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Short Communication

The bovine CXCR1 gene is highly polymorphic

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ABSTRACT

Several single nucleotide polymorphisms (SNP) in the bovine CXCR1 gene have been implicated in resistance to mastitis and milk somatic cell counts in several sample populations of Holstein dairy cows. As such, a more thorough understanding of SNP present in and near the bovine CXCR1 gene is needed. This study identified 36 SNP in the coding region and surrounding sequences of CXCR1 in 88 Holstein dairy cows. Four SNP induced amino acid changes and 1 SNP an early stop codon. Two amino acid changes occur in the third intracellular loop and C-terminus in locations tied to intracellular signaling. The 36 SNP could be subdivided into 4 separate linkage groups. Using representative or 'tag' SNP from each linkage group, haplotypes or the combination of SNP found on a single allele were generated to increase the specificity of an animal's genetic background. Four haplotypes were identified that represented 99% of the sample population. The haplotypes generated using tag SNP agreed with haplotypes generated from SNP causing amino acid changes. In conclusion, the CXCR1 gene is highly polymorphic and has potential implications towards genetic selection and understanding host factors that increase the risk of infection.

1. Introduction

The bovine CXCR1 gene is a high affinity receptor for the chemokine interleukin (IL)-8 or CXCL8 (Lahouassa et al., 2008). The CXCR1 ligand, IL-8 is released during infection by epithelial cells, macrophages, endothelial cells, fibroblasts and other cells in response to invading pathogens or stressors such as low oxygen, oxidative stress, and low glutamine (DeForge et al., 1993; Desbaillets et al., 1997; Shuster et al., 1997; Rambeaud et al., 2003; Bannerman et al., 2004; Bobrovnikova-Marjon et al., 2004). Subsequent binding of IL-8 to CXCR1 or the more promiscuous CXCR2 induces migration, regulates cell survival, modifies cytokine production, and increases phagocytosis, and reactive oxygen species generation (Kettritz et al., 1998; Mitchell et al., 2003; Lahouassa et al., 2008). The primary population influenced by IL-8/CXCR1 binding has traditionally been attributed to neutrophils that have the greatest expression of CXCR1 (Grob et al., 1990). However, recent studies have implicated this ligand–receptor combination in migration and survival of metastatic breast cancer, melanoma, and prostate cancer cells (Charafe-Jauffret et al., 2009; Gabellini et al., 2009; Shamaladevi et al., 2009). CXCR1 also has been observed on NK, CD4+ T, CD8+ T, dendritic, epithelial, and endothelial cells and presumably would also influence cellular migration and survival (Sallusto et al., 1998; Murdoch et al., 1999; Takata et al., 2004; Berahovich et al., 2006; Gasser et al., 2006). With the significant rise in IL-8 typically observed during periods of inflammation and stress, the presence of CXCR1 on multiple cell types that influence immunity, and the ability to modify cellular migration and survival, suggests the IL-8/CXCR1 axis could have a significant impact on disease resistance.

Recent studies have demonstrated the bovine CXCR1 gene contains at least 8 single nucleotide polymorphisms (SNP) (Youngerman et al., 2004b; Leyva-Baca et al., 2008). A SNP at position CXCR1+777 (G>C) relative to

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the mRNA sequence (U19947) leads to a Q245H amino acid replacement that has been significantly associated with alterations in bovine neutrophil migration, apoptosis, adhesion molecule expression, and reactive oxygen species generation (Rambeaud and Pighetti, 2005, 2007; Rambeaud et al., 2006). In a sample Holstein population, cows with a CC genotype experienced a higher incidence of chronic intramammary infections (37%) versus cows with either a GG (22%) or GC (22%) genetic background (Youngerman et al., 2004a). Later studies examining the relationship of this SNP to a common indicator of intramammary infection, somatic cell count (SCC) or score (SCS) have provided mixed results. Two large-scale studies that examined SCS in daughters or granddaughters of bulls, revealed no significant association with SCS (Leyva-Baca et al., 2008; Goertz et al., 2009; Beecher et al., 2010). However, an on-farm study with approximately 250 cows across 5 breeds revealed an association between CXCR1+777 SNP and SCS ($P < 0.10$) (Beecher et al., 2010). In contrast to CXCR1+777, the SNP CXCR1−1768 relative to NM_001557.2 was significantly associated with SCS (Leyva-Baca et al., 2008). The association of at least two SNP with bovine neutrophil function and measures of mastitis susceptibility suggests this gene is critical to disease resistance. Thus a better understanding of the genomic structure and polymorphisms present in the bovine CXCR1 gene, along with a common numbering system are needed.

