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Evaluation of breed-dependent differences in the innate immune responses of Holstein and Jersey cows to *Staphylococcus aureus* intramammary infection

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Mastitis is one of the most prevalent diseases of cattle. Various studies have reported breed-dependent differences in the risk for developing this disease. Among two major breeds, Jersey cows have been identified as having a lower prevalence of mastitis than Holstein cows. It is well established that the nature of the initial innate immune response to infection influences the ability of the host to clear harmful bacterial pathogens. Whether differences in the innate immune response to intramammary infections explain, in part, the differential prevalence of mastitis in Holstein and Jersey cows remains unknown. The objective of the current study was to evaluate several parameters of the innate immune response of Holstein and Jersey cows to intramammary infection with *Staphylococcus aureus*, a common mastitis-inducing pathogen. To control for non-breed related factors that could influence these parameters, all cows were of the same parity, in similar stages of milk production, housed and managed under identical conditions, and experimentally infected and sampled in parallel. The following parameters of the innate immune response were evaluated: acute phase protein synthesis of serum amyloid A and lipopolysaccharide-binding protein; total and differential circulating white blood cell counts; milk somatic cell counts; mammary vascular permeability; milk N-acetyl-beta-D-glucosaminidase (NAGase) activity; and production of the cytokines, interferon (IFN)-γ, interleukin (IL)-12, tumour growth factor(TGF)-α, and TGF-β1. The temporal response of all of these parameters following infection was similar between Holstein and Jersey cows. Further, with the exception of changes in circulating neutrophils and NAGase activity, the overall magnitude of these parameters were also comparable. Together, these data demonstrate that the innate immune response of Holstein and Jersey cows to *Staph. aureus* intramammary infection remains highly conserved despite previously reported differences in mastitis prevalence, as well as genotypic and phenotypic traits, that exist between the two breeds.

Keywords: Breed, cytokines, dairy cow, innate immunity, mastitis.

Mastitis is the inflammation of the mammary gland and most commonly occurs in dairy cows as a result of a bacterial infection. This disease has a high prevalence and remains among the most frequently cited reasons for the culling of cows (Esslemont & Kossaibati, 1997; Bascom & Young, 1998). Mastitis is also one of the most costly diseases to the dairy industry and is estimated to result in worldwide economic losses of $35 billion annually (Wellenberg et al. 2002).

Staphylococcus aureus is one of the most prevalent bacteria to cause mastitis (Fox et al. 1995; Wilson et al. 1997). Although *Staph. aureus* commonly establishes a chronic subclinical infection, this pathogen is also a leading cause of clinical mastitis (Wilesmith et al. 1986; Barkema et al. 1998). *Staph. aureus* intramammary

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infections are often refractory to antibiotic therapy (Deluyker et al. 2001; Gillespie et al. 2002) and can persist throughout the lifetime of the animal (Sutra & Poutrel, 1994).

Breed-dependent genetic differences are known to influence the disease resistance of food producing animals (Kelm et al. 2001). In regard to two popular dairy breeds, Jersey cows have been reported to have a lower prevalence of, and risk for, mastitis than Holstein cows (Erb & Martin, 1978; Motie et al. 1985; Morse et al. 1987; Washburn et al. 2002; Dego & Tareke, 2003; Youngerman et al. 2004; Biffa et al. 2005; Berry et al. 2007). Large-scale surveys have also reported that Jersey cows tend to have higher milk somatic cell counts (SCC) than Holstein cows (Sewalem et al. 2006; Paape et al. 2007). Because milk SCC increase dramatically in response to infection and play a protective role, it remains unclear whether the breed differences in SCC reported in these latter studies reflect a differential prevalence of underlying intramammary infection or influence the nature of the response to the infection.

Previous studies have established that differential innate immune responses elicited to bacteria influence the outcome of intramammary infection. Whereas *Escherichia coli* elicits a heightened inflammatory response characteristically leading to its rapid elimination in mid- and late-lactation cows, *Staph. aureus* evokes a more modest response and establishes a chronic infection (Riollet et al. 2000; Bannerman et al. 2004). Induction of a heightened inflammatory response early after *Staph. aureus* infection results in a ten-fold reduction in bacterial recovery from infected glands (Kauf et al. 2007). Thus, differences in the nature of the inflammatory response influence the ability of the host to control intramammary infections. Because breed-dependent differences in the prevalence of mastitis between Holstein and Jersey cows have been reported, we studied whether there were differences in the innate immune response of these breeds to *Staph. aureus* intramammary infection that may explain the differential prevalence of mastitis.

Materials and Methods

Animals

Clinically healthy Holstein and Jersey cows, all of which were in their first lactation, were selected from the USDA National Animal Disease Center herd. The mean (±SE) number of days in milk was comparable between the Holstein (132±12 d) and Jersey (152±10 d) cows (P= 0·2145). None of the cows were pregnant at the time of the study. Experimental infection was induced in the right front quarter of each animal so as to eliminate potential quarter-dependent effects that could arise if different quarters on the various cows were infected. Milk SCC of <200 000 cells/ml and the absence of specific bacterial growth in two independent milk samples collected from these quarters within the 1-week period prior to the study established that these quarters were free of pre-existing infection and mastitis. The use and care of all animals in this study was approved by the USDA National Animal Disease Center's Animal Care and Use Committee.

