogenesis) and harmful (e.g., acidosis) processes. Several studies have demonstrated that the diet is the major conditioner for ruminal disruption.

Some studies have associated ruminal epithelium damage (extensive papillae clumping) with compromised rumen integrity in cattle fed high grain diets, which may enable the translocation of microbes, their products, or both to the bloodstream (Haskins et al., 1969; Vance et al., 1972; Liu et al., 2013; Devant et al., 2016). Nevertheless, the major detrimental effect of a high-grain diet appears to be its effect on ruminal pH and its potential to induce acute or subacute ruminal acidosis (Nagaraja and Lechtenberg, 2007; Nagaraja and Titgemeyer, 2007). The detrimental effect of ruminal acidosis on ruminal epithelium integrity and animal performance has been the subject of considerable research. However, ruminal acidosis is still one of the major challenges in the cattle industry (McCann et al., 2016). Further, high production or reduced absorption of ruminal VFA may induce increments in osmotic pressure, disturb Na transport, and impair ruminal barrier function, increasing the risk of microbial translocation (Schweigel et al., 2005). The negative effect of high osmotic pressure on rumen integrity can be exacerbated by ruminal acidosis and further damage the ruminal epithelium, initiating rumenitis, microbial translocation, inflammation, and disease (Thomson, 1967; Owens et al., 1998).

Dysbiosis

"Dysbiosis" refers to changes in the composition of the resident microbial population of the GIT compared with microbial composition commonly observed in healthy animals. In a state of dysbiosis, populations of microbes that are normally tolerated at the gut surface are diminished, whereas populations of a small number of pathobiont species dramatically increase, ultimately leading to a decrease in microbial diversity (Petersen and Round, 2014). Hawrelak and Myers (2004) provided the more complete definition: "Dysbiosis is a state in which the microbiota produces harmful effects via: (1) qualitative and quantitative changes in the gut flora; (2) changes in their metabolic activities; and (3) changes in their local distribution." Hence, the harmful effects of an altered microbial population can be elicited directly by the microorganisms or by their products, and can have local or systemic effects or both. Ruminal microorganisms under steady-state conditions coordinate to enable feed fermentation and maintain immunological tolerance; in contrast, shifts in microbial populations can not only impair the efficiency of feed fermentation but may lead to harmful effects, conditioning cattle to several diseases (Nagaraja and Titgemeyer, 2007; Jacob et al., 2009; Bradford et al., 2015; Amachawadi and Nagaraja, 2016).

Perturbations in the GIT may increase the release of MAMP or endogenous products released by tissue damage, known as damage-associate molecular patterns (**DAMP**; Janeway, 1992; Medzhitov and Janeway, 2002; Neish, 2009). Those, after being recognized by the host immune system, can trigger a local inflammatory response (Sansonetti, 2004; Colaço and Moita, 2016). In conditions when the local GIT inflammatory response fails to resolve the disturbance, the gastrointestinal barrier is disrupted, potentially leading to systemic infection or inflammation (Cerf-Bensussan and Gaboriau-Routhiau, 2010; Burcelin, 2016). Frequent perturbations of the normal microbial population in the GIT can lead to an inappropriate activation of the host immune system, promoting chronic inflammation and subsequent disease (Carney, 2016).

CROSSTALK AMONG RUMINAL MICROBES, EPITHELIAL CELLS, AND IMMUNE CELLS

The GIT represents the largest interface between the animal and the environment, not only responsible for facilitating nutrient uptake by the host but also functioning as a barrier preventing the uptake of harmful microbes and some harmful products (Kraehenbuhl and Neutra, 1992; Farhadi et al., 2003). The balance between immune tolerance and inflammation is regulated through the crosstalk between innate immune cells and the intestinal microbiota, and dendritic cells are the main intermediates in this immunomodulatory process (Schiavi et al., 2015). The effect of ruminal microbes on animal performance and health was long ago recognized due to their critical role in feed fermentation. Still, the key mechanisms by which gut-microbe interactions are regulated remain to be elucidated (Lee, 2009; Burcelin, 2016). The use of gnotobiotic or germ-free animals has advanced the understanding of the array of contributions of gut microbiota to health and performance. Microbiota effects observed in such studies that may be of relevance for ruminants include (a) gut epithelial cell renewal is slower in germ-free animals (Sommer and Bäckhed, 2013); (b) microbes induce development, maturation, and activity of immune cells (Macpherson and Harris, 2004; Hajishengallis and Lamont, 2016); and (c) microbial dysbiosis promotes immunometabolic diseases (Cani and Knauf, 2016; Li et al., 2016). The manipulation of ruminal microbes by early intervention is a research area initiated long ago, but with the current microbiome and metagenomic advances, research in this area has been refocused (Eadie, 1962; Yáñez-Ruiz et al., 2015).

Differences Between the Ruminal and Intestinal Epithelia

The gut epithelium functions as a barrier separating the luminal contents from the circulation. However, the mechanisms employed likely differ somewhat throughout the GIT, given the dramatically different epithelial structures of the foregut (rumen-reticulum and omasum) and mid or lower gut (abomasum and small and large intestine) epithelia (Dobson et al., 1956; Graham and Simmons, 2005). The ruminal epithelium is a 4-layer stratified squamous (Figure 1, left) structure, whereas epithelium in the intestines is a single layer of columnar epithelial cells protected by 2 mucous layers (Figure 1, right). Underlying the stratum basale in the rumen and the single

layer epithelium in intestines is the lamina propria. The intestinal lamina propria is constituted by organized lymphoid tissues called Peyer's patches as well as by diffused lymphatic follicles rich in lymphocytes, macrophages, and dendritic cells (Sigurethardóttir et al., 2004; Forchielli and Walker, 2005). Moreover, other specialized cells (Dobson et al., 1956; Pyarokhil et al., 2012) are present in the lower gut of mammals but are absent in the foregut, as part of the epithelial layer (i.e., endocrine cells, goblet, paneth, and M cells; Figure 1). The ruminal epithelium and lamina propria lack organized lymphoid tissues, but the lamina propria appears to have nonorganized immune cells (Singh et al., 1984; Josefsen and Landsverk, 1996, 1997; Fuertes et al., 2015a,b). The intestinal mucosa is composed of the epithelium, lamina propria, and a thin layer of muscularis mucosa, which separates the lamina propria from the submucosa (Figure 1, right). However, in the rumen, the lamina propria appears to be merged with the subepithelium due to the lack of a layer of muscularis mucosa (Poonia et al., 2011; Scala et al., 2011). Mahesh et al. (2014) described the lamina propria in the goat rumen as constituted by loose connective tissue that, together with the subepithelium, contains a mixture of fibers, cells, blood capillaries, and nerve bundles.

