MOLECULAR STUDIES OF DRB RELATIVE TO *STAPHYLOCOCCUS AUREUS* MASTITIS

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SUMMARY

Using a human cDNA probe to DRB of the major histocompatibility complex, five DNA patterns were identified in 110 Holstein cattle in three herds in which mastitis status was found in relatively high levels. One of these DNA patterns appears to be associated with resistance to mastitis caused by *S. aureus* but not by mastitis caused by other pathogens. The pattern associated with resistance is not a homozygous pattern based on family studies but can still be useful for selection of heifer calves which should remain in the milking herd.

PCR primers (Ellegren et al. 1993) were used to identify alleles at the DRB locus of these cows. One allele showed significant association to susceptibility to *S. aureus*.

INTRODUCTION

Mastitis is recognized worldwide as one of the most costly diseases afflicting dairy cows. Reduced milk production from cows with subclinical infections appears to be responsible for even greater losses than acute mastitis (Blosser, 1979). The major pathogen associated with subclinical infections is *Staphylococcus aureus* (Bramley et al., 1981). Approximately 15% of humans are persistent carriers of *S. aureus* (Sheagren, 1984) which appears to be associated with the DR3 gene of the major histocompatibility complex (MHC) (Kinsman et al. 1983).

Initially southern blot analysis was conducted to identify RFLPs at the DRB locus in Holstein cattle in which mastitis status was determined in order to seek possible allele associations with resistance or susceptibility (Schmutz et al. 1992). Subsequently PCR analysis was used to identify specific alleles of these cattle. Comparison of these two approaches was therefore possible and could be used to confirm or refute our original findings.

MATERIALS AND METHODS

The cow population

Three herds with relatively high *S. aureus* infections were chosen among supervised Dairy Herd Analysis Service (DHAS) herds within a 40 km radius of Saskatoon. Rates of infection varied between these herds. The *S. aureus* infection rate per cow was 2.8% for herd A, 14.3% for herd B, and 34.2% for herd C. Only herds with relatively high *S. aureus* infections were studied because the analysis required that exposure to this organism was obtained prior to classifying animals as "resistant".

Information regarding the sire and dam was available on 38 registered animals. Thirty-one different sires were involved with no bull the sire of more than 3 of the cows in this sample.

Bacteriology

Quarter milk samples were collected aseptically from every lactating cow on each of two occasions. Samples were collected in sterile plastic vials and stored on ice until they reached the laboratory where 0.01 ml samples were immediately streak plated onto 5% blood agar plates and incubated for 24 hours at 37° C. At 24 hours a presumptive identification was made based on colony appearance. All presumptive staphylococci in mixed culture were further typed if they were hemolytic or if they were present in significant numbers (i.e. $\geq$ 4 colonies). Presumptive staphyloccoci which were Gram posotove and catalase positive (Research Committee of the National Mastitis Council, 1987) were further typed for tube coagulase, DNAse, hyaluronidase, and maltose and mannitol fermentations (Carter and Cole, 1980). Isolates which were coagulase positive but anomalous for at most one of DNAse, hyaluronidase or mannitol, were identified as *S. aureus*.

DNA Studies

Blood samples were drawn from the tail vein from the cows at the time of the second milk sample collection. In addition to the 110 cows tested for *S. aureus*, a small group of familes were also DNA typed. The Greenbrae dairy herd of the University of Saskatchewan was used for family studies to determine the inheritance of the DNA patterns.

DNA was extracted from 10 ml of whole blood using an ABS automated DNA extractor and stored at -70° C until analyzed. 8 µg aliquots of DNA were digested with either *Hind* III or *Bgl* II (BRL) at the conditions appropriate for each enzyme. The samples were run on agarose gels (0.9%) at 55 volts for 16 hours in 1X TBE. Capillary blotting was done onto Hybond N+ (Amersham) overnight. Prehybridizations and hybridizations were carried out in 0.263M Na_2HPO_4-$7H_20$, 7% SDS, 1% BSA and 1 mM EDTA at 60 °C for 16-20 hours. Two washes were done in 2X SSC and 0.1% SDS at room temp. for 15 min., and one wash in 0.5X SSC and 0.5% SDS, at 60°C for 30 min.

