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Research paper

Differential innate immune responses of bovine peripheral blood leukocytes to *Salmonella enterica* serovars Dublin, Typhimurium, and Enteritidis

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ABSTRACT

The majority of *Salmonella* serovars cause no clinical disease in cattle, while some are associated with severe disease. The objective of the current study was to determine the innate immune responses of bovine peripheral blood leukocytes exposed to *Salmonella enterica* serovar Dublin (bovine-specific), *Salmonella typhimurium* (murine adapted, but zoonotic), and *Salmonella enteritidis* (poultry host-adapted) in 3-week-old calves. All *Salmonella* exposures increased cell surface CD14 and CD18 regardless of serovar. The greatest CD14 marker mean fluorescence was in monocytes and the greatest mean fluorescent of the marker mean was in neutrophils. Phagocytosis increased with all serovars, but was not different among them. Neutrophils had the greatest marker mean fluorescence for phagocytosis, with all serovars being equal. Oxidative burst increased in all serovars compared to control cells, but were not different among the serovars. Neutrophils and monocytes were similar in the oxidative burst, with limited oxidative burst detected in the primarily lymphocyte population. mRNA expression of TNF-α, IL-8, and IL-12, increased above the control cells whereas none of these serovars affected mRNA expression of TLR4. TNF-α was greatest in *S. enterica* and *S. typhimurium*, compared to *Salmonella dublin*. In contrast, IL-8 was expressed more in *S. dublin* than *S. typhiurium*, with *S.* Enteriditus intermediary. These results show while cell surface markers, phagocytosis, and oxidative burst were largely unaffected by serovar, cytokine and chemokine expression differed among the *Salmonella* serovars. It appears that internal responses of the cells differ, rather than cell recognition, creating pathogenicity differences among of the serovars, even in the neonate with developing immunity.

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1. Introduction

The majority of *Salmonella* serovars cause no clinical disease in cattle, while some serovars may cause severe disease. *Salmonella typhimurium* is a murine-host-adapted serovar, but it also infects a wide range of domesticated or wild animals, as well as humans (Lax et al., 1995). In calves less than 2 months of age, *S. typhimurium* is often associated with outbreaks of enteric diseases (Wray et al., 1987; Gelberg, 2001). In general, it causes a self-limiting acute gastroenteritis, with severe scouring developing about

Abbreviations: SD, *Salmonella enterica* serovar Dublin; SE, *Salmonella enterica* serovar Enteritidis; ST, *Salmonella enterica* serovar Typhimurium; ATCC, American Type Culture Collection; RPMI, Roswell Park Memeorial Institute; RPE, R-phycoerythrin; HBSS, Hank's balanced salt solution; TLR, toll-like receptor; CR3, macrophage-1 antigen; RT-PCR, reverse transcription-polymerase chain reaction.

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12–48 h post-infection, and is usually cleared by about 8 days. However, this gastroenteritis can be lethal, at high infective doses (up to 10^{10} CFU), due to extensive intestinal lesions and extreme fluid loss (Wray, 1991). Unlike *S. typhimurium*, *Salmonella dublin* is a bovine-host-specific serovar which is associated with disease of similar frequencies in young and adult cattle (Sojka and Field, 1970). Compared to *S. typhimurium*, *S. dublin* is more invasive, and causes both enteric and severe systemic infections (Wray, 1991). Some cattle infected with *S. dublin* fail to clear the infection and become chronically infected, thus remain as a source of *S. dublin* infection in the herd (House et al., 1993). Chronic *Salmonella* infections with other serovars have been documented, but appear to be less common (Evans and Davies, 1996). The difference in pathogenesis between *S. typhimurium* and *S. dublin* may result from serotype-specific virulence factors, the route of infection, the dose of the pathogen, and the innate and acquired host immune response against *Salmonella* infection (Gray et al., 1996; Meyerholz and Stabel, 2003).