Activation of Inflammatory Response

When the steady-state conditions of the GIT are disrupted, and changes in microbial populations occur, the tolerant state of the mucosal immune system is changed to a more aggressive stance. Research with rodents has demonstrated that commensal bacteria are required for the development of a mature, tolerant immune system by promoting the development of regulatory immune cells under steady-state conditions (Hajishengallis and Lamont, 2016). Although it is clear that changes in the microbial population leading to the overgrowth of pathogenic microbes promote disease development, the mechanisms involved are less clear. Oxygen availability has emerged as a potential mechanism initiating and perpetuating dysbiosis, leading to the loss of tolerated commensal bacteria and overgrowth and phenotype switching of pathobionts (Zechner, 2017).

Inflammation is the first response of specialized cells in response to the recognition of pathogenic microbes or tissue damage (Takeuchi and Akira, 2010). Expression of pattern recognition receptors (**PRR**) in the rumen and immune cells allows the recognition of MAMP and DAMP (Janeway, 1992; Medzhitov and Janeway, 2002; Neish, 2009). The most numerous and best-characterized PRR are toll-like receptors (**TLR**), and others are nucleotide-binding oligomerization domain–like receptors (**NLR**), retinoid acid–inducible gene-I, and C-type lectin receptors. Intestinal epithelial cells express PRR on plasma and endosomal membranes (TLR, C-type lectin receptors) and in the cytoplasm (NLR, retinoid acid–inducible gene-I, [RIG-I]-like-receptors); the latter act as sensors of microbial products injected by extracellular pathogens (Fukata and Arditi, 2013). After recognition of MAMP or DAMP, immune cells use an orchestrated signaling cascade to trigger an immune response by initiation of inflammation

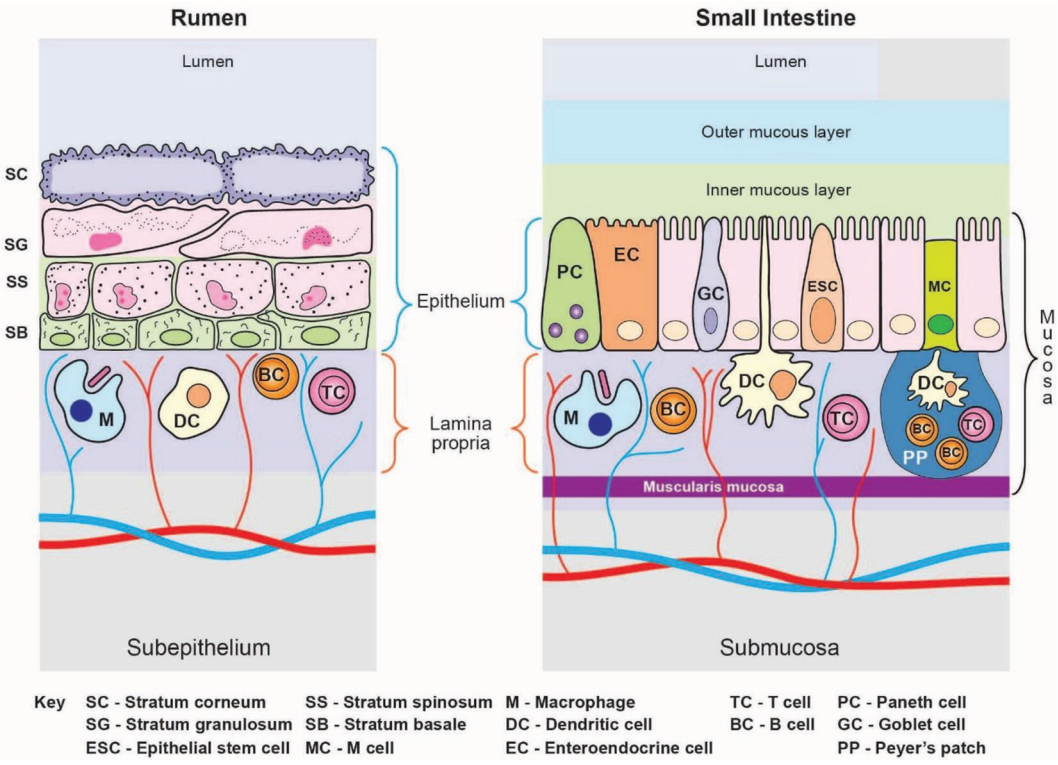

Figure 1. Structural differences between ruminal and intestinal tissues. Color version available online.

(Janeway, 1992; Neish, 2009). Because bacteria are the major ruminal microbial population and their cell wall constituents, including LPS and lipoteichoic acids, are primarily recognized by TLR, we will center our discussion on the activation of TLR as a mechanism to trigger an inflammatory response.

Expression of TLR recognizing both gram-positive (**TLR2**) and gram-negative (**TLR4**) bacteria have been identified in the ruminal tissue from cows (Trevisi et al., 2014; Minuti et al., 2015), steers (Chen and Oba, 2012), and goats (Liu et al., 2013, 2015). In murine intestinal epithelium, TLR are strategically localized in basolateral rather than apical surfaces, which may contribute to the tolerance of intestinal epithelial cells to commensal microbes (Kelly et al., 2005). The expression of TLR in the intestinal epithelium appears to be mediated by the presence of microbes; germ-free mice had lesser expression of TLR2 and TLR4 in colon and expression of these TLR only increased after microbiota from a wild-type mouse were transplanted (Wang et al., 2010). Intriguingly, in ruminants, 10 TLR were expressed in ruminal tissue (the epithelium and lamina propria) of calves less than 6 mo of age, but their expression decreased with age (Malmuthuge et al., 2012). The authors hypothesized that newborn calves are more dependent on TLR as the major mechanism of defense against invading pathogens, and as they mature, other mechanisms (e.g., peptidoglycan recognition protein 1 and β-defensin) may become more critical in maintaining homeostasis. Abundant expression of TLR in early life may be a response to a rapid ruminal colonization, and the decrease in TLR after 6 mo may represent an evolutionary mechanism permitting tolerance to commensal ruminal microbes to avoid an excessive immune response.