Experimental intramammary infection

Staph. aureus strain 305 (American Type Culture Collection, Manassas VA, USA), which was originally isolated from a clinical case of mastitis (Newbould, 1977), was used to induce experimental intramammary infection. Preparation of the bacterial inoculum was performed as previously described (Bannerman et al. 2004). On experimental day 0, immediately following the morning milking, the right front quarters of ten Holstein and ten Jersey cows were infused with 3 ml of the inoculum. Plating of an aliquot of the inoculum on blood agar confirmed that each quarter was infused with 68 colony forming units (cfu) of *Staph. aureus*.

Quantification of intramammary Staph. aureus *growth*

Milk samples were aseptically collected from all infused quarters at various time points throughout the study and serially diluted in sterile phosphate-buffered saline. One-hundred microlitres of the resulting dilutions were spread on blood agar plates, the latter of which were subsequently incubated for 24 h at 37 °C. Following incubation, the plates were examined for bacterial growth and colonies enumerated.

Quantification of milk SCC and circulating white blood cells (WBC)

To quantify somatic cells, milk samples were heated to 60 °C for 15 min and subsequently maintained at 40 °C until counted on an automated cell counter (Bentley Somacount 150; Bentley Instruments, Inc., Chaska MN, USA). For the determination of total and differential WBC counts, blood was collected from the jugular vein into Vacutainer® glass tubes containing EDTA (Becton-Dickinson Corp, Franklin Lakes, NJ, USA). The tubes were inverted ten times, and analysed using a HEMAVET® 1500 multi-species haematology system (CDC Technologies/Drew Scientific, Inc., Oxford CT, USA).

Whey and plasma preparation

Milk samples were centrifuged at 44 000 ***g*** at 4 °C for 30 min and the fat layer removed with a spatula. The skimmed milk was decanted into a clean tube, re-centrifuged for 30 min, and the translucent whey supernatant collected and stored at −70 °C. For the preparation of plasma, blood samples were collected as above, inverted

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ten times, centrifuged at 1500 ***g*** for 15 min, and the clear plasma supernatant collected and stored at −70 °C.

Enzyme-linked immunosorbent assays (*ELISAs*)

ELISAs for bovine serum albumin (BSA), interferon (IFN)-γ, interleukin (IL)-12, LPS-binding protein (LBP), serum amyloid A (SAA), transforming growth factor (TGF)-α, and TGF-β1 were all performed as previously described (Bannerman et al. 2003, 2004, 2005, 2006).

Milk N-acetyl-beta-D-glucosaminidase (*NAGase*) *activity assay*

The NAGase activity assay was performed as previously described (Mattila & Sandholm, 1985) with slight modification. Isolated milk whey samples were diluted 1 : 2 in deionized H_2O, and 10 µl of the resulting dilutions were added to the wells of a black, clear bottom 96-well plate. Forty microlitres of substrate solution (2·25 mM-4-methylumbelliferyl N-acetyl-β-D-glucosaminide, 0·25 M-sodium citrate, pH 4·6; Sigma Chemical Co., St. Louis MO, USA) were added to each well and the plate was shaken in the dark at 150 rpm for 15 min at 25 °C. Immediately at the end of the incubation period, 150 µl of stop buffer (0·2 M-glycine, pH 10·8; Sigma Chemical Co.) were added to each well, and the fluorescence measured on a Synergy HT™ multi-modal plate reader (Bio-Tec Instruments, Inc., Winooski VT, USA) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. NAGase activity of the samples was calculated by extrapolation from a standard curve consisting of a series of 1 : 2 dilutions of 200 µM−4-methylumbelliferone (Sigma Chemical Co.) dissolved in stop buffer. To determine the amount of activity (i.e., nmoles/ml per min) the extrapolated values were multiplied by the dilution factor (i.e., 2), multiplied by a factor of 100 to calculate the activity in 1 ml of sample, and the resulting product divided by 15 to calculate the activity per min.

Statistical analysis

Repeated measures ANOVA was performed using SAS PROC MIXED® (SAS Version 9.1.3., SAS Institute, Cary NC, USA) to compare the mean responses of variables to control (time 0) values and to compare breed-dependent differences for a given variable. Milk bacterial counts, SCC, and IFN-γ concentrations were transformed to $\log_{10}$ values to satisfy distributional requirements of ANOVA. Correlations among repeated measurements across time within cows and between breeds were modelled using appropriate covariance structures for each parameter analysed. The Spearman rank correlation coefficient (r_s) for the relationship between mean $\log_{10}$ SCC and mean NAGase values was calculated using GraphPad InStat® (Version 3.06, GraphPad Software, Inc., San Diego CA, USA). A *P*-value of <0·05 was considered significant.