Macrophages and neutrophils play important roles in *Salmonella* recognition and elimination. Two receptors important for *Salmonella* recognition are cluster of differentiation (CD)14, and TLR 4. The lipopolysaccharide (LPS) of Gram-negative bacteria is recognized by a complex of TLR4 and CD14 on the cell surface, and subsequently activates macrophages to produce and secrete cytokines (Leung et al., 2005; Burkey et al., 2007; Gioannini and Weiss, 2007). Important cytokines produced by activated macrophages in response to *Salmonella* infection include TNF-α, IL-12, and IL-8. TNF-α activates the vascular endothelium and increases vascular permeability, which leads to increased entry of antibody, complement, and leukocytes to the site of infection. It plays a critical role in inducing the acute-phase response, which favors effective host defense in various ways (Möller and Villiger, 2006). Interleukin-12 activates NK cells and induces the differentiation of T cells (Gee et al., 2009; O'Garra and Murphy, 2009). Interleukin-8 directs the flow of neutrophil traffic, recruiting neutrophils from the blood into the site of infection (Thelen, 2001). Circulating neutrophils express chemokine receptors CXCR1 and CXCR2, which bind IL-8 emanating from the infected tissue (Lee and Corry, 2004). Neutrophils have another important surface molecule designated CD18, which is essential for recruitment of cells to site of infection or inflammation. CD18 on neutrophils binds adhesion molecules on the endothelium, thus forming a tight interaction needed for diapedesis and migration to the center of infection along the IL-8 gradient (Lee and Corry, 2004). After its arrival at the site of infection, the neutrophil, along with the macrophage, acts as potent killer of pathogens through phagocytosis and oxidative burst. Neutrophils and macrophages use similar mechanisms to phagocytize microorganisms. After a neutrophil or macrophage engulphs a pathogen, oxidative burst occurs, in which the pathogen is killed by several toxic reactive oxygen or nitric species (*e.g.* hydrogen peroxide) rapidly released by these two phagocytes (Mayer, 2006; Elbim and Lizard, 2009).

In vitro studies regarding differential responses of host cells to different *Salmonella* serovars have been conducted in human, mice, and farm animals, such as swine and chickens (Burkey et al., 2007; Weinstein et al., 1998; Okamura et al., 2005). However, little attention has been given to the differential innate immune responses of bovine leukocytes to different *Salmonella enterica* serovars. Therefore, the present study was conducted to compare various parameters of bovine leukocyte innate immune response against murine-host-adapted serovar Typhimurium, bovine-host-adapted serovar Dublin, and chicken-host-adapted serovar Enteritidis at time that calves are susceptible to disease. Such differences may help us to understand the mechanisms of host susceptibility to *Salmonella*.

2. Materials and methods

2.1. Animals, housing, diets, and vaccinations

The experimental protocol was approved by the Purdue Animal Care and Use Committee. Ten Holstein calves (heifers) from the Purdue Dairy Teaching and Research Center were assigned to the experiment and were allowed to stay with the dams for up to 2 h before being transferred to individual calf housing. A commercial dry feed (Vita Plus, Madison, WI) was fed to the calves during the 3-week study period in addition to a commercial milk replacer (Land O'Lakes, Shoreview, MN) twice a day. At 1 day of age, calves were vaccinated with ENDOVAC-Bovi (Immvac, Columbia, MO) against Gram-negative endotoxemic diseases and *Clostridium perfringens* Type A Toxoid (Novartis, Larchwood, IA) at 3 days of age. At 2 weeks of age, calves were vaccinated with Bovi-Shield GOLD 5 (Pfizer, Exton, PA) as an aid in the prevention of disease caused by infectious bovine rhinotracheitis (IBR) and bovine virus diarrhea (BVD).

2.2. Bacterial strains and culture

S. enterica serovar Dublin (SD) strain BAA-1514, *S. enterica* serovar Enteritidis (*Salmonella enteritidis*, SE) strain 13076, and *S. enterica* serovar Typhimurium (*S. typhimurium*, ST) strain 1331 were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Bacteria were grown overnight at 37 °C in Luria Bertani broth cultures (BD, Sparks, MD) and adjusted to 10^8 CFU/mL. Numbers of bacteria were determined by colony plating on MacConkey agar (Neogen, Lansing, MI) incubated at 37 °C for 24 h, under aerobic conditions. Bacteria were heat killed (20 min at 65 °C) in a hot water bath, pelleted (3 min at 1157 × *g*) and resuspended in Roswell Park Memorial Institute medium (RPMI) 1640 (Gibco, Grand Island, NY) at equal volume, before the *ex vivo* exposure.

2.3. Sample collection and analyses

At approximately 3 weeks of age, jugular blood from each calf was collected into one 5-mL EDTA tube (BD, Franklin Lakes, NJ), and four 10-mL tubes containing acid citrate dextrose (ACD; BD, Franklin Lakes, NJ). Blood from the EDTA tube was used to determine the counts of monocytes, neutrophils, eosinophils, basophils, lymphocytes,