GENETICS AND BREEDING

Genetic Association Between Parameters of Innate Immunity and Measures of Mastitis in Periparturient Holstein Cattle[1]

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ABSTRACT

Relationships between genetic measures of mastitis (somatic cell score, score for clinical mastitis, and scores for IMI with major or minor pathogens) and immunological parameters (physiological and molecular markers) were examined for periparturient Holstein cows. Physiological markers included 11 in vitro immunological assays. Molecular markers included the second exon of the DRB3 locus of the bovine major histocompatibility complex, the IgG2 isotype genotype, and the CD18 genotype (the locus responsible for bovine leukocyte adhesion deficiency). A gene substitution model was used to estimate the additive genetic effects of alleles of the three molecular markers on estimated breeding value (EBV) for mastitis measures. Pearson correlation coefficients between EBV for immunological assays and EBV for mastitis measures were computed. Molecular markers explained up to 40% of the variation in EBV for measures of mastitis. The presence of allele *DRB3.2*16* was associated with higher EBV for SCS. Allele *DRB3.2*8* was associated with increased EBV for clinical mastitis, as was the $IgG2^b$ allele and the normal CD18 allele. Alleles *DRB3.2*11*, **23*, $IgG2^a$, and the recessive allele for bovine leukocyte adhesion deficiency were associated with decreased clinical mastitis. A positive genetic association was found between allele *DRB3.2*24* and EBV for IMI by major pathogens and between *DRB3.2*3* and IMI by minor pathogens. Several correlations between EBV for immunological assays and EBV for mastitis measures were significantly different from 0. Cows with low EBV for SCS tended to have neutrophils that had greater functional ability at maximal immunosuppression, low serum IgG_1, and high numbers of circulating mononuclear cells. Immunological parameters, including physiological and molecular markers, are useful aids to understand the genetics of resistance to mastitis.

(**Key words**: genetic, mastitis, immunology, cattle)

Abbreviation key: **BLAD** = bovine leukocyte adhesion deficiency, **BoLA** = bovine major histocompatibility complex, **CM** = clinical mastitis, **IR** = immune response, **MHC** = major histocompatibility complex, **RFLP** = restriction fragment length polymorphism.

INTRODUCTION

Animal health is one of many factors that affect the economic efficiency of a dairy herd (31). A large variety of mechanisms exist to protect an individual from the harmful effects of pathogenic organisms. Innate resistance to infectious diseases reflects the physiological attributes of an animal that make it more or less susceptible to the development of disease caused by a particular pathogen. Genes governing the immune response (**IR**) to an antigen may be classified into those that control the quality and quantity of the innate IR (such as genes encoding nonspecific opsonins, receptors and enzymes involved in phagocytosis) and those that control the specificity of the adaptive IR (such as major histocompatibility complex (**MHC**), T-cell receptor, and Ig genes).

Suppression of several aspects of the immune system has been observed in periparturient cattle, and the most severe immunosuppression occurred near parturition (5, 7, 17, 25, 27). Neutrophil function has been shown to be impaired at this time (5, 7, 25, 27) as has lymphocyte function (7, 17). Genetic variation

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in IR differed before, during, and after immunosuppression (8); thus, genetic mechanisms of control may be different during each time period.

Immune assays can be used to measure aspects of the quality and quantity of innate IR. These assays are in vitro correlates of in vivo immune functions, and genetic variation in these assays has been reported (22). The EBV for these immunological assays collectively represent the additive effects of all genes involved in the effector functions of immune cells.

Molecular DNA markers may be used to identify the haplotypes that are involved with the specificity of the IR. Alleles at numerous loci within the bovine MHC have been associated with various diseases, including mastitis (1, 26). Previous studies found that serotype *w14(w18)* of Class I MHC was associated with decreased California Mastitis Test scores for quarter milk samples and decreased California Mastitis Test scores and Wisconsin Mastitis Test scores for composite milk samples (37). Serotype *w11* was associated with decreased clinical mastitis (**CM**), discarded milk, and udder health costs (37). Similarly, Aarestrup et al. (1) found that Class I allele *A11* (*w11*) was associated with lowered SCS. Other studies have found class I serotypes *w16* and *w6* associated with increased susceptibility to mastitis (33), and Class II DQ^{1A} restriction fragment length polymorphisms (**RFLP**) associated with a low EBV for CM (24).