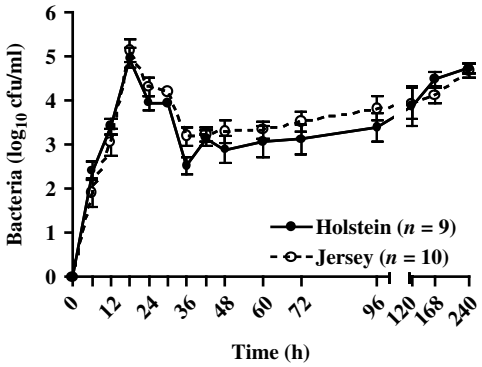

Fig. 1. Recovery of *Staph. aureus* from experimentally infected quarters. Sixty-eight colony forming units (cfu) of *Staph. aureus* were infused into one mammary quarter of 9 Holstein and 10 Jersey cows. Milk samples, which were aseptically collected immediately prior to (time 0) and at various times after infusion, were spread on blood agar plates. Following overnight incubation of the plates, bacterial colonies were enumerated. In those quarters where *Staph. aureus* were recovered, the mean (±SE) $\log_{10}$ milk bacterial concentration is reported in cfu/ml.

Results

Recovery of Staph. aureus *from experimentally-infected quarters*

The right front quarters of ten Holstein and ten Jersey cows were infused with 68 cfu of *Staph. aureus*. One of the Holstein cows developed a mixed infection in the infused quarter, which was characterized by the recovery of more than one genus of bacteria. The colonies that grew on plated milk samples collected from this cow differed morphologically from those of the plated *Staph. aureus* infusate, as well as the plated samples from the other experimentally infected cows that were confirmed to have a *Staph. aureus* intramammary infection. Further, none of the colonies isolated from the cow with the mixed infection demonstrated the characteristic haemolysis of *Staph. aureus*. Therefore, because an intramammary infection other than *Staph. aureus* developed, the cow was dropped from the study. For the cows that remained in the study, *Staph. aureus* was recovered from six of nine and seven of ten infused Holstein and Jersey quarters, respectively, within 6 h of infusion. From 18 to 120 h after infusion, *Staph. aureus* was recovered from 100% of the infused quarters of both breeds. Milk samples obtained from one Holstein and one Jersey cow at both 168 h and 240 h after infusion were free of *Staph. aureus*. Thus, only one cow of each breed was able to successfully clear *Staph. aureus* during the experimental period. Maximal mean (±SE) milk concentrations of *Staph. aureus* were detected 18 h after infusion in both Holstein (4·97±0·23 $\log_{10}$ cfu/ml) and Jersey (5·14±0·27 $\log_{10}$ cfu/ml) cows, and overall concentrations in the infected quarters were equivalent (*P*=0·3741) between breeds throughout the study (Fig. 1).

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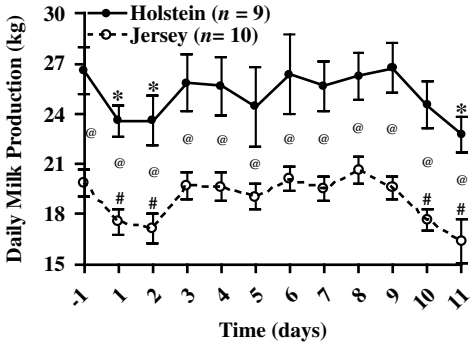

Fig. 2. Changes in milk production following *Staph. aureus* intramammary infection. Total daily milk weights were recorded prior to (day −1) and following (days 1–11) experimental *Staph. aureus* intramammary infection of Holstein and Jersey cows. Mean (±SE) daily milk production is reported in kg.

*,#Decreased ($P<0{\cdot}05$) compared with pre-infection (day −1) production amounts of Holstein or Jersey cows, respectively.
@Differences ($P<0{\cdot}05$) in milk production between breeds at a given time point.

Holstein and Jersey cow milk production following Staph. aureus *intramammary infection*

Daily milk production, defined as the sum of the morning and evening milk weights, was recorded prior to and for several days following intramammary infusion of *Staph. aureus* (Fig. 2). Mean (±SE) pre-infection (day −1) milk production was higher ($P=0{\cdot}0007$) in Holstein cows (26·56±1·42 kg) than in Jersey cows (19·87±0·84 kg), as was overall milk production throughout the experimental period ($P=0{\cdot}0003$). Relative to pre-infection amounts, milk production was significantly lower in both breeds for the first 2 d after infection. Within 3 d of infection, the milk production of both breeds returned to levels comparable to those observed prior to infection. By day 10 and 11, respectively, decreased milk production was once again detected in the Jersey (17·69±0·62 kg; $P=0{\cdot}0005$) and Holstein (22·78±1·06 kg; $P=0{\cdot}0150$) cows relative to pre-infection (day −1) production levels.

Systemic responses of Holstein and Jersey cows to Staph. aureus *intramammary infection*

To determine whether there were breed-dependent differences in the systemic response to *Staph. aureus* intramammary infection, changes in body temperature, acute phase protein synthesis and differential WBC counts were evaluated. Fever is generally defined as a >1–1·5 °C increase in body temperature (Cotran et al. 1999; Ryan & Levy, 2003), and in cattle, has been characteristically defined as body temperatures that exceed 39·5 °C (Drillich et al. 2006). Throughout this study, the mean body temperatures of either breed did not fluctuate by ⩾0·6 °C from basal (pre-infection) body temperatures nor did they exceed 39 °C (data not shown). Thus, *Staph. aureus* failed