In murine studies, strategic microbiota colonization can induce transcriptional and epigenetic programming and regulate the activity of immune cells, making them either more tolerant or over-reactive (Thaiss et al., 2016). Therefore, colonization of the rumen with a variety of microbes in early life may program a better recognition of commensal or pathogenic microbes and optimize homeostasis and animal performance.

Recognition of MAMP or DAMP by a TLR complex leads to the activation of inflammatory mediators and their subsequent translocation to the nucleus, where they upregulate the expression of extracellular mediators including cytokines (Takeuchi and Akira, 2010). Cytokines are a diverse group of small proteins secreted as intercellular signaling molecules with a variety of roles depending on the type of cytokine and the target cell (Tisoncik et al., 2012). Under a proper immune cell activation, the nuclear-translocated transcription factor NF-κB triggers the expression of proinflammatory cytokines including tumor necrosis factor (**TNF**)-α, interleukin (**IL**)-1β, and IL-6. These cytokines regulate apoptosis of damaged cells, modify vascular endothelial permeability, recruit blood cells to inflamed tissue, and induce production of acute phase proteins (**APP**, Takeuchi and Akira, 2010). In contrast, activation of the transcription factor IRF3 and its subsequent translocation into the nucleus drives the expression of interferon-β, a cytokine with antiviral and anti-inflammatory roles, which inhibits the production of TNF-α, IL-1, IL-8, and interferon-γ (Weiss et al., 2012). Depending on the context, this anti-inflammatory response could also lead to a detrimental microbial persistence. In the ruminal epithelium or the lamina propria, a proper immune response activates epithelial and immune cells to initiate an inflammatory response that aids tissue repair and healing, aiming to recover homeostasis. However, an unchecked release of proinflammatory cytokines, often referred as a cytokine storm, can result in an unresolved inflammatory state that may fail to induce tissue repair and healing. Subsequently, cytokines are spread throughout the body via the systemic circulation, inducing systemic inflammation (Tisoncik et al., 2012).

Acute phase proteins are typically in very low concentrations in blood, but their concentration peaks after the liver senses elevated concentrations of proinflammatory cytokines or other inflammatory signals, triggering hepatic expression of APP (Bode et al., 2012). Although APP were initially thought to be produced only by the liver, it has been reported that several extrahepatic tissues can produce APP in response to a local inflammation (Ceciliani et al., 2012). Among the major bovine APP are LPS binding protein, haptoglobin, serum amyloid A (**SAA**), and α-1 acid glycoprotein. Lipopolysaccharide binding protein binds to LPS or bacteria and facilitates presentation to immune cells expressing the cluster of differentiation (**CD**)14, which then activates TLR4 and enhances the inflammatory response of these immune cells, neutralizing the endotoxic effect of LPS or bacteria (Zweigner et al., 2006). Haptoglobin binds hemoglobin, limiting iron availability for bacteria, and appears to have an immunomodulatory effect by promoting the production of anti-inflammatory mediators when the haptoglobin–hemoglobin complex binds to the CD163 receptor on monocytes and macrophages (Philippidis et al., 2004). Alpha-1 acid glycoprotein also possesses anti-inflammatory and immunomodulatory properties by modulating the inflammatory response of neutrophils and platelets (Hochepied et al., 2003). Serum amyloid A has a major proinflammatory effect; it mediates migration and infiltration of monocytes and neutrophils and opsonizes bacteria to enhance their recognition (Ceciliani et al., 2012).

Ruminal Epithelium as a Selective Defense Barrier

The keratinized outer layer of the ruminal epithelium is sloughed at a high rate (Dobson et al., 1956; Graham and Simmons, 2005), indicating a persistently rapid cell turnover. Earlier research confirmed the proliferative capacity of the stratum basale by in vivo measures of mitosis (Sakata and Tamate, 1978; Goodlad, 1981; Baldwin et al.,

2004a), but these studies did not aim to identify stem cells in the ruminal epithelium. Xiang et al. (2016) evaluated the transcriptome of GIT tissues, some stratified squamous epithelial tissues (skin, tonsil), and spleen from sheep. The authors reported that the rumen transcriptome clustered with skin and tonsil epithelium but not with other GIT tissues (abomasum, duodenum, colon, cecum, rectum) or organs (liver, spleen). Furthermore, skin, tonsil, and GIT tissues were enriched in genes involved in cell cycle control, which, for rumen, may indicate a higher turnover rate of this tissue compared with that of liver and muscle, and may represent a selective adaptability of the ruminal epithelium to harsh conditions (Xiang et al., 2016). The authors also found genes from the family of mammalian epidermal development complex enriched in the epithelia–rumen–tonsil cluster, with *TCHHL2* and several genes from the PRD-SPRRII protein family being exclusively enriched in the rumen cluster, as previously reported by the same laboratory (Jiang et al., 2014). According to Xiang et al. (2016), the TCHHL2 protein may be involved in cross-linking keratins at the ruminal surface, and the PRD-SPRRII proteins may be involved in cornification of the keratin-rich surface of the rumen. Furthermore, *KRT36* was present in the rumen cluster, whereas *KRT14* was also expressed in all epithelial organs, but they were 500 and 10 times, respectively, more highly expressed in the rumen as compared with skin, which had the second greatest expression of these 2 genes. Yohe et al. (2016) measured the presence of 6 potential markers of stem and progenitor cells in ruminal sections (containing the outer keratin layer, the 4 strata, and the dermis) and found that *KRT14* was the most highly expressed gene. These studies highlight the importance of keratin proteins (keratins 14 and 36) for rumen integrity.