Unlike laboratory animals, markers for genetic variants of Ig for cattle have rarely been defined. However, allotypic variants ($IgG2^a$ and $IgG2^b$) can be distinguished on the Fc portion of the bovine IgG2 molecule (14, 19). Bovine IgG2 constitutes one-half of the total serum IgG and is an important opsonin in phagocytosis (14).

Cells of the immune system work in concert with accessory cells to activate the host response to infection. Three families of adhesion receptors mediate these interactions: the Ig superfamily, the integrin family, and the selectins (34). A genetic disorder of one family of integrins has been described for cattle (21). The disorder causes bovine leukocyte adhesion deficiency (**BLAD**) and is due to a mutation of the β-subunit (CD18) of the β_2 integrin family of adhesion molecules (32). The effects of this allele on the health of heterozygous carriers have not previously been examined.

The main objective of this study was to measure the genetic association between measures of mastitis prevalence and genotypes for the MHC class II DRB3 locus, the IgG2 locus, and the CD18 mutation responsible for BLAD. Additionally, we investigated genetic association between measures of mastitis prevalence and 11 in vitro immunological assays measuring the functional capability of the IR.

MATERIALS AND METHODS

Cows and Experimental Design

Periparturient Holstein cows from the Iowa State University breeding herd were used in this study. Cows calving between May 1, 1990 and September 30, 1991 (n = 137) were available for use. All cows were managed identically and fed for high production. For immunological assays, weekly blood samples were collected starting 35 d before calving until 35 d after calving. Cows that were known to be twins were excluded from the study to avoid using leukochimeric cows. Sires had, on average, 3 daughters (range 1 to 13) included in the study. Five steers were used as laboratory standards to reduce the daily variability that is typical for assays of immune cell function.

Immunological Markers

Molecular markers. Class II alleles of the bovine MHC (**BoLA**) were assigned by a polymerase chain reaction-RFLP method (36). Genomic DNA was amplified with primers specific for exon 2 of the BoLA DRB3 locus. The reaction products were digested in three separate reactions with the restriction endonucleases *Rsa*I, *Hae*III, and *Bst*YI. After nondenaturing acrylamide gel electrophoresis of digested DNA, the gels were stained with ethidium bromide, photographed, and used to genotype the cows as described (36). Data from cows with alleles found at frequencies less than 2% were pooled and denoted as "other".

Immunodiffusion analysis was conducted to determine the IgG2 isotypic subclass genotype of the cows using antisera against the whole IgG_2 molecule or its Fc fragment (14).

To genotype the mutation responsible for BLAD, genomic DNA was isolated from peripheral blood leukocytes, amplified, and subjected to restriction endonuclease digestion by *Taq*I or *Hae*III. The mutation was detected by the presence or absence of different RFLP patterns that were visualized under ultraviolet light in agarose gels containing ethidium bromide (32).

Immunological assays. In vitro immunological assays were performed as described (7, 21). These assays were neutrophil migration under agarose, neutrophil phagocytosis-associated native chemiluminescence, neutrophil cytochrome *c* reduction, neutrophil

myeloperoxidase-catalyzed iodination, neutrophil ingestion of bacteria, and lymphocyte blastogenic response to the mitogen concanavalin A. Serum Ig (IgG1, IgG2, and IgM) concentrations and leukograms were also determined.

Data obtained from each immunological assay were used to calculate EBV for individual cows. Raw data from each assay were handled in five stages: 1) individual cow results were expressed as a ratio of laboratory standards (i.e., the percentage of the average value of steers for each sample day) to account for the variability inherent to neutrophil and lymphocyte assays; 2) a $\log_{10}$ transformation was applied to these ratios, and a square root transformation was applied to the number of mononuclear cells to approximate a normal distribution; 3) a nonlinear regression model was applied to the adjusted values to describe the changes observed in the values near calving, and the three extremes of the nonlinear curve were retained: the maximum before, the minimum during, and the maximum after immunosuppression at parturition; 4) for each assay separately, animal models including parity and season of calving as fixed effects were used to obtain REML estimates of heritability for the maxima before and after immunosuppression and for the minimum at immunosuppression; and 5) EBV for each cow were computed for assays with heritability estimates different from 0 [Table 1, adapted from (8)].