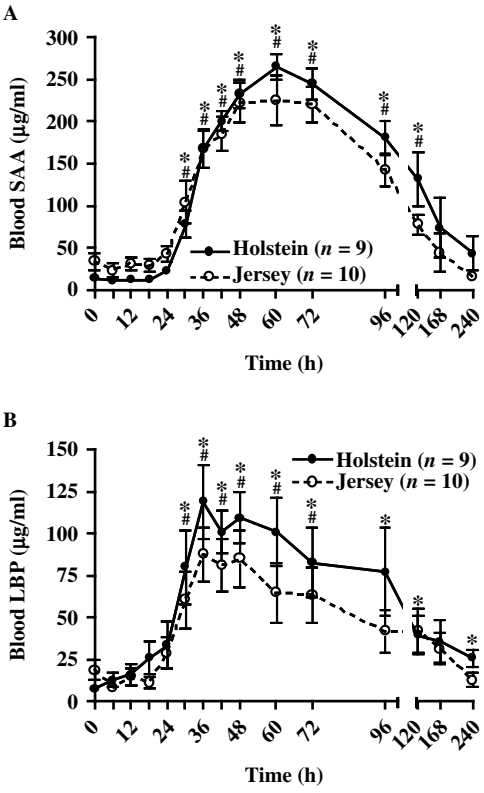

Fig. 3. Induction of acute phase protein synthesis following intramammary *Staph. aureus* infection. Blood samples were collected immediately before (time 0) and at various time points after intramammary infusion of *Staph. aureus* into Holstein and Jersey cows. Plasma derived from the blood samples was assayed for serum amyloid A (SAA; A) and lipopolysaccharide-binding protein (LBP; B). Mean (±SE) concentrations of SAA and LBP are reported in µg/ml.

*,#Increased ($P<0{\cdot}05$) compared with pre-infection (time 0) measurements in Holstein or Jersey cows, respectively.

to elicit a febrile response in either Holstein or Jersey cows.

In contrast to the absence of an effect on body temperature, intramammary infection with *Staph. aureus* did evoke the synthesis of the acute phase proteins, SAA and LBP. From 30 to 120 h after infection, increased circulating blood concentrations of SAA were detected in Holstein and Jersey cows (Fig. 3A). In both breeds, maximal increases in SAA were observed 60 h after infection when mean (±SE) concentrations reached 265±15 and 225±30 µg/ml, respectively, in Holstein and Jersey cows. The overall SAA response, as well as the maximal concentrations detected, were comparable between the two breeds ($P=0{\cdot}5192$). Similarly to SAA, increases in circulating LBP concentrations were detected within 30 h of infection (Fig. 3B). Maximal mean (±SE) increases in LBP were detected in both Holstein (119±22 µg/ml) and Jersey (88±16 µg/ml) cows within 6 h of the initial increase. Both

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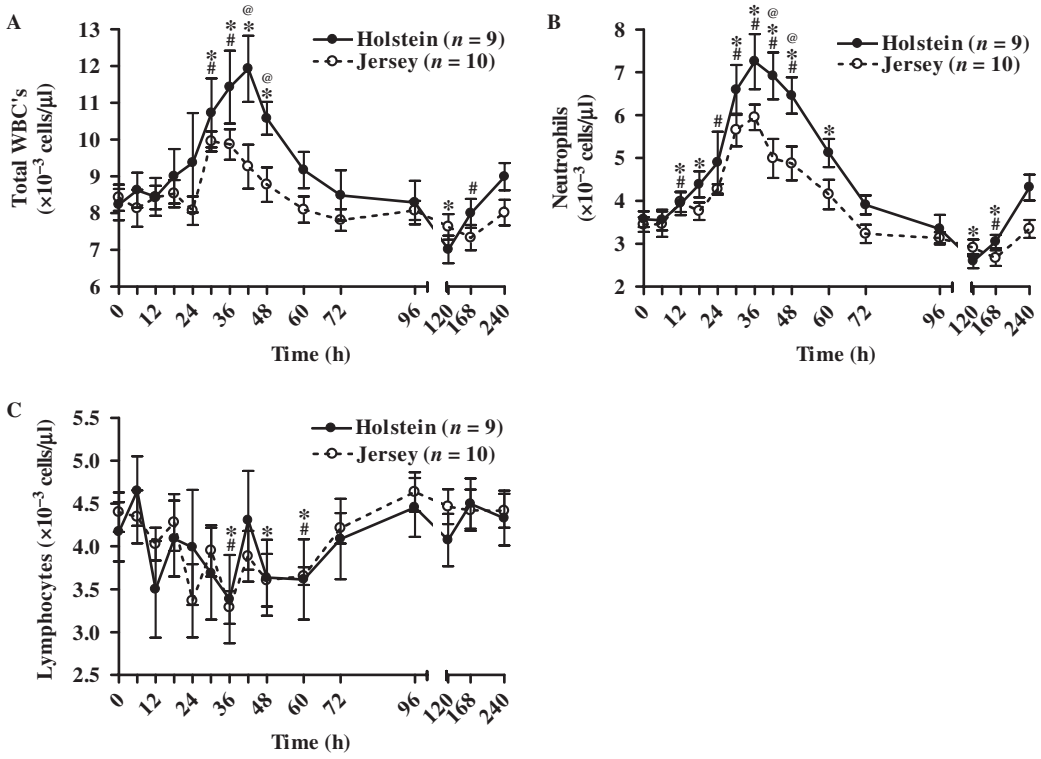

Fig. 4. Alterations in circulating white blood cell (WBC) counts following intramammary *Staph. aureus* infection. Blood samples were collected immediately before (time 0) and at various time points following intramammary infusion of *Staph. aureus* into Holstein and Jersey cows. Blood was analysed for total (A) and differential WBC counts (B, C). Mean (±SE) cell counts are reported in thousands/µl.

