Table 1: Assays of immune function performed in the present study.

Neutrophil assays:
Random migration under agarose
Ingestion of bacteria
Cytochrome C reduction
Myeloperoxidase-catalyzed iodination
Resting chemiluminescence
Stimulated chemiluminescence
Serum concentration:
IgM
IgG_1
IgG_2
Lymphocyte blastogenic response to concanavalin A
Number of blood monocytes

Table adapted from the following publication:
Kelm, S.C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:(*Accepted 2/26/97*).

Table 2: Observed numbers and frequencies for DRB3.2 alleles, IgG2 isotypes, and BLAD genotypes.

	Number	Frequencies
DRB3.2 allele		
*8	52	21.3
*11	43	17.6
*23	21	8.6
*24	21	8.6
*22	20	8.2
*16	16	6.6
*27	13	5.3
*12	11	4.5
*26	7	2.9
*3	7	2.9
*28	6	2.5
others	27	11.1
IgG2 allotype (genotype)		
AA ($IgG2^a/IgG2^a$)	48	39.0
AB ($IgG2^a/IgG2^b$)	59	48.0
BB ($IgG2^b/IgG2^b$)	16	13.0
BLAD[1]		
CD18 / CD18	110	88.7
CD18 / D128G	14	11.3
D128G / D128G	0	0.0

[1] Normal allele: *CD18*. Mutant allele: *D128G*.

Table adapted from the following publication:
Kelm, S.C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:(*Accepted 2/26/97*).

TABLE 3. Gene substitution effects for DRB3.2 alleles of the bovine major histocompatibility complex, for alleles designated by IgG2 isotype, and for alleles at mutation responsible for the bovine leukocyte adhesion deficiency (BLAD) on EBV for mastitis indicators.

	Somatic cell score	Clinical mastitis score	IMI caused by major pathogen	IMI caused by minor pathogen
DRB3.2 alleles				
*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 alleles				
IgG2[a]	-0.024	-0.035*	0.002	-0.011
IgG2[b]	0.024	0.035*	-0.002	0.011
BLAD alleles[2]				
CD18	0.024	0.077*	-0.040	-0.018
D128G	-0.024	-0.077*	0.040	0.018

† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$

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

[2] Normal allele: *CD18*. Mutant allele: *D128G*.

Table adapted from the following publication:
Kelm, S.C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:(*Accepted 2/26/97*).

Table 4. 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	n.a.[1]	-0.036
Ingestion	-0.091	n.a.	-0.002
Cytochrome C reduction	-0.080	-0.173†	-0.094
Iodination	-0.146	-0.115	n.a.
Chemiluminescence - resting	-0.053	-0.067	n.a.
Chemiluminescence - stimulated	n.a.	-0.259*	0.148
IgG_1	n.a.	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

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

[1] Breeding value not available as estimated heritability is less than or equal to zero.

Table adapted from the following publication:
Kelm, S.C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:(*Accepted 2/26/97*).

Table 5. Pearson correlation coefficients between EBV for CM and EBV for IR measures taken before, during, and after immunosuppression.

	Time relative to immunosuppression		
	Before	During	After
Random migration	-0.321*	n.a.[1]	-0.013
Ingestion	0.004	n.a.	0.292*
Cytochrome C reduction	0.202	-0.086	-0.022
Iodination	0.058	-0.035	n.a.
Chemiluminescence - resting	0.204	0.227†	n.a.
Chemiluminescence - stimulated	n.a.	-0.171	0.080
IgG_1	n.a.	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

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

[1] Breeding value not available as estimated heritability is less than or equal to zero.

Table adapted from the following publication:
Kelm, S.C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:(*Accepted 2/26/97*).

G00069

Association of molecular and physiological markers of immunity with measures of mastitis in periparturient Holstein cattle.

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Abstract:
Relationships between estimated breeding values (EBV) for mastitis measures and molecular markers of immune function were examined. Relationships between EBV for mastitis measures and EBV for 11 *in vitro* immunologic assays were also investigated. Data were available from 137 periparturient Holsteins. Mastitis measures included somatic cell score (SCS), clinical mastitis, and intramammary infection with major or minor pathogens. Molecular markers included alleles of the DRB3 locus of the bovine major histocompatibility complex, IgG2 isotypes, and bovine leukocyte adhesion deficiency (BLAD) genotypes. Immune assays measured neutrophil function, serum immunoglobulins, leukocyte counts, and lymphocyte blastogenesis. A gene substitution model was used to estimate the effects of molecular markers on EBV for mastitis measures. Markers explained up to 40% of the variation in genetic measures of mastitis. Presence of allele *DRB3.2*16* was associated with higher EBV for SCS, thus this allele may indicate greater mastitis susceptibility. Alleles *DRB3.2*8*, *IgG2^b*, and normal CD18 allele were associated with increased EBV for clinical mastitis. Alleles *DRB3.2*11*, **23*, and *IgG2^a* were associated with decreased clinical mastitis. Carriers of the mutant CD18 allele, *D128G*, also had decreased EBV for clinical mastitis. Significant associations were found between DRB3.2 alleles and EBV for intramammary infection by major and minor pathogens. Pearson correlation coefficients between EBV for mastitis and EBV for immunologic assays were calculated. Several correlations were significantly different from zero. In general, cows with low EBV for mastitis measures had high EBV for measures of neutrophil function, low serum IgG[1], and high numbers of circulating mononuclear cells.