2. Materials and methods

Total RNA was isolated from bovine neutrophils (TriZol, Invitrogen, Carlsbad, CA). The 5′ and 3′ ends were identified by rapid amplification (GeneRacer; Invitrogen), cloning into pGEMTeasy vectors, and sequencing by the University of Tennessee core facility. The 5′ and 3′ cDNA ends were assembled using Invitrogen Vector NTI (v 10.0) to generate the full length cDNA sequence. The full length cDNA sequence was then aligned with the Bovine UCSC browser (Bos taurus, build 4.0) to identify exon and intron boundaries and submitted to Genbank (accession HM367082).

To identify polymorphisms in CXCR1, genomic DNA was isolated from 88 cows located at the Middle Tennessee Research and Education Center (Springfield, TN) that had completed at least one full lactation. Genomic DNA spanning the coding region and sequences 5′ and 3′ to the coding region were amplified by PCR, sequenced by the University of Tennessee core facility, and polymorphisms detected using Sequencher 4.2 software (Gene Codes Corp., Ann Arbor, MI). Selected areas of the CXCR1 gene were amplified as follows: 40 ng of bovine genomic DNA was used as template in a 20 μl reaction containing specific primers and Eppendorf HotMaster Mix (Eppendorf North America; Westbury, NY) according to manufacturer's guidelines. The conditions for amplification were as follows: an initial hot-start denaturation occurred at 94 °C for 2 min, followed by 37 cycles of 94 °C denaturation for 30 s, 58–61 °C annealing for 30 s, and 68 °C extension for 45 s. After the last cycle, a 10 min final extension step at 68 °C was added before reactions were chilled to 4 °C. Amplified products were purified to remove primer and excess nucleotides by treatment with exonuclease-shrimp alkaline phosphatase (Exosap-it, USB Corporation; Cleveland, OH) as per the manufacturer's guidelines. Haplotypes were generated using PHASE (Stephens et al., 2001; Stephens and Scheet, 2005). Using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), Bovine CXCR1 (HM367082/ADK47157.1) also was compared against human (NM_000634.2/NP_000625.1), mouse (NM_178241.4/NP_839972.1), rat (NM_019310.1/NP_062183.1), rabbit (NM_001171082.1/NP_001164553.1), and swine (XM_003133655.2/XP_003133703.2) CXCR1 protein sequences to determine amino acid conservation and similarity among species (Table 1 and Fig. 1). Swine currently does not have a reference sequence for CXCR1 but the sequence chosen for comparison (XM_003133655.2) was based on chromosomal position on Sscrofa9.2 (UCSC genome browser).

Table 1
Similarity scores among CXCR1 protein sequences evaluated through ClustalW2.

	Bovine	Human	Swine	Mouse	Rat	Rabbit
Bovine	100	72	86	62	61	76
Human		100	75	64	66	83
Swine			100	63	63	76
Mouse				100	89	66
Rat					100	67
Rabbit						100

3. Results and discussion

The current full length cDNA sequences available for bovine CXCR1 in Genbank indicate a gene with a single exon (NM_001105038.1, U19947.1, NM_174360.2). However, in humans CXCR1 contains two exons (Sprenger et al., 1994), suggesting the full length bovine mRNA sequence has not yet been determined. Therefore, in order to accurately reflect the nature of polymorphisms identified, as well as conform to naming standards, it was necessary to clone the full length cDNA sequence. An 1800 bp cDNA sequence was generated from bovine neutrophils (Genbank HM367082) which shares 99% identity with the bovine CXCR1 reference sequence (NM_001105038.1). Subsequent alignment to genomic DNA (AC150887.4) using BLAST (http://blast.ncbi.nlm.nih.gov) revealed CXCR1 has two exons and 1 intron, which corresponds to the genomic structure of human CXCR1 (NG_011814.1) (Sprenger et al., 1994). Exon 1 is 87 bp (149,023–149,109 bp of AC150887.4), exon 2 is 1713 bp (151,327–153,041 bp of AC150887.4) with an open reading frame of 1081 bp, and the intervening intron is 2219 bp, which is consistent with predicted bovine CXCR1 (Pighetti and Rambeaud, 2006).

In CXCR1, 36 nucleotide polymorphisms were identified (Table 2). Eleven were located in the coding region: 4 introduced amino acid changes, 1 introduced a stop codon, and 6 were synonymous. The remaining 25 polymorphisms were located in the first intron, 3′ untranslated region, and 3′ to the gene. Naming of the polymorphisms is based on the Human Genome Variation Society guidelines (http://www.hgvs.org/mutnomen/recs.html), with the A of the ATG translation start site serving as position 1. Six of