*,#Significantly different ($P<0{\cdot}05$) than pre-infection (time 0) cell counts of Holstein or Jersey cows, respectively.
@Differences ($P<0{\cdot}05$) in cell counts between breeds at time points where cell counts differed from those at time 0 in one or both breeds.

the maximal concentrations ($P=0{\cdot}2537$) and the magnitude of the overall LBP response ($P=0{\cdot}2072$) were comparable between the two breeds. Temporal differences existed, however, as LBP concentrations remained elevated, relative to pre-infection (time 0) levels, for 168 h longer in the Holstein cows.

Changes in the total and differential circulating WBC counts were evaluated as another aspect of the systemic response to *Staph. aureus* intramammary infection. For both breeds, a transient leucocytosis was observed within 30 h of infection (Fig. 4A). Relative to pre-infection (time 0) counts, the duration of leucocytosis was greater in Holstein cows, and the overall magnitude of the response observed in these cows approached a level ($P=0{\cdot}0921$) that significantly differed from that of Jersey cows. Analysis of differential WBC counts revealed a sustained increase in circulating neutrophils (Fig. 4B) and highly variable fluctuations in circulating lymphocytes (Fig. 4C) following infection. Although there were no breed-dependent difference in pre-infection neutrophil counts, the magnitude of the overall neutrophil response that accompanied infection was higher in Holstein than in Jersey cows ($P=0{\cdot}016$). In contrast, there were no breed-dependent differences in the overall lymphocyte response ($P=0{\cdot}881$).

Localized inflammatory responses of Holstein and Jersey cows to Staph. aureus *intramammary infection*

As a local indicator of inflammation, milk SCC were enumerated prior to and after infection (Fig. 5). Pre-infection (time 0) milk SCC were equivalent ($P=0{\cdot}4877$) between Holstein (11 333 ± 1869 cells/ml) and Jersey cows (14 250 ± 2923 cells/ml). In both breeds, transient increases in milk SCC were observed 6 h after infection and sustained increases detected from 18 h after infection until the end of the study. Maximal mean (±SE) SCC were detected in both the Holstein ($28{\cdot}23 \times 10^6 \pm 5{\cdot}43 \times 10^6$ cells/ml) and Jersey cows ($34{\cdot}06 \times 10^6 \pm 6{\cdot}33 \times 10^6$ cells/ml) at 42 h after infection and did not statistically differ ($P=0{\cdot}5813$). The overall SCC responses of the two breeds were similar ($P=0{\cdot}2116$) although SCC in Holstein cows exceeded those in Jersey cows at 96 h and 240 h after infection.

Two other indicators of local inflammation, increases in mammary vascular permeability and NAGase activity, were also evaluated. Initial increases in mammary vascular permeability, as evidenced by increased milk concentrations of BSA, were detected in both breeds at 30 h after *Staph. aureus* intramammary infection (Fig. 6A). Similarly, initial increases in NAGase activity over pre-infection

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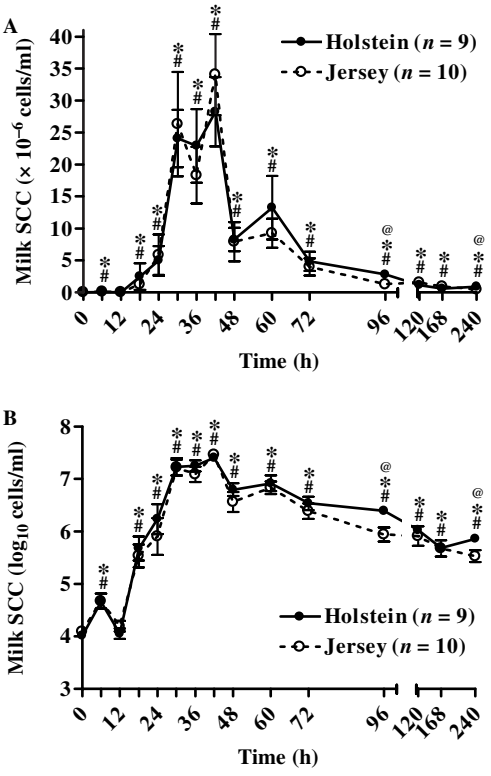

Fig. 5. Changes in milk somatic cell counts (SCC) following intramammary *Staph. aureus* infection. SCC were enumerated in milk samples collected immediately before (time 0) and at various time points following intramammary infusion of *Staph. aureus* into Holstein and Jersey cows. Mean (±SE) milk SCC are reported in millions of cells/ml (A) and $\log_{10}$ cells/ml (B).

*,#Increased ($P<0{\cdot}05$) compared with pre-infection (time 0) concentrations in Holstein or Jersey cows, respectively.
@Differences ($P<0{\cdot}05$) in SCC between breeds at time points where SCC differed from those at time 0 in one or both breeds.