The DRB cDNA (ATCC 57080) insert was removed from the plasmid by *Bam* HI digest and electroelution. The complete DRB fragment was labelled to high activity using the random primer extension procedure (Pharmacia). Films were exposed for 3 days at -70°C. Autoradiographs were read and the RFLP pattern for each cow was recorded.

PCR analysis was conducted according to the method outlined by Ellegren et al. (1993) using primers LA53 and LA54. Alleles were identified based on comparisons with known stardards from the BoLA workshop. Data were analyzed using Chi-square analysis.

RESULTS

Five DNA patterns were identified using *Bgl*II digestions utilizing three variable bands at approximately 4.9, 3.6, and 3.0 Kb. The DNA pattern in which all three of these bands was present, designated 1, was found to be significantly associated with cows which did not have *S. aureus* infections (Table 1, $X^2 = 4.3$, $P = 0.037$). It would therefore appear that the cows with DNA pattern 1 are more resistant to *S. aureus* mastitis with a relative incidence (Woolf, 1955) of 0.24 compared to 1.0 for other DNA patterns. However, this DNA pattern did not appear to be associated with resistance to all types of mastitis ($X^2 = 0.2$, $P = 0.62$), nor to other Staphylococci infections ($X^2 = 0.5$, $P = 0.46$). Analysis of family data would suggest that *Bgl* II pattern 1 is not the result of a single allele in a homozygous state. Two pair of cattle wherein both the cow and bull had DNA pattern 1 produced calves with a DNA pattern other than pattern 1.

Table 1. Number of Holstein cows with *Bgl* II pattern 1 detected by DRB probing, versus other DNA patterns in relation to *S. aureus* infection.

	Staph Positive	*Staph* Negative
DNA pattern 1	3	20
Other DNA patterns	12	19

Since DNA pattern 1 was the most common DNA pattern found and may result as a heterozygote of several other combinations, we conducted PCR analysis to identify alleles which might more specifically be the cause of this resistance. Fourteen alleles were identified with only three cows homozygous. Only one allele, 207 (Ellegren et al. 1993), showed a significant association ($X^2 = 5.7$, $P = 0.017$) to *S. aureus* infection and this allele imparts susceptibility rather than resistance. No single allele showed a signficant association with resistance. There was no correlation evident between the genotypes identified using PCR analysis and patterns using southern blot analysis ($r=0.1$, $P=0.44$). No genotype occurred in more than three individuals and therefore associations based on genotype were not tested.

Table 2. Number of Holstein cows with each allele,defined by fragment size, detected by PCR analysis in relation to *S. aureus* infection.

Frament Size (bp)	*Staph* Positive	*Staph* Negative
159	2	3
163	0	2
171	1	3
179	3	8
181	0	2
183	3	11
185	3	7
187	2	3
189	2	9
193	4	12
195	0	7
203	1	1
207	8	7
219	1	3
TOTAL	30	78

DISCUSSION

The results of this study suggest that DRB is associated with *S. aureus* mastitis resistance/susceptibility. Cows carrying *Bgl*II pattern 1 revealed with southern blotting are resistant to *S. aureus* in the population studied. It is not believed that the BglII pattern 1 association to *S. aureus* resistance is caused by a sire effect since a wide variety of sires (n=58) were represented in this study. Although PCR analysis could more precisely identify genotypes, only one allele was found to be associated with susceptibility. The two analyses lend support to a role for DRB but do not reveal the same levels of polymorphism nor do they detect polymorphism in the same portion of the gene.

Lunden et al. (1990) also found an association between DQ^{1A} (detected using a human DQB cDNA probe) and mastitis susceptibility in Swedish Red and White cattle. The bovine DQA, DQB, DRA, and DRB genes are all closely linked (Andersson et al. 1988) and therefore these two studies corroborate the hypothesis that the class II MHC genes are involved in mastitis resistance and susceptibility.

Resistance only to *S. aureus* mastitis appears to be conferred by the heterozygous *Bgl* II pattern 1. Such specificity is not surprising since the MHC loci are highly polymorphic. *S. aureus* is quite different from the other major pathogens which are freqently associated with mastitis, such as *Streptococcus dysgalactiae*, *Klebsiella bovis*, and *Escherichia coli*. Mastitis caused by *S. aureus* is more resistant to antibiotic therapy than other forms (Nickerson 1987) and therefore a marker for resistance to this form should be useful to the dairy industry.

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