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CXCR