In the fully developed rumen, differentiated epithelial cells form a stratified squamous epithelium whose major function is to absorb VFA. In preweaned calves, the rumen is neither active nor developed, particularly when they are fed primarily liquid feed; however, following initiation of solid feed intake, the rumen undergoes both physical and metabolic development that confers its absorptive capacity (Baldwin et al., 2004b). Graham and Simmons (2005) examined the functional organization of the ruminal epithelium using electron and light microscopic techniques combined with immunohistochemistry. The authors found that the stratum granulosum is rich in tight junctions, decreasing toward the stratum spinosum, and the stratum basale, which is a single columnar epithelial layer, is rich in Na^{+}-K+ pump proteins and mitochondria. The particular structure of the ruminal epithelium confers a selective barrier capacity to transfer small molecules (e.g., VFA) from the lumen and across the upper strata to the stratum basale while preventing translocation and systemic dissemination of microbes and their products. Light and scanning electron microscope studies (Mahesh et al., 2014) found differences in thickness and cell number of each ruminal strata, except the stratum basale, across different rumen sections.

Research evaluating the immunological and inflammatory capacity of strictly isolated ruminal epithelial cells is lacking. Recently, a few studies aimed to evaluate the role of ruminal epithelial cells on immune function by measuring the mRNA and protein abundance of PRR and other immune-related genes (Chen and Oba, 2012; Malmuthuge et al., 2012; Liu et al., 2013, 2015; Trevisi et al., 2014; Minuti et al., 2015). Most of those studies do not provide details on which ruminal cells or tissues, in addition to the epithelium, were included in the analyzed ruminal sample. Because of the difficulty (e.g., laser capture microdissection; Steele et al., 2013) of separating the epithelium from the lamina propria and subepithelium, it is likely these studies did not exclusively evaluate ruminal epithelium. The lamina propria underlying the stratum basale and the subepithelial layer is inhabited by distributed immune cells (Josefsen and Landsverk, 1996, 1997). Although there is no direct evidence to date, recent findings support the idea that ruminal epithelial cells express PRR and can initiate inflammatory responses. Zhang et al. (2016) isolated bovine ruminal epithelial cells by serial digestion and then pooled cells corresponding to the stratum spinosum and basale. The authors found that LPS, but not low pH, increased the expression of proinflammatory cytokines, indicating the capacity of cells from the stratum spinosum and basale to respond to LPS, presumably via TLR4. Furthermore, mRNA of several APP (haptoglobin, SAA, LPS binding protein, α-1 acid glycoprotein) was detected in ruminal tissue of healthy cattle, although by immunohistochemistry, only haptoglobin and α-1 acid glycoprotein were localized in the superficial layers of ruminal epithelium (Rahman et al., 2010; Dilda et al., 2012). This recent evidence indicates that the ruminal epithelium may have immune sentinal functions in addition to its role as a physical barrier preventing microbial invasion. However, much remains to be clarified, including whether functional PRR are found in the stratum granulosum.

Immune Cells as Sentinels of the GIT

In addition to the physical epithelial barrier, immune cells provide a second line of defense against invading pathogens. In contrast to the lower gut, where many of the immune cells can be found as organized resident cells in mesenteric lymph nodes and Peyer's patches (Peterson and Artis, 2014), in the foregut, the lamina propria apparently contains only dispersed immune cells. Several studies have reported the presence of immune cells (macrophages and lymphocytes) in the lamina propria and their subsequent infiltration of the stratified epithelium in cattle and goats undergoing parasitic infestation (Singh et al., 1984; Fuertes et al., 2015a,b). In addition, the presence of dendritic cells and multiple subpopulations of T cells within the stratum basale and lamina propria have been detected by immunohistochemistry in fetal and adult

sheep and reindeer (Josefsen and Landsverk, 1996, 1997). Most of the research regarding the functionality of immune cells has been executed using mice and some human and murine intestinal cells lines. Therefore, the discussion regarding immune cells will mostly refer to these nonruminant studies but will highlight recent research regarding immune cells associated with the rumen and its immune response.

Professional Antigen-Presenting Cells. Resident macrophages of the GIT are the most abundant among body tissues and are characterized for having a noninflammatory phenotype, downregulating the release of proinflammatory cytokines (Smythies et al., 2010). Although these macrophages express TLR and retain phagocytic function, they do not express the LPS co-receptor, the IgA receptor, or the IgG receptor, and do not promote inflammation (Smith et al., 2005). Resident macrophages also induce the differentiation of anti-inflammatory T-regulatory cells, helping to maintain gut tolerance (Kelsall, 2008). Monocytes are continuously recruited into the gastrointestinal tissue to replace resident macrophages, and their differentiation into a pro- or anti-inflammatory phenotype depends upon the integrity of the epithelium (Zigmond and Jung, 2013).

Dendritic cells are the most important professional antigen-presenting cells distributed in the lamina propria, mesenteric nodes, Peyer's patches, and intraepithelially in the intestines (Rumbo et al., 2004; Artis, 2008). Dendritic cells are critical for maintaining a dynamic balance between tolerance of commensal microbes and suppression of pathogens by bridging the innate and adaptive immune systems to maintain homeostasis (Manicassamy et al., 2010; Chang et al., 2014). A unique subset of dendritic cells is present across the GIT, and their functionality is regulated by the presence of gut microbes (Uematsu et al., 2008). In mice, dendritic cells use a paracellular mode to penetrate the single intestinal epithelial layer by extending their dendrites to sample microbes, MAMP, and microbial products (Rescigno et al., 2001). In the lamina propria, a subset of dendritic cells, called tolerogenic or regulatory cells, are responsible for maintaining gut tolerance for commensal microbes by sensing the presence of products such as butyrate, which interacts with a specific receptor leading to an IL-10 mediated induction of T-regulatory cells (Steimle and Frick, 2016). In addition, immature dendritic cells strongly express TLR, RIG-I-like-receptors, and NLR, and their activation induces rapid dendritic cell maturation. This maturation enhances capacity to initiate an immune response, including the upregulation of major histocompatibility complex (**MHC**)-II receptors and T cell costimulatory molecules, secretion of proinflammatory cytokines, and further activation of innate immunity (e.g., macrophage and neutrophil activation) as well as adaptive responses, particularly of T cells (Steimle and Frick, 2016).

