

Effects of a Single Nucleotide Polymorphism in the Interleukin-8 Receptor on Susceptibility of Dairy Cattle to Mastitis

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Story in Brief

Mastitis costs about 2 billion dollars annually in the U.S., due to milk loss, increased culling, veterinary services, and treatment. There are genetic differences among cows in their susceptibility to mastitis, suggesting that marker-assisted selection might be effective in reducing the incidence of mastitis. The present study was conducted to investigate the effect of a polymorphism in the interleukin-8 (IL-8) receptor on mastitis susceptibility, milk yield, and quality. Blood samples were collected from 75 Holstein cows, DNA was recovered, and polymerase chain reaction procedures were used to amplify a 311 base pair segment of the IL-8 receptor, followed by digestion with a restriction enzyme and electrophoresis. Three IL-8 receptor genotypes (GG, GC and CC) were identified. Cows with the GG or GC genotypes for the IL-8 receptor had lower mean somatic cell counts ($P = 0.01$) than cows with the CC genotype. Genotype had no effect on 305-day adjusted milk ($P = 0.40$), protein ($P = 0.57$), or fat ($P = 0.84$) production. Results suggest selection based on genotypes for the IL-8 receptor polymorphism would be effective in reducing the incidence of mastitis in dairy cattle.

Introduction

Mastitis is an inflammatory response of the mammary system to an infection, commonly caused by various strains of *Staphylococcus* and by *E. coli*. In response to this inflammation, neutrophils migrate to the mammary gland and become predominant. Interleukin-8 (IL-8) is a chemokine produced by a number of cell types, including mammary epithelial cells, that is critical in regulating the inflammatory response of neutrophils by inducing their migration to the site of infection and then, enhancing their killing ability (Barber and Yang, 1998).

There are genetic differences among cows in their susceptibility to mastitis. Considering the importance in the IL-8 in the immune response, it would be a good candidate for a gene marker to select cows that are less susceptible to mastitis infection. A single nucleotide polymorphism (SNP) has been reported in the IL-8 receptor (CXCR2) resulting in genotypes with increased or decreased susceptibility of Holstein and Jersey cows to mastitis (Youngerman et al., 2004b). Of the 3 genotypes associated with the IL-8 receptor, GG and CC are associated with low and high somatic cell counts, respectively, while GC is intermediate for somatic cell count. The present study was conducted to further investigate the effect of IL-8 genotypes on mastitis susceptibility, as well as, milk quantity and quality

Experimental Procedures

Blood samples were collected from 75 Holstein cows at a local dairy in which Dairy Herd Improvement (DHI) records were available for somatic cell count (SCC) and milk production. DNA was recovered from each blood sample, quantified and frozen at -20°C until use. The polymerase chain reaction-restriction length polymorphism (PCR-RFLP) procedures were based on those reported by Youngerman et al. (2004a). Forward (5'-CTTCCGTGAGGCC-

TATCAAC-3') and reverse (5'-AGGTCTCAGCAATCAC-ATGG-3') primers were used to amplify a 311 base pair segment of the bovine IL-8 receptor locus.

The reaction mixture consisted of 2 μl of 10x buffer, 1.5 mM MgCl_2 , 200 μM of each deoxynucleotide triphosphate (dNTP), 10 pmoles of each primer, 2 units of DNA polymerase and 50 ng of DNA in a total volume of 20 μl . Thermal cycler conditions were an initial DNA denaturing of 95°C for 2 min, followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 30 sec and extension at 68°C for 1 min. After the last thermal cycle, one-half (10 μl) of each product was placed in a separate pcr tube with 5 units of Bme1580I restriction endonuclease and digested at 37°C .

The digested and undigested product for each sample were loaded side-by-side into a 1.5% agarose gel, and DNA fragments in each sample separated by electrophoresis in Tris (hydroxymethyl) aminomethane - boric acid - EDTA (TBE buffer). The GG genotype was recognized by the presence of 19 and uncut 292 base pair (bp) DNA fragments. With the CC genotype, the restriction endonuclease recognizes a G to C polymorphism at position 777 of the IL-8 receptor locus, resulting in 19, 103, and 189 bp DNA fragments. The heterozygous (GC) genotype was recognized by the presence of all four (19, 103, 189, and 292 bp) DNA fragments. Cows were grouped by genotype, and analysis of variance was used to compare mean somatic cell count, 305-day milk, protein, and fat data obtained from monthly DHI records.

Results and Discussion

The frequency of GG, GC, and CC genotypes of the 75 Holstein cows evaluated in this study were 0.33, 0.47 and 0.20, respectively. The allele frequency was 0.57 and 0.43 for G and C, respectively. This allele frequency was the same as previously reported for Holstein cows (Youngerman et al., 2004a). In order to select for or against a polymorphism, it must occur at a high

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enough frequency in a given population. With over half of the Holstein cows in the current study having the G allele for this IL-8 receptor polymorphism, selection should be effective. The allele frequency appears to vary with breed of dairy cattle. Jerseys are reported to have an allele frequency of 0.87 and 0.13 for G and C, respectively (Youngerman et al., 2004a).

The inflammation caused by mastitis infection causes large numbers of leukocytes (somatic cells) to be shed into the udder to kill bacteria. Therefore, somatic cell counts are used as an indicator of the presence and severity of mastitis. A somatic count of 200,000 or greater is considered the threshold for mastitis infection. Cows with the GG or GC genotypes for the IL-8 receptor had lower mean somatic cell counts ($P = 0.01$) than cows with the CC genotype (Table 1). Genotype had no effect on 305-day adjusted milk ($P = 0.40$), protein ($P = 0.57$), or fat ($P = 0.84$) production.

According to the DHI Dairy Records Management System website (<http://www.drms.org/dhia.htm>), there is increasing milk loss during lactation with increasing somatic cell count. Cow with a somatic cell count between 284,000 and 565,000 (as is the case for the cows with the CC genotype in the present study) would be expected to produce about 1,200 pounds less milk during their lactation than cows with very low somatic cell counts. In the present study, cows with the CC genotype produced 1,234 pounds less milk than the average for cows with the GG and GC genotypes.

The National Mastitis Council (www.nmconline.org) estimates the annual cost in milk loss, increased culling, veterinary services, and treatment averages about \$180 per cow per year, or about 2 billion dollars annually in the U.S. Antibiotic resistance of strains of bacteria responsible for most incidences of mastitis has increased for several years making treatment less effective (Rajala-Schultz et al., 2004). Results of the present study suggest selection based on genotypes for the IL-8 receptor polymorphism would be effective in reducing the incidence of mastitis in dairy cattle.

Literature Cited

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Table 1. Effects of Interleukin-8 receptor polymorphism on somatic cell count, milk yield and quality.

IL-8 receptor genotype	No. of cows	Mean SCC (thousands)	305-d avg milk, lb	305-d avg milk protein, lb	305-d avg milk fat, lb
GG	25	81 ± 14 ^b	27,427 ± 691	789 ± 22	1,007 ± 29
GC	35	114 ± 24 ^b	26,328 ± 505	773 ± 15	995 ± 23
CC	15	374 ± 147 ^a	25,643 ± 948	751 ± 22	983 ± 43

^{a,b}Mean somatic cell count differed among genotypes ($P = 0.01$). Genotype had no effect on 305-day average milk ($P = 0.40$), protein ($P = 0.57$), or fat ($P = 0.84$) production.