Mastitis Measures

For each cow and each lactation, measures of mastitis prevalence were SCS, number of quarters with CM, and scores for cultural evidence of IMI by major and minor pathogens. The SCS were obtained from DHIA. For cows born between 1985 and 1991, CM incidence and monthly SCS were recorded during the cumulative life of the cow. Bacteria that were isolated from culture were categorized as major pathogens (all streptococci, *Staphylococcus aureus*, coagulase-positive staphylococci other than *S. aureus*, and coliforms), and minor pathogens (coagulase-negative staphylococci and *Corynebacterium bovis*). The IMI status was determined with samples collected in duplicate from each quarter of each cow 35 d prepartum, at parturition, and 35 d postpartum. In addition, if a cow developed CM during this same period, duplicate quarter milk samples were collected from all four quarters for microbiological evaluation. Scores for IMI by major pathogens were defined as the number of quarters that were infected with major pathogens or the number of times the same quarter was infected with major pathogens during the sampling period. Similar scores were created for IMI by minor pathogens (6).

Animal models that included environmental effects influencing the occurrence of IMI were used to obtain REML estimates of heritability for IMI scores as

TABLE 1. The REML estimates of heritability and approximate standard errors for immunological assays before, during, and after immunosuppression.

Immunological assays	Week[1]	Periparturient immunosuppression					
		Before		During		After	
		$\hat{h}^2$	SE	$\hat{h}^2$	SE	$\hat{h}^2$	SE
Serum concentration							
IgM[2]	–2, 0, +2	0.74	0.30	0.55	0.29	0.85	0.33
IgG_1[2]	–3, –1, +3	0.00		0.29	0.23	0.43	0.27
IgG_2[2]	–2, 0, +3	0.25	0.26	0.63	0.23	0.24	0.26
Neutrophil							
Random migration[3]	–2, –1, +2	0.30	0.30	0.00		0.47	0.47
Ingestion[3]	–2, 0, +3	0.26	0.32	0.00		0.27	0.42
Cytochrome *c* reduction[3]	–2, 0, +3	0.22	0.31	0.88	0.35	0.99	0.22
Iodination[3]	–2, 0, +2	0.51	0.38	0.25	0.20	0.00	
Resting chemiluminescence[3]	–2, +1, +3	0.53	0.36	0.19	0.35	0.00	
Stimulated chemiluminescence[3]	–2, +1, +3	0.00		0.71	0.37	0.30	0.56
Lymphocyte blastogenesis[3,4]	–2, 0, +3	0.16	0.30	0.40	0.31	0.01	0.32
Number of blood monocytes[5]	–2, 0, +3	0.10	0.27	0.40	0.38	0.07	0.36

[1]Week when maximum values were observed before immunosuppression, minimum at maximal immunosuppression, and maximum after immunosuppression.

[2]Logarithm$_{10}$ concentration in nanograms per milliliter of serum.

[3]Logarithm$_{10}$ ratio of laboratory standards.

[4]Blastogenic response to the mitogen concanavalin A.

[5]Square root of number of cells per milliliter of blood.

TABLE 2. Total number of individuals for which EBV were calculated for mastitis measures and immunological assays before, during, and after immunosuppression.

Trait	n	Trait	n
SCS	104	CM	72
Major IMI	73	Minor IMI	73
Immunological assays before immunosuppression			
Random migration	101	IgG_1	NA
Ingestion	90	IgG_2	96
Cytochrome *c* reduction	105	IgM	94
Iodination	100	Concanavalin A	45
Chemiluminescence		Mononuclear cells	80
Resting	107		
Stimulated	NA[1]		
Immunological assays during immunosuppression			
Random migration	NA	IgG_1	119
Ingestion	NA	IgG_2	110
Cytochrome *c* reduction	116	IgM	118
Iodination	119	Concanavalin A	114
Chemiluminescence		Mononuclear cells	109
Resting	109		
Stimulated	91		
Immunological assays after immunosuppression			
Random migration	88	IgG_1	101
Ingestion	96	IgG_2	103
Cytochrome *c* reduction	106	IgM	101
Iodination	NA	Concanavalin A	40
Chemiluminescence		Mononuclear cells	71
Resting	NA		
Stimulated	65		

[1]No EBV was calculated for trait because heritability estimates were not different from 0.

described (6). Heritability estimates of 10% were then used as priors to compute the EBV for IMI scores. Heritability estimates of 10% for SCS and CM were obtained from the literature (3, 6) and used to compute the BLUP of additive genetic effects for each measure of mastitis for each cow (13). Low EBV for SCS, CM, major IMI, and minor IMI indicate greater resistance to mastitis, and high EBV for the same mastitis measures indicate greater susceptibility to mastitis.