In ruminants, research evaluating the capacity of dendritic cells to migrate or extend dendrites into the ruminal stratum basale is lacking. Using immunohistochemistry, Josefsen and Landsverk (1996, 1997) found that the ruminal epithelium, mainly the stratum basale, had MHC-II$^+$ cells that were concluded to be dendritic cells. The oresophageal epithelium, a 4-layer stratified epithelium similar to the rumen, is populated by Langerhans-type dendritic cells with the capacity to extend their dendrites through the upper strata of the epithelium to reach the oral cavity (Hertel, 2014). This characteristic of the oresophageal epithelium, coupled with the identification of MHC-II$^+$ dendritic cells (Josefsen and Landsverk, 1996, 1997) in the stratum basale of ruminants, opens the possibility that these immune cells may at least have the capacity to extend toward the luminal layers of the ruminal epithelium.

Lymphocytes. Josefsen and Landsverk (1996, 1997) identified the presence of CD4$^+$, CD8$^+$, and γδT cells in the stratum basale and lamina propria of ruminal tissue, and Trevisi et al. (2014) detected markers of B and T cells infiltrating the ruminal fluid. Multiple lymphocyte subtypes in the GIT are modulated by gut microbiota. Among them are B cells, which are primarily responsible for mediating humoral immunity by the production of antibodies to recognize specific antigens. Effector B cells can recognize pathogenic antigens and are responsible for the production and secretion of IgA into the intestinal lumen (Gutzeit et al., 2014). Intraepithelial γδT cells account for up to 60% of the small intestine epithelial lymphocytes and provide the first immuno-cellular line of defense at this vulnerable point for pathogen invasion (Cheroutre et al., 2011). Unique functions of γδT cells, considered a component of innate immunity, are being uncovered, such as their long-lasting memory and antigen presentation capacities (Lalor and McLoughlin, 2016). The ruminal epithelium and lamina propria contain γδT cells, with greater numbers accumulated in areas with greater absorption capacity (Josefsen and Landsverk, 1996). Additionally, deer with ruminal lesions had a greater number of γδT cells (Josefsen and Landsverk, 1997), suggesting that γδT cells in the rumen wall are responsive to absorptive flux or disruptions in the epithelial barrier.

From rodent studies, it is known that intestinal mucosal T cells are important regulators of intestinal homeostasis, not only by defending against intestinal pathogens but also by promoting wound healing, barrier repair, and regeneration (Sommer and Bäckhed, 2013). Among the T cells subsets that drive a proinflammatory response are T helper- 1, 2, and 17 cells, and these differentiate in response to a specific cytokine microenvironment and, in turn, secrete cytokines that regulate their differentiation (interferon-γ, IL-4, and IL-17, respectively; Stockinger and Veldhoen, 2007; Sommer and Bäckhed, 2013). The T cell subsets driving anti-inflammatory responses include T regulatory (CD4$^+$, CD25$^+$, and FOXP3$^+$) and T regulatory type 1 (CD4$^+$, CD25$^+$, and FOXP3$^-$) cells; these cell types are responsible for secretion of cytokines to suppress immune activation and prevent self-reactivity, making them

critical mediators of gut tolerance (Sommer and Bäckhed, 2013; Chistiakov et al., 2015).

Recently, a new term has been coined for a class of lymphocytes: "innate lymphoid cells" (**ILC**). This class does include the well-known natural killer cells, which recognize and kill infected or tumorous cells and also express receptors to activate B cells, T cells, and lymphoid tissue inducer cells, responsible for promoting the formation of secondary lymphoid tissues (Eberl et al., 2015). The ILC are classified into ILC1 (ILC1 and NK cells), ILC2, and ILC3 (ILC3 and lymphoid tissue inducer cells) reflecting phenotypical and functional characteristics of T helper cells (Walker et al., 2013). Several subsets of ILC have been identified in mucosal surfaces of rodents and humans, where they secrete several cytokines to promote inflammation and provide immunity to infections (Walker et al., 2013). A new GIT subset of ILC, with regulatory roles similar to that of T regulatory cells, have also been recently identified. These ILC regulatory cells have a unique inhibitory role during the innate immune response, thereby contributing to the resolution of the innate intestinal inflammation (Wang et al., 2017).

Phagocytic Cells. Macrophages and dendritic cells possess phagocytic ability, but neutrophils are the prototypical phagocytic cells. In homeostatic conditions, neutrophils are not present in the epithelium or lamina propria of the GIT but are circulating, surveilling for chemoattractants produced by gut resident macrophages and specialized epithelial cells. Upon recruitment, they cross the endothelial barrier or even, under pathological conditions, enter the intestinal (and potentially the ruminal) lumen (Fournier and Parkos, 2012; Trevisi et al., 2014). In the lamina propria, neutrophils kill microbes via intracellular and extracellular mechanisms and contribute to the further recruitment of immune cells by production of cytokines and other inflammatory mediators (Fournier and Parkos, 2012). The prevailing view is that neutrophil intestinal epithelial recruitment leads to pathologic inflammation due to leakage of the epithelial barrier, widely referred to as "leaky gut." However, a recent study found that migrated neutrophils bind to epithelial cells expressing ICAM-1 and promote mucosal homeostasis and wound healing (Sumagin et al., 2016).

RUMINAL MICROBES, INFLAMMATION, AND DISEASE

Liver Abscesses Caused by F. necrophorum

The presence of liver abscesses is the primary abnormality of cattle at slaughter, with an incidence ranging from 10 to 20% (Amachawadi and Nagaraja, 2016). As emphasized before, feeding high-grain diets may induce dysbiosis and ruminal epithelial damage as well as chronic ruminal acidosis and rumenitis, which in turn can increase the risk of animals developing liver abscesses (Nagaraja and Chengappa, 1998). The pathology of liver abscesses can involve different causes that are not necessarily related to disruption of ruminal epithelium and subsequent rumenitis. Rezac et al. (2014) reported that only 32% of slaughter cows with severe and mild rumenitis had liver abscesses, whereas 19% of cows with an apparently healthy rumen (epithelium with thick, lush papillae and no signs of inflammation, ulceration, or another insult) also had liver abscesses. Although a previous study (Jensen et al., 1954) reported a greater incidence of liver abscess (43 vs. 23%, respectively) in cattle with ruminal lesions compared with a healthy rumen, another study reported no relationship between liver abscesses and ruminal lesions (Wieser et al., 1966). However, as pointed out by Brent (1976), a small breach of the ruminal epithelium can allow bacterial translocation, and transport via the portal vein may cause liver damage and a systemic response, even if the rumenitis is then resolved.