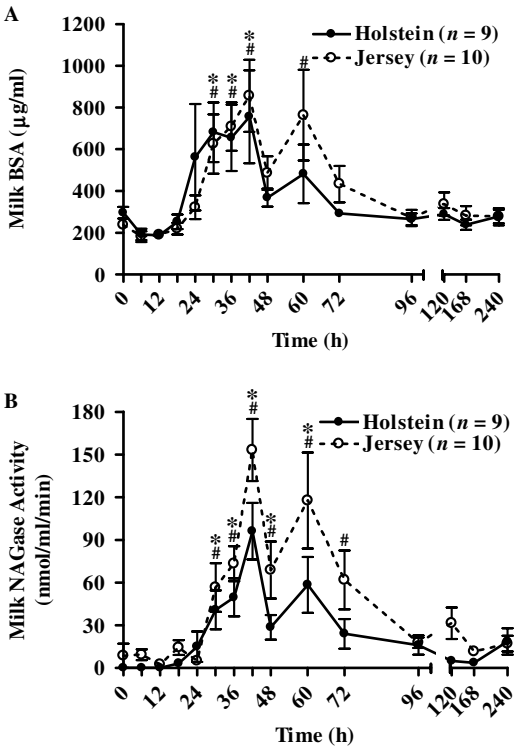

Fig. 6. Effect of *Staph. aureus* intramammary infection on milk bovine serum albumin (BSA) concentrations and N-acetyl-beta-D-glucosaminidase (NAGase) activity. Milk samples were collected immediately before (time 0) and at various time points after experimental *Staph. aureus* intramammary infection of Holstein and Jersey cows. Milk whey was assayed for BSA (A) and NAGase activity (B). Mean (±SE) BSA concentrations are indicated in µg/ml and mean (±SE) NAGase activity reported in nmol/ml/min.

*,#Increased ($P<0{\cdot}05$) compared with pre-infection (time 0) concentrations in Holstein or Jersey cows, respectively.

(time 0) levels were detected in both breeds at 30 h after infection (Fig. 6B). Both parameters of inflammation remained elevated, relative to pre-infection levels, for longer durations in Jersey cows. The overall change in milk concentrations of BSA in response to infection were comparable ($P=0{\cdot}5830$) between the two breeds, whereas, overall NAGase activity was higher ($P=0{\cdot}0082$) in Jersey cows than in Holstein cows. A significant correlation between SCC and NAGase was identified in both Jersey ($r=0{\cdot}8821$; $P<0{\cdot}0001$) and Holstein ($r_s=0{\cdot}9642$; $P<0{\cdot}0001$) cows consistent with reports that NAGase is an indicator of cell injury, and that high SCC are associated with mammary tissue injury (Kitchen et al. 1978, 1980; Capuco et al. 1986).

Cytokine response of Holstein and Jersey cows to Staph. aureus *intramammary infection*

To evaluate breed-dependent differences in the cytokine response to *Staph. aureus* intramammary infection, cytokines known to be up-regulated in response to this pathogen (Bannerman et al. 2004, 2006) were evaluated in milk samples collected prior to and following infection. Relative to pre-infection (time 0) concentrations, initial increases in the type 1 helper T cell (Th_1-Type) cytokines, IFN-γ and IL-12, were detected at 30 h after infection in both breeds (Fig. 7A and B). Maximal mean (±SE) concentrations of IFN-γ were detected in Jersey (961±260 pg/ml) and Holstein (1830±903 pg/ml) cows at 42 h after infection and were higher in the latter ($P=0{\cdot}0165$). In contrast, the maximal concentrations of IL-12, which were detected 30 h after infection, were comparable between the two breeds ($P=0{\cdot}5081$). The duration of the increase of each cytokine was similar between breeds as was the overall magnitude of the IFN-γ ($P=0{\cdot}8585$) and IL-12 ($P=0{\cdot}3369$) response.

Within 6 h of infection, transient increases in milk concentrations of TGF-α and TGF-β1 were detected in Jersey, but not in Holstein cows (Fig. 7C and D). Initial