Statistical Analyses

Data. A total of 137 periparturient cows were used during the 16 mo of this study. The IgG2 allotype of 5 cows and the genotype of a sixth cow at the DRB3 locus could not be determined accurately, excluding these individuals from subsequent analyses. Table 2 lists the total number of cows from which molecular marker information was gathered. Table 2 also contains the number of individuals for which EBV for immunological assays and mastitis measures were computed. Data used in the calculation of EBV for immunological assays and mastitis measures were not complete for all cows because of short or incomplete lactations or technical errors in the immunological assays.

Gene substitution model. The effect of each molecular marker on the four measures of mastitis was estimated with the following gene substitution model:

$$D_i = MHC_i + IgG_i + BLAD_i + e_i \qquad [1]$$

where

D_i = EBV of cow i for SCS, CM, major IMI, or minor IMI;

MHC_i = $\Sigma_m b_m n_{m_i}$ where m = 1, 2, . . . 12, b_m = gene substitution effect of each allele m in the *DRB3.2* genotype, n_{m_i} = number of copies of allele m in the genotype of cow i (n_{m_i} = 0, 1, or 2), and $\Sigma_m b_m = 0$;

IgG_i = $\Sigma_g b_g n_{g_i}$ where g = 1 or 2, b_g = gene substitution effect of each allele g in the isotype IgG2, n_{g_i} = number of copies of allele g in the isotype of cow i (n_{g_i} = 0, 1, or 2), and $\Sigma_g b_g = 0$;

$BLAD_i = \Sigma_c b_c n_{c_i}$ where c = 1 or 2, b_c = gene substitution effect of each allele c in the genotype for BLAD, n_{c_i} = number of copies of allele c in the genotype of cow i (n_{c_i} = 0, 1, or 2), and $\Sigma_c b_c = 0$; and

e_i = residual random term that is assumed $\mathbf{N}(0, \sigma^2)$.

With this model, cows that are heterozygous at a locus for a particular allele are assigned one copy of that allele, and cows that are homozygous at the locus are assigned two copies of that allele. Because the sum of additive effects over all alleles at one locus was null, an estimable gene substitution effect for each allele was expressed as the difference from the mean of all alleles for each locus (26). Tests of significance were computed using a two-tailed Student's *t* test; $P < 0.10$ were reported as significant. Computations were done using the GLM procedure of SAS software (28).

Correlations. Pearson correlations between EBV for SCS, CM, IMI from major pathogens, IMI from minor pathogens, and EBV for immunological assays before, at, and after immunosuppression were obtained using the CORR procedure of SAS software (28).

RESULTS AND DISCUSSION

Table 3 summarizes the number and relative allelic frequencies for the DRB3 locus, the IgG2 isotypic subclasses, and the BLAD genotypes. Nearly 40% of *DRB3.2* alleles found in this herd were *DRB3.2*8* or **11*. Rare alleles with frequencies less than 2% accounted for 11% of the herd. The 11.3% carrier rate for the BLAD CD18 allele was expected based on the carrier rate among AI bulls (32), and the frequencies of the IgG2 allotypes were similar to those in previous reports for the Holstein breed (2).

All mastitis and immunological measures were not taken over the same period. Immune assays and scores for IMI were taken during the periparturient period, but the data that were used in the calculation of EBV for SCS and CM were taken over the entire herd life of the cow; thus, interpretations may not be the same across all measures of mastitis.

Molecular Markers

Alleles at the DRB3 locus of the bovine MHC, IgG2 genotype, and BLAD genotype are all at the genotypic level; thus, EBV for measures of mastitis were used as dependent variables in the analysis. Model [1], which included substitution effects of the MHC, IgG2, and BLAD genes, explained 17, 41, 28, and 20% of the variation in EBV for SCS, CM, IMI from major pathogens, and IMI from minor pathogens, respectively. Thus, the molecular markers constituted part of the genetic variability in susceptibility to mastitis found in this study.

Gene substitution effects of the DRB3 locus on measures of mastitis are summarized in Table 4. A positive gene substitution effect indicates that presence of the allele is unfavorable (i.e., the allele is associated with greater EBV for mastitis measures). Reciprocally, a negative effect is associated with lower EBV for mastitis measures. The DRB3 locus was originally chosen for genetic characterization because it is highly polymorphic and is the most actively transcribed gene in the Class II DRB region (36).

