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

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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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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.