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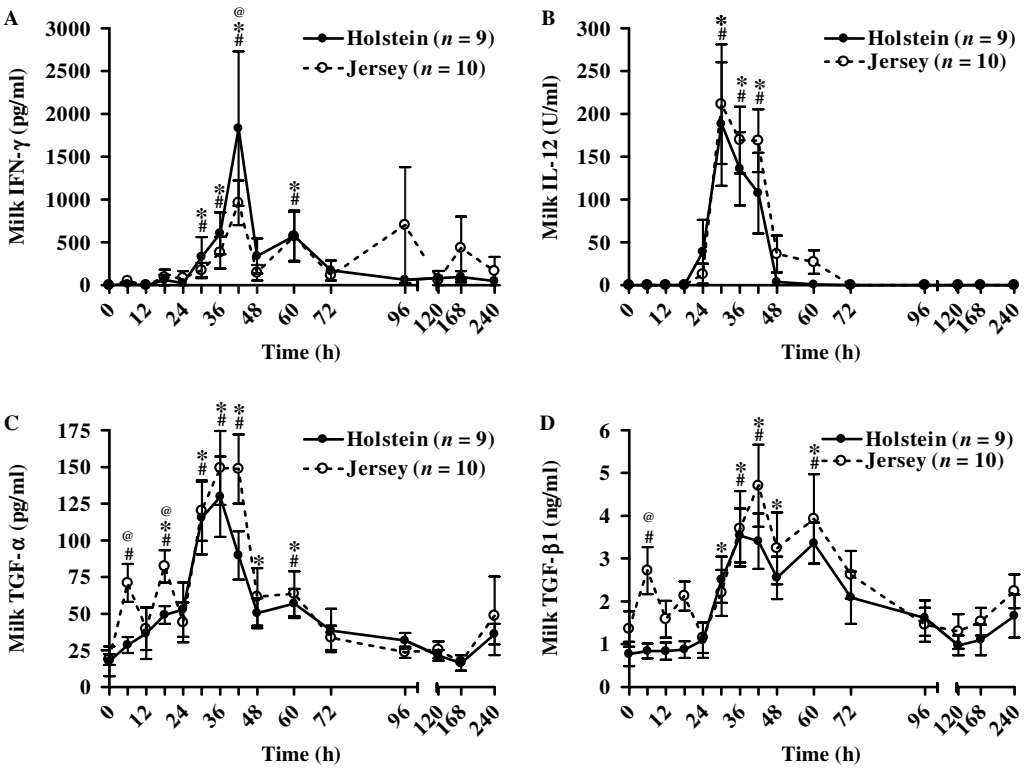

Fig. 7. Cytokine response following intramammary *Staph. aureus* infection. Milk samples were collected immediately before (time 0) and at various time points following intramammary infusion of *Staph. aureus* into Holstein and Jersey cows. Milk whey was assayed for interferon (IFN)-γ (A), interleukin (IL)-12 (B), transforming growth factor (TGF)-α (C), and TGF-β1 (D). Mean (±SE) concentrations of IFN-γ and TGF-α are expressed in pg/ml; mean (±SE) concentrations of IL-12 are expressed in units (U)/ml; and mean (±SE) TGF-β1 concentrations are reported in ng/ml.

*,#Increased ($P<0{\cdot}05$) compared with pre-infection (time 0) concentrations in Holstein or Jersey cows, respectively.
@Differences ($P<0{\cdot}05$) in concentrations between breeds at time points where concentrations differed from those at time 0 in one or both breeds.

sustained increases in TGF-α were observed in both breeds at 30 h after infection and increases were detected as late as 60 h post-infection. Maximal mean (±SE) concentrations were detected at 36 h post-infection and were comparable between Holstein (130±27 pg/ml) and Jersey (149±25 pg/ml) cows. Similarly to TGF-α, elevated TGF-β1 concentrations were detected in both breeds as late as 60 h after infection and the maximum concentrations detected were comparable. There was no overall difference in the magnitude of the TGF-α ($P=0{\cdot}1850$) or TGF-β1 ($P=0{\cdot}1779$) response between the two breeds.

Discussion

The innate immune responses of Holstein and Jersey cows during *Staph. aureus*-induced mastitis were evaluated in the current study. In contrast to other parameters (e.g, milk production, milk composition, longevity, conception rates, etc.), there are few published reports comparing breed-dependent immune responses to intramammary infection in a controlled manner. A previous study investigated the response of Holstein and Jersey cows to *Escherichia coli* intramammary infection following immunization, however, because only three Jersey cows were included in that study, breed-dependent differences were not examined (Todhunter et al. 1991). Another study investigated the innate immune response of a group of five Holstein and five Jersey cows during experimentally induced *Streptococcus uberis* mastitis (Rambeaud et al. 2003). The main objective of that study did not focus on investigating breed-dependent differences and the investigators presented combined data for all cows. In a subanalysis of the data, the investigators reported no significant differences between the two breeds in the innate immune parameters evaluated. Several factors were not controlled in that study, however, including: (1) the bacterial inoculum preparation used to infect the two breeds differed by ~60%; (2) each breed was housed on a different farm and subjected to different management practices; and (3) the breeds were infected, sampled, and monitored at different times of the year. In contrast, the current study was designed to control for these factors by housing all cows in a single barn and subjecting them to the same management

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practices, infecting the same quarter on each cow with the same preparation and amount of bacterial challenge inoculum, and infecting and sampling all cows in parallel. A larger sample size was used in the current study and several additional innate immune responses were assessed, including: induction of acute phase protein synthesis; changes in total and circulating WBC; and induction of the cytokines IFN-γ, IL-12, TGF-α, and TGF-β1. Because innate immune responses to intramammary infection can be influenced by parity (Gilbert et al. 1993; Van Werven et al. 1997; Mehrzad et al. 2002) and stage of lactation (Lescourret & Coulon, 1994; Shuster et al. 1996; Vangroenweghe et al. 2005), only cows of the same parity and in similar stages of lactation were evaluated.

With the exception of changes in circulating neutrophils and NAGase activity, the overall magnitude of all other innate immune responses was comparable between the two breeds. Although Holstein cows demonstrated an increased number of circulating neutrophils after infection relative to Jersey cows, the overall milk SCC response was equivalent between the breeds. Because neutrophils compose >90% of milk somatic cells during acute mastitis (Saad & Ostensson, 1990) it is interesting that the elevated number of circulating neutrophils did not correspond with an overall SCC response that was higher in Holstein cows. One may speculate that rate-limiting steps, such as adhesion molecule expression and transendothelial migration, limit the overall number of neutrophils that can be recruited to the mammary gland. Alternatively, cytokines and lipid mediators of inflammation with chemoattractant properties may have been produced in equivalent amounts in the two breeds, similarly to the other cytokines that were measured. The increased circulating numbers of neutrophils did not appear to confer an advantage to Holstein cows as the overall number of quarters infected and the bacterial concentrations within these quarters were comparable between breeds.