One *DRB3.2* allele was shown to affect significantly the EBV for SCS (Table 4). The presence of allele *DRB3.2*16* was associated with a significant increase in EBV for SCS ($P < 0.05$). This finding is in agreement with a larger study (9) in which *DRB3.2*16* was associated with increased risk of acutely elevated SCS. A companion study (10) has also found that *DRB3.2*16* was negatively associated with mononuclear cell counts and was positively associated with serum IgM, complement, and conglutinin.

Allele *DRB3.2*24* was associated with a decrease in EBV for SCS that approached significance (P =

TABLE 3. Observed number and frequencies for alleles for IgG2 isotype, DRB3 locus, and for the CD18 locus responsible for bovine leukocyte adhesion deficiency (BLAD) genotype.

	Number	Frequencies
		(%)
IgG2 Isotypic subclass genotype		
$IgG2^a$ $IgG2^a$	48	39.0
$IgG2^a$ $IgG2^b$	59	48.0
$IgG2^b$ $IgG2^b$	16	13.0
DRB3.2 Allele		
**8*	52	21.31
**11*	43	17.62
**23*	21	8.61
**24*	21	8.61
**22*	20	8.20
**16*	16	6.56
**27*	13	5.33
**12*	11	4.51
**26*	7	2.87
**3*	7	2.87
**28*	6	2.46
Others	27	11.07
BLAD genotype		
A_1A_1	110	88.7
A_1A_2[1]	14	11.3
A_2A_2	0	0.0

[1]Cows heterozygous (*BL or carriers of the *D128G* allele at the *CD18* locus) for the mutation responsible for BLAD

0.1082) but was also associated with a significant increase in EBV for IMI caused by major pathogens (Table 4). An interpretation of this apparent paradox is that cows with *DRB3.2*24* could have been more susceptible to the establishment of infection with major pathogens, but, once they were infected, cows were more resistant to the pathogenic effects of the bacteria. In a companion study (10), *DRB3.2*24* was associated with increased neutrophil counts and increased IgG_2.

The three most frequent alleles in this group of cattle were associated with EBV for CM (Table 4). The presence of *DRB3.2*8* was associated with a significant increase in EBV for CM ($P < 0.10$). The presence of *DRB3.2*23* was associated with the largest decrease in EBV for CM ($P < 0.05$). Allele *DRB3.2*11* was associated with a smaller decrease in EBV for CM. Together, these three alleles accounted for over 47% of the total allelic frequency. Alleles *DRB3.2*8* and *11 have been positively associated with serum concentrations of Ig and complement and negatively associated with circulating levels of mononuclear cells (10). Only *DRB3.2*3* was shown to have a significant effect on IMI caused by minor pathogens ($P < 0.10$). Interestingly, no studied allele in this group was demonstrated to cause a consistent directional effect on all measures of mastitis, indicating the complexity of the immune system and the disease itself.

Effects of allotypic variants of IgG2 and BLAD genotype on EBV of measures of mastitis were statistically significant only in the case of CM (Table 4). The presence of the more frequent $IgG2^a$ allele was associated with significant decreases in EBV for CM ($P < 0.05$). This allele was also associated with decreases in EBV for SCS that approached significance ($P < 0.122$). The presence of the CD18 mutant allele in heterozygous cows was significantly associated with decreased EBV for CM ($P < 0.05$). The mutant CD18 allele tended to decrease EBV for SCS, although not significantly.

Immunological Assays

Tables 5, 6, 7, and 8 summarize the correlations between the EBV for immunological assays and EBV for SCS, CM, IMI from major pathogens, and IMI from minor pathogens, respectively. The design of this study does not prove a cause and effect relationship between the ability of a cow to mount an effective

TABLE 4. Gene substitution effects for alleles at locus DRB3 of the bovine major histocompatibility complex, for IgG2 isotype alleles, and for alleles responsible for the bovine leukocyte adhesion deficiency (BLAD) on EBV for mastitis indicators.