Introduction:
Identification of individuals that are resistant to disease is desirable but difficult in commercial populations. Estimated breeding values (EBV) are based on phenotypic information from an individual and its family members and can be used to identify resistant individuals in lieu of direct challenge. After identification of resistant individuals, the biological causes of disease resistance can be examined. Potential causes include superior immune response capabilities or the possession of single genes with large effects.

The major objective of this study was to measure the associations between EBV for mastitis measures and three molecular markers in dairy cattle; the MHC class II DRB3 genotype, the IgG2 allotype, and the BLAD genotype. As a second objective, the genetic association between measures of mastitis prevalence and 11 *in vitro* immunological assays measuring the functional capability of the immune system was investigated.

Materials and Methods:
Periparturient Holstein cows (n=137) were available for use in this study. Cows were managed as a commercial herd and fed for high production.

Molecular markers: Animals were genotyped at the Class II DRB3.2 locus using a PCR-RFLP method as described

(1). Alleles found at low frequencies (< 2%) were pooled. Immunodiffusion analysis was used to determine IgG2 isotypes as described (2) and genotypes were deduced from isotypes. Animals were genotyped for the BLAD mutation at the CD18 locus using a PCR-RFLP method as described (3).

Immunologic assays: Weekly blood samples were taken from all cows from 5 weeks before to 5 weeks after calving. Five steers served as laboratory controls. Eleven *in vitro* assays were performed as described (4,5) (see Figure 1). Immunosuppression (IS) is well-documented at calving (6,7), thus, immune response was categorized as before IS, during IS, or after IS. Correction for environmental factors was accomplished by calculating EBV for each cow for individual assays, as described (6).

Mastitis measures: Four measures of mastitis were used; somatic cell score (SCS), clinical mastitis (CM), and intramammary infection by major pathogens (IMI Major) or minor pathogens (IMI Minor). Lifetime CM incidence and monthly SCS records were used for each cow. The IMI status of each cow was based on cultural evidence with duplicate quarter milk samples taken at -35, 0, and 35 days relative to calving. The EBV for mastitis measures were computed as described (8).

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

D[i] = MHC[i] + IgG2[i] + BLAD[i] + e[i].

In this model, D[i] represents the EBV of cow i for SCS, CM, IMI Major, or IMI Minor. Also, MHC[i] represents the sum of the following product from m=1 to 12: gene substitution effects for allele m in the DRB3.2 genotype * number of copies (n) of allele m in the genotype of cow i, where n = 0, 1, or 2. The sum of gene substitution effects is restricted to zero. The variables IgG2[i] and BLAD[i] are defined similarly. An estimable gene substitution effect was expressed as the difference from the mean of all alleles for each locus (9). Tests of significance were computed using a two-tailed Student's *t* test. Pearson correlations between EBV for SCS, CM, IMI Major, IMI Minor and EBV for immunological assays before, at, and after IS were calculated. Computations were completed using procedures of SAS software (10).

Results:

Table 2 summarizes the frequencies of DRB3.2 alleles, IgG2 allotypes, and BLAD genotypes (see Figure 2). Estimates of the gene substitution effects are summarized in Table 3. Note that a positive gene substitution effect is unfavorable, i.e. the allele is associated with undesirable or high EBV for mastitis measures.

The presence of *DRB3.2*16* was associated with a significant increase in EBV for SCS (see Figure 3). Allele *DRB3.2*8* was associated with an increase in EBV for CM while *DRB3.2*23* and **11* were associated with decreases in EBV for CM. Allele *DRB3.2*24* was shown to have a significant, undesirable effect on IMI Major and *DRB3.2*3* was associated with IMI Minor. The presence of the CD18 mutant allele and *IgG2^a* were associated with significant decreases in EBV for CM (see Figure 3).

Tables 4 and 5 summarize the correlations between EBV for immunological assays and EBV for SCS and CM, respectively. Mononuclear cell counts and two neutrophil measures were associated with decreased EBV for SCS (see Figure 4). Several correlations between EBV for CM and EBV for immune response were significantly different from zero but were not readily interpretable. This was also true for correlations involving EBV for IMI Major and EBV for IMI Minor (data not shown).

Discussion and Conclusions:

The results of this study confirm earlier research on potential use of DRB3.2 alleles as markers of disease susceptibility (11,12). The agreement between results of the three reports is noteworthy; *DRB 3.2*8* and *DRB3.2*16* were associated with greater disease susceptibility in all three studies. Furthermore, *DRB3.2*24* was associated with increased EBV for IMI Major in this study and was associated with increase susceptibility to bovine leukosis (12), while alleles *DRB3.2*11* and **23* were favorably associated with disease status (12), in agreement with the present study.

Carriers of the mutant CD18 allele had significantly lower EBV for CM, however, sample size was small as only 14 heterozygotes were identified. Further studies involving larger populations are necessary to rule out sampling error. Carriers of *IgG2^a* also had significantly lower EBV for CM. We believe this is the first report of functional differences in IgG2 allotypes in dairy cattle.

Interpretation of the relationship between EBV for immunological assays and EBV for mastitis measures is problematic because a cause-and-effect relationship between the variables can not be established. It is clear, however, that animals with a favorable EBV for SCS possessed neutrophils with greater functional ability and greater numbers of circulating monocytes (see Figure 4).

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Comments:

The contents of this poster will be presented in greater detail in the following manuscript accepted for publication in the Journal of Dairy Science:

Kelm, S.C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. J. Dairy Sci. 80:(*Accepted 2/26/97*).

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Questions and Answers