In contrast to cytokines such as TNF-α and IL-8, intramammary infection with *Staph. aureus* has been shown to induce the production of IL-12 and IFN-γ (Bannerman et al. 2004). These cytokines contribute to the immunological response of the host by activating neutrophils and macrophages and promoting a T_h1-type immune response (Trinchieri, 1997). The finding that both IFN-γ and IL-12 increased at the same time (i.e., 30 h after infection) in both breeds is consistent with reports that these cytokines positively regulate the production of each other (Collins et al. 1998; Munder et al. 1998; Ma, 2001). The overall concentrations of these cytokines in the milk of the two breeds after *Staph. aureus* intramammary infection were comparable with those reported in a previous study (Bannerman et al. 2004). Similarly to IFN-γ and IL-12, the concentrations of TGF-α and TGF-β1 have been reported to increase in milk following *Staph. aureus* intramammary infection. TGF-α and TGF-β1 are pleiotropic cytokines that are structurally and functionally distinct from one another. TGF-α has pro-inflammatory properties that are conferred by its ability to up-regulate the production of prostaglandins and synergistically enhance the effects of other pro-inflammatory cytokines (Bry, 1993; Unemori et al. 1994; Subauste & Proud, 2001). In contrast, TGF-β1 suppresses inflammation in a variety of disease states (Johns et al. 1991; Santambrogio et al. 1993; Neurath et al. 1996; Powrie et al. 1996; Williams et al. 2005) and it is believed that its anti-inflammatory properties are partly governed by its ability to down-regulate the pro-inflammatory responses of macrophages and other cell types (Ayoub & Yang, 1997; Letterio & Roberts, 1998; Ashcroft, 1999). Both TGF-α and TGF-β1 are expressed in the healthy mammary gland and the relative increases in the expression of these cytokines following *Staph. aureus* intramammary infection are comparable with a previous report (Bannerman et al. 2006). Although it was beyond the scope of the this study to perform an exhaustive evaluation of all cytokines known to influence the inflammatory response, the finding that the overall production of these four cytokines were equivalent across the two breeds precludes that their differential induction is responsible for the previously reported differences in mastitis susceptibility between Holstein and Jersey cows.

In addition to the evaluation of cytokine production, two markers of the systemic acute phase response, SAA and LBP, and two markers of localized inflammation, BSA and NAGase, were evaluated. Similarly to the cytokine responses, overall changes in the circulating concentrations of the acute phase proteins were comparable across breeds. The temporal expression of LBP from 30–72 h after *Staph. aureus* intramammary infection is consistent with a previous report (Bannerman et al. 2004). Further, the concentrations of circulating SAA induced by *Staph. aureus* were comparable with other studies (Gronlund et al. 2003; Eckersall et al. 2006). The temporal change in milk BSA concentrations, as well as the magnitude of the change, were consistent with a previous report (Bannerman et al. 2006). Similarly to LBP and SAA, overall changes in BSA were comparable across breeds. In contrast, milk NAGase activity was significantly higher in the infected quarters of Jersey cows relative to those of Holstein cows. Increased NAGase activity is an indicator of mammary tissue injury and elevated SCC are associated with mammary tissue injury (Bogin & Ziv, 1973; Kitchen et al. 1978, 1980, 1984; Mattila et al. 1986; Chagunda et al. 2006). Correspondingly, a significant correlation between SCC and NAGase was identified in both breeds. Interestingly, however, there were no overall differences in the SCC response between the two breeds. Thus, if the higher level of NAGase activity in the milk of Jersey cows is truly representative of greater mammary tissue injury, then factors other than elevations in SCC must be operative in the induction of this injury.

The risk of clinical mastitis is positively correlated with milk production (Oltenacu & Ekesbo, 1994; Waage et al. 1998; Fleischer et al. 2001). Despite the fact that milk

production was higher in Holstein than in Jersey cows, all cows developed an intramammary infection with *Staph. aureus* that was sustained for several days. This finding is consistent with a previous study reporting that the severity of the inflammatory response to intramammary infection is not affected by differences in milk production within a given breed (Kornalijnslijper et al. 2003). Thus, the correlation between risk of clinical mastitis and milk production may be related to differences in susceptibility to initial infection rather than differences in the response to infection.

To our knowledge, the present report is the first controlled study to compare the innate immune responses of Holstein and Jersey cows to *Staph. aureus* intramammary infection. Overall, the magnitude, temporal onset, and duration of these responses were highly conserved between the two breeds. Because innate immunity encompasses evolutionarily ancient and highly conserved host defence mechanisms (Hoffmann et al. 1999; Janeway & Medzhitov, 2002) perhaps it is not surprising that Holstein and Jersey cow innate immune responses to intramammary infection are similar despite the genetic and phenotypic differences that exist between the two breeds.

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