Alleles	Somatic cell score	Clinical mastitis score	IMI caused by major pathogen	IMI caused by minor pathogen
DRB3.2				
*8	0.002	0.040†	0.001	–0.031
*11	–0.013	–0.038†	0.006	–0.021
*23	–0.039	–0.070*	0.007	0.041
*24	–0.042	–0.024	0.108**	0.002
*22	0.032	0.028	–0.055	0.028
*16	0.084*	0.001	–0.004	–0.039
*27	0.017	0.042	–0.019	–0.041
*12	0.006	–0.038	–0.001	–0.023
*26	–0.057	0.029	–0.015	0.049
*3	0.016	0.044	–0.033	0.068†
*28	–0.034	–0.005	–0.047	–0.025
Others[1]	0.028	–0.009	0.054†	–0.009
IgG2				
$IgG2^a$	–0.024	–0.035*	0.002	–0.011
$IgG2^b$	0.024	0.035*	–0.002	0.011
CD18				
A_1	0.024	0.077*	–0.040	–0.018
A_2[2]	–0.024	–0.077*	0.040	0.018

[1]*DRB3.2* alleles designated as "others" have frequencies below 2%.

[2]Recessive allele responsible for BLAD.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

TABLE 5. Pearson correlation coefficients between EBV for SCS and EBV for immune response measures taken before, during, and after immunosuppression.

	Time relative to immunosuppression		
	Before	During	After
Random migration	–0.158	NA[1]	–0.036
Ingestion	–0.091	NA	–0.003
Cytochrome *c* reduction	–0.080	–0.173†	–0.094
Iodination	–0.146	–0.115	NA
Chemiluminescence			
Resting	–0.053	–0.067	NA
Stimulated	NA	–0.259*	0.148
IgG_1	NA	0.152	0.263*
IgG_2	–0.108	–0.043	0.138
IgM	0.045	0.127	0.023
Concanavalin A	–0.074	–0.056	–0.056
Mononuclear cells	–0.203†	–0.227*	–0.198

[1]No EBV was calculated for trait because heritability estimates were not different from 0.

†$P < 0.10$.

*$P < 0.05$.

immune response and the ability to resist IMI or disease in the mammary gland. The analysis from this study only allows statistical association of EBV for IR measures with EBV for mastitis measures. Indeed, the presence of IMI or CM may have altered the values of the immunological assays, but it is equally possible that values for an individual cow for the various immunological assays altered susceptibility to mastitis. In fact, both situations possibly existed. Although the sample size of our study was large compared with other immunological studies, only 137 cows were used; therefore, our estimates are subject to large sampling errors. Only general trends in the genetic association between immunological and mastitis traits are discussed.

Several correlations between EBV for measures of IR and EBV for measures of mastitis were significantly greater than 0. Two measures of neutrophil function at maximal immunosuppression were negatively correlated with EBV for SCS (Table 5). Also, nearly all assays of neutrophil function were negatively correlated with EBV for SCS; thus, cows with low EBV for SCS tend to have neutrophils that function at a higher capacity, especially when cows are stressed at parturition and are at maximal immunosuppression. The EBV for circulating mononuclear cell numbers is also negatively correlated with EBV for SCS. Mononuclear cell populations are primarily lymphocytes in addition to monocytes. Cows with low EBV for SCS tend to have higher concentrations of circulating mononuclear cells. The interpretation of Ig measures is not straightforward, although EBV for IgG_1 were positively correlated with EBV for SCS (Table 5). A cow would be expected to produce increased concentrations of IgG_1 following an IMI and would also be expected to have an elevated SCS; therefore, the results of this study are not surprising.

TABLE 6. Pearson correlation coefficients between EBV for clinical mastitis and EBV for immune response measures taken before, during, and after immunosuppression.

	Time relative to immunosuppression		
	Before	During	After
Random migration	–0.321*	NA[1]	–0.013
Ingestion	0.004	NA	0.292*
Cytochrome *c* reduction	0.202	–0.086	–0.022
Iodination	0.058	–0.035	NA
Chemiluminescence			
Resting	0.204	0.227†	NA
Stimulated	NA	–0.171	0.080
IgG_1	NA	0.215†	0.373**
IgG_2	0.060	–0.062	0.194
IgM	0.167	0.029	0.049
Concanavalin A	–0.165	–0.127	0.143
Mononuclear cells	–0.205	–0.052	–0.062

[1]No EBV calculated for trait as heritability estimates were not different from 0.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

TABLE 7. Pearson correlation coefficients between EBV for IMI caused by major pathogens and EBV for immune response measures taken before, during, and after immunosuppression.