The bacterial population in liver abscesses is dominated by gram-negative anaerobes (Nagaraja and Chengappa, 1998). Most studies conclude that *F. necrophorum* is the primary causative agent, followed by *Trueperella* (formerly *Arcanobacterium*) *pyogenes* (Amachawadi and Nagaraja, 2016). Recently, the occurrence of *Salmonella enterica*, particularly of the serotype Lubbock, has been reported; however, the etiologic role of *Salmonella* in liver abscesses needs to be investigated (Amachawadi et al., 2017). *Fusobacterium necrophorum* is a normal resident of the rumen, whose major fermentative role is to use lactic acid for VFA production (Tan et al., 1994a) as well as breakdown of protein and AA, particularly lysine, derived from feed and ruminal epithelium (Russell, 2006; Elwakeel et al., 2013). The population of *F. necrophorum* in the rumen of forage-fed cattle is low ($<1 \times 10^5$/g of ruminal contents), but it increases ($>1 \times 10^6$/g of ruminal contents) when feeding more concentrates (Coe et al., 1999), likely because of increased availability of lactate (Tan et al., 1994a). The organism has some competitive advantages allowing it to migrate to and become established in the liver. First, it can adhere to the keratinized stratum of the ruminal epithelium, mediated by outer membrane proteins (Kumar et al., 2013, 2014). Second, *F. necrophorum* can colonize the ruminal epithelium due to its oxygen tolerance (Hofstad, 1984) and its ability to proliferate at the slightly basic pH (Tan et al., 1994a) commonly observed at the ruminal epithelial surface. Third, it elaborates certain virulence factors to facilitate survival and proliferation in ruminal epithelial tissue and the liver parenchyma (Nagaraja et al., 2005).

Virulence Factors Produced by F. necrophorum Affect Immune Activation and Function

As a gram-negative bacterium, *F. necrophorum* possesses LPS as its primary MAMP, which can be recognized by TLR4 expressed in the ruminal epithelium and by innate immune cells (Neal et al., 2006). However, the primary pathogenicity of *F. necrophorum* resides in the capacity of

this bacterium to produce virulence factors, which have evolved to avoid or to overcome host recognition (Medzhitov, 2001). Of the 2 subspecies of *F. necrophorum*, subspecies *necrophorum* is more virulent than *fundiliforme* and is the primary subspecies causing liver abscesses (Amachawadi and Nagaraja, 2016).

Leukotoxin is the major virulence factor produced by *F. necrophorum* and is toxic for neutrophils, macrophages, hepatocytes, and potentially for ruminal epithelial cells (Tan et al., 1994b; Nagaraja et al., 2005). The population of *F. necrophorum* in the rumen produces little leukotoxin, which may indicate that *F. necrophorum* has evolved to activate its pathogenic phenotype opportunistically (Tan et al., 1994b; Narayanan et al., 2002). Cattle and sheep polymorphonuclear leukocytes (**PMN**; neutrophils, eosinophils, and basophils) are the most susceptible targets for leukotoxin cytotoxicity compared with PMN from horses, swine, and rabbits (Tan et al., 1994b). The expression of specific *F. necrophorum* leukotoxin receptors in PMN has not been confirmed but is suggested by enhanced phagocytic and killing capacity and subsequent apoptosis of PMN exposed to low concentrations of leukotoxin (20 U/mL, Narayanan et al., 2002). Leukotoxin from *F. necrophorum* is strongly immunogenic, and high antibody titer against this leukotoxin following vaccination reduced the prevalence of liver abscesses in feedlot cattle (Jones et al., 2004).

Other virulence factors produced by *F. necrophorum* include extracellular proteins (hemagglutinin, hemolysin, platelet aggregation factors, and adhesin) with primary roles in erythrocyte agglutination, platelet aggregation, and enhancement of bacterial adherence to ruminal epithelium and hepatocytes (Kanoe and Iwaki, 1987; Kanoe et al., 1998; Tadepalli et al., 2009). Additional virulence factors are extracellular enzymes targeting ruminal epithelium, hepatocytes, and immune cells. Amoako et al. (1993) found that the most expressed enzymes in *F. necrophorum* were DNase, alkaline phosphatase, and lipase, with DNase exclusively produced by the subspecies *necrophorum*. The potent cytotoxicity of *F. necrophorum* for PMN may be related to the ability of this bacterium to release DNase to degrade extracellular DNA, which is a structural component of neutrophil extracellular traps, thereby impairing the killing ability of PMN and contrarily using these traps as reservoirs for proliferation.

Protozoa Mediate Bacterial Virulence Enhancement

Ciliated protozoa predominantly digest the bacterial cells they ingest. However, some pathogenic bacterial species can survive and replicate within protozoa, induce gene transfer, and undergo virulence enhancement (McCuddin et al., 2006; Ricard et al., 2006). Earlier studies showed that *Mycobacterium avium* and *Legionella pneumophila* grown in aquatic protozoa had greater pathogenic capacity compared with same strains grown in agar media (Cirillo et al., 1994, 1997). The pathogenicity of a *Salmonella enterica* serotype Typhimurium was enhanced by exposure to protozoa (Rasmussen et al., 2005). Similarly, the pathogenicity of *Escherichia coli* O157:H7 and its export and transmission were enhanced when this strain proliferated within ruminal protozoa (Steinberg and Levin, 2007).