	Time relative to immunosuppression		
	Before	During	After
Random migration	–0.048	NA[1]	–0.141
Ingestion	0.079	NA	–0.149
Cytochrome *c* reduction	–0.327*	0.117	0.247†
Iodination	–0.101	–0.022	NA
Chemiluminescence			
Resting	–0.041	–0.093	NA
Stimulated	NA	–0.001	–0.151
IgG_1	NA	–0.125	0.050
IgG_2	–0.105	0.193	0.112
IgM	–0.266†	0.198	–0.023
Concanavalin A	0.205	0.043	–0.236
Mononuclear cells	0.055	0.174	–0.014

[1]No EBV calculated for trait as heritability estimates were not different from 0.

†$P < 0.10$.

*$P < 0.05$.

Several correlations between EBV for IR and EBV for CM, IMI from major pathogens, and IMI from minor pathogens were significantly different from 0. Many of these correlations are not readily interpretable. In general, EBV for random migration of neutrophils was negatively correlated with EBV for CM and IMI; thus, neutrophils with greater inherent mobility are apparently needed to combat IMI. In general, EBV for circulating IgG_1 was positively correlated with EBV for mastitis measures.

Our hypothesis was that cows with high EBV for the innate IR traits are more resistant to mastitis because earlier studies demonstrated the role of an efficient IR against IMI (16, 18, 29, 30). Phenotypically, resistance to mastitis pathogens was less for cows in which neutrophils had a low capacity to undergo a respiratory burst of oxidative metabolism associated with phagocytosis (15, 38). A pattern of occurrence of CM for cows with the most severe immunosuppression for neutrophil iodination was present in this study and earlier reports from other herds (5, 20). Although not conclusive, the correlations between EBV for neutrophil function and EBV for SCS add more evidence to the conclusion that high levels of inherent neutrophil function are needed for combating mastitis.

Immunoglobulin M is very efficient at activating the classic pathway of complement (4), IgG1 is the predominant Ig isotype in milk (4), IgG2 is very important in bacterial opsonization of pathogens for phagocytosis by neutrophils and antibody-dependent neutrophil cytotoxicity (4), and IgG2 has been associated with an increased incidence of pyogenic mastitis (23). The correlation coefficients of EBV for serum IgG_1 and IgM concentrations with EBV for mastitis indicators tended to be positive in this study. This result suggests that serum Ig concentrations might be elevated as a result of subclinical or chronic disease, which has been observed under other conditions with repeated antigenic stimulation from recurrent infections (11, 12, 35).

TABLE 8. Pearson correlation coefficients between EBV for IMI caused by minor pathogens and EBV for immune response measures taken before, during, and after immunosuppression.

	Time relative to immunosuppression		
	Before	During	After
Random migration	0.108	NA[1]	–0.253[†]
Ingestion	0.039	NA	0.214
Cytochrome *c* reduction	0.236[†]	0.012	–0.125
Iodination	–0.128	–0.128	NA
Chemiluminescence			
Resting	0.132	–0.005	NA
Stimulated	NA	–0.101	0.065
IgG_1	NA	–0.012	0.093
IgG_2	–0.024	0.083	0.130
IgM	0.043	–0.010	0.103
Concanavalin A	–0.055	0.060	0.203
Mononuclear cells	0.001	0.014	–0.007

[1]No EBV calculated for trait as heritability estimates were not different from 0.

[†]$P < 0.10$.

CONCLUSIONS

Genetic association was found between alleles at the DRB3 locus, especially between measures of mastitis and alleles *DRB3.2*16*, **23*, and **24*. The *DRB3.2* alleles have considerable promise as potential markers for mastitis resistance or susceptibility. We also found a genetic association between IgG2 alleles and EBV for CM as well as an association between the BLAD alleles and CM score.

Several significant genetic associations were found between the mastitis measures and the assays that measure the innate capability of neutrophils to respond to an infection and the serum concentration of Ig. We could not determine whether immunocompetence conveys resistance to mastitis. More studies are necessary to determine whether genetic markers of innate immunity control genetic resistance to mastitis. To determine causation, cows that are free of mastitis should be genetically selected for an immunocompetence marker and subsequently challenged with a mastitis pathogen. It should then be possible to determine whether cows with a particular immunological marker are more resistant to mastitis than cows without the marker. More research is also necessary to find new measures of udder infection that describe precisely the pathogenic stages of the disease and that consider the effects of the animal, the environment, and the pathogen, each of which being involved in the outcome of this complex disease.

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