The virulence of bacteria associated with ruminal protozoa is a major concern due to the risk of human diseases caused by foodborne pathogens transmitted by ruminant products. However, as evaluated by Rasmussen et al. (2005), newborn calves are also an important target of these pathogenic bacteria. The rearing practices for dairy and beef cattle differ and may affect the risk of calves being exposed to protozoa containing pathogenic bacteria. Protozoa can be present in the rumen of offspring raised with their dam as early as 2 wk of age, but in dairy calves separated from dams at birth, protozoa may take longer to establish (Yáñez-Ruiz et al., 2015).

RUMINAL ENDOTOXIN, INFLAMMATION, AND DISEASE

Cattle fed energy-dense diets are predisposed to ruminal acidosis and subsequent rumenitis, conditions that are commonly exacerbated in feedlot cattle and dairy cows during the transition to lactation (Nagaraja and Titgemeyer, 2007). In the last 5 yr, several reviews have highlighted the effect of ruminal endotoxins and other antigens, translocated to the circulation, on inflammatory and acute phase responses (Plaizier et al., 2012; Zebeli and Metzler-Zebeli, 2012; Bradford et al., 2015; Sato, 2015), and others have highlighted the effect on periparturient diseases and fertility (Ametaj et al., 2010; Dong et al., 2011; Zebeli et al., 2015; Eckel and Ametaj, 2016). This section aims to compile recent research to briefly explain the fates of ruminally derived LPS and to discuss the nutritional cost of systemic inflammation.

Toxicity of Ruminal LPS

The injection of cell-free ruminal fluid was lethal for mice and chick embryos, induced a pyrogenic response and leukocytosis in rabbits, and enhanced the susceptibility to bacterial infection in mice (Nagaraja et al., 1978a,b,c). These studies suggested that the detrimental effects of ruminal fluid in laboratory animals may be due to the presence of endotoxin produced by gram-negative bacteria that predominate in the rumen. Lipopolysaccharides can be released either by bacterial lysis or by shedding during bacterial growth (Nagaraja and Titgemeyer, 2007). High concentrations of LPS in cell-free ruminal fluid have long been associated with the feeding of high-grain diets; ruminal LPS concentrations were twice as great in grain-fed compared with hay-fed cattle (Nagaraja et al., 1978b). Furthermore, LPS varies in chemical composition, and LPS from *E. coli* appears to have more potent endotoxic activity than LPS from the commensal bacteria *Mega-*

sphaera elsdenii and *Selenomonas ruminantium* (Nagaraja and Titgemeyer, 2007).

Ruminal Responses to Acidosis and Endotoxin

Several studies have reported the expression of TLR in ruminal tissue and the potential to respond to ruminal LPS (Chen and Oba, 2012; Trevisi et al., 2014; Minuti et al., 2015). Ruminal tissue (immune cells present in the stratum basale and lamina propria and potentially epithelial cells) appears to respond to LPS via expression of genes coding for downstream signaling molecules such as NF-κB and TNF-α (Minuti et al., 2015). The feeding of high-concentrate diets resulted in a reduction of ruminal pH, increased osmolarity, and increased ruminal LPS concentration (Mao et al., 2013). The same laboratory (Zhang et al., 2016) found that cows fed high-concentrate diets upregulated the expression of several inflammatory genes in ruminal papillae, including IL-1β and IL-6. Furthermore, cells isolated from the stratum spinosum and basale of the rumen were exposed to conditions mimicking acidosis, and LPS, but not low pH, triggered an inflammatory response (Zhang et al., 2016). The effect of acidosis may be primarily structural damage of the ruminal epithelium, enabling translocation of microbes and LPS (Nagaraja and Titgemeyer, 2007).

Translocation and Transport of LPS

Even though several studies have reported associations between increased ruminal LPS concentration and its translocation into circulation (Khafipour et al., 2009; Dong et al., 2011; Chang et al., 2015a,b), the mechanisms by which ruminal LPS reaches circulation are not fully understood. Postulated mechanisms of paracellular and transcellular transport of LPS from the GIT of cattle to the circulation are based on model animal studies (Eckel and Ametaj, 2016). These postulated mechanisms would apply to cattle intestine, which shares a similar structure and functionality with intestines of animal models; however, due to the stratified squamous epithelium of the reticulum-rumen, these mechanisms may not apply to ruminal translocation of LPS. If LPS is translocated from the rumen to circulation, the most plausible mechanism of transport appears to be simple passive diffusion via physical damage of the ruminal epithelium, leaving exposed areas for LPS and microbial translocation. The finding (Lassman, 1980) that ^{51}Cr–labeled LPS was absorbed neither through lymph nodes nor the portal vein in normal or acidotic ruminal environments, in the absence of ruminal lesions, supports the hypothesis above. Moreover, the lack of physical damage to the ruminal epithelium (e.g., ulceration) after induction of lactic acidosis (pH <4.6) in steers subjected to repeated i.v. administration of LPS (Anderson, 1984) further suggests that LPS alone is insufficient to disrupt the ruminal epithelial barrier. In vitro studies found that the ruminal and colonic tissues of ruminants cultured with LPS under acidic conditions (pH = 4.5) allow translocation of LPS, whereas under the condition of high pH (≥5.5), LPS translocation did not occur (Emmanuel et al., 2007). Further, the development of systemic inflammation in acidosis-induced sheep was linked to increased ruminal permeability; however, authors were not able to exclude the potential role of intestinal translocation (Minuti et al., 2014). In the absence of ruminal epithelial damage, it appears that the major route of LPS translocation is the lower gut, due to disruption of intestinal tight junctions (Turner, 2009). In addition to a paracellular route, enterocyte TLR4-mediated LPS internalization (Neal et al., 2006) and the translocation capacity of M cells (Hathaway and Kraehenbuhl, 2000; Kucharzik et al., 2000) provide additional routes for intestinal LPS entry.

Regardless of whether LPS is translocated via the rumen or the lower gut, it rapidly enters the portal vein. High ruminal concentration of LPS was linked with a high concentration of LPS in the portal vein and with greater LPS uptake by the liver (Andersen et al., 1994; Chang et al., 2015a,b), consistent with the pivotal role of the liver for uptake and metabolism of LPS. Based on studies with animal models, LPS associated with chylomicrons can be transported via lymph (Ghoshal et al., 2009); however, the contribution of this route of entry in cattle is not known.

Peripheral Responses to LPS-Induced Inflammation

Lymph Nodes and Circulating Immune Cells. After translocation from the gut, LPS typically circulates bound to LPS binding protein or apolipoprotein E, associated with chylomicrons (Kell and Pretorius, 2015). In the lymph node, LPS is recognized by resident immune cells such as macrophages and dendritic cells, triggering a classical inflammatory response leading to the production of proinflammatory cytokines (Ghoshal et al., 2009; Petersen and Round, 2014). Monocytes isolated from blood of healthy heifers and stimulated with LPS in vitro secreted greater amounts of IL-1β, IL-6, and TNF-α (Corripio-Miyar et al., 2015). Isolated PMN from the blood of healthy lactating dairy cows increased the expression of genes coding for proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) and increased secretion of TNF-α when stimulated with LPS (Garcia et al., 2015a,b).

Activated immune cells increase their demand for nutrients to perform their inflammatory response. Bovine PMN challenged with LPS upregulated the expression of genes coding for glucose transporter 3 (Garcia et al., 2015b), consistent with the increased needs of PMN for glucose, as glucose has been reported to be the primary fuel for PMN in rodent models (Pithon-Curi et al., 2004). As reported in humans, another important fuel supporting the inflammatory response of PMN is the AA glutamine (Ogle et al., 1994; Furukawa et al., 2000). Bovine PMN challenged with LPS and cultured with a pool of essential and nonessential AA, except glutamine, did not alter the apparent utilization of any of the included AA, suggesting no preferential use of AA other than glutamine (Garcia et al., 2016a).

Liver. The liver has a central role in nutrient metabolism and utilization. Moreover, the liver plays a critical role during inflammation and detoxification, as it can regulate its inflammatory response depending on the availability of nutrients that may impair or exacerbate inflammation (Garcia et al., 2016b). The enhanced production of APP and diminished production of other proteins (e.g., albumin) in the liver is an acute response to localized or systemic inflammation (Ceciliani et al., 2012). The liver can directly mount an inflammatory response to LPS via TLR4 expressed in hepatocytes (Mamedova et al., 2013), stellate cells (Friedman, 2008), and Kupffer cells (Su et al., 2000). The ability of liver from lactating dairy cows to respond to an LPS challenge was evaluated via in vitro culture of liver explants (Garcia et al., 2015a). The authors found that LPS challenge increased the mRNA abundance and protein secretion of TNF-α as well as haptoglobin and SAA mRNA. These responses were coupled with decreased gluconeogenesis, measured by fluxomic estimation of phosphoenolpyruvate carboxykinase activity (Garcia et al., 2015a).

Ruminal acidosis and laminitis, induced by an oligofructose overload (17 g/kg of BW), appeared to provoke systemic inflammation; white blood cell count increased at 18 to 24 h and plasma concentrations of SAA and haptoglobin were elevated at 72 h after challenge (Danscher et al., 2011). Systemic inflammation, induced after daily injection of TNF-α, promoted acute phase responses and altered the hepatic metabolism of dairy cows (Bradford et al., 2009; Yuan et al., 2013). Although Yuan et al. (2013) found no effect of TNF-α infusion on hepatic glucose and lipid metabolism in early lactation, Bradford et al. (2009) found that TNF-α altered hepatic metabolism in late lactation, including increased triglyceride content and reduced hepatic mRNA abundance of gluconeogenic genes. Other models of systemic inflammation resulted in downregulation of the hepatic expression of genes associated with AA, glucose, and fatty acid metabolism (Jiang et al., 2008).

Nutritional Cost of Systemic Inflammation

Ensuring the availability of limiting nutrients during an inflammatory response is critical for proper resolution of inflammation after elimination of the pathogenic invasion. However, the nutritional requirements of an activated bovine immune system are still unknown. Research in chickens concluded that the acute phase response is markedly costlier than the adaptive immune response (Iseri and Klasing, 2013, 2014). In ruminants, the high demand for glucose during an acute phase response may be the most problematic metabolic shift to adapt to, particularly during early lactation, when balancing glucose supply and demand is already a challenge. Kvidera et al. (2016, 2017) measured the glucose demand of cattle during the 12 h after an LPS challenge and found 1.00 and 0.66 g of glucose/kg of $BW^{0.75}$ per h was required to maintain euglycemia in growing steers and lactating cows, respectively. A recent review by Klasing (2016) discussed ongoing research revealing that the direct use of nutrients by immune cells and liver during inflammation is less than the amount of nutrients needed to address the increased metabolic rate, nutrient imbalances, and digestive inefficiencies occurring during inflammation. Klasing (2016) concluded that all these associated costs lead to reduced productivity that cannot be reversed by the supply of additional nutrients. Indeed, glucose infusion to keep euglycemia did not reverse the decrease in milk production of LPS-challenged cows (Kvidera et al., 2017). Ideally, prevention of pathogenic or antigenic challenges leading to inflammatory responses is the key to avoiding detrimental effects on animal productivity. However, cattle at critical physiological stages (e.g., weaning, transition) are highly susceptible to inflammatory challenges, and this susceptibility is not necessarily replicated with short-term feed restriction models (Moyes et al., 2009). Hence, uncovering the nutritional demands of an activated immune system and the imbalance of nutrients that may be generated is worthy of further investigation in our efforts to improve animal well-being and productivity.

IMPLICATIONS

The cattle industry has to deal with several inflammation-associated disorders, including fatty liver, liver abscesses, mastitis, and metritis, all leading to significant economic losses. The ability of the immune system to mount an inflammatory response with appropriate intensity and resolution is critical for mitigating the most severe consequences of these production disorders. Therefore, uncovering the roles and mechanisms of ruminal epithelial cells and how they may differ in each stratum, as well as the regulatory mechanisms of subepithelial resident immune cells, are critical areas of research. Furthermore, a clear understanding of the complex crosstalk of the ruminal microbes with the gastrointestinal epithelium and immune cells would allow for the development of direct nutritional and management interventions to ensure a proper inflammatory response and improved animal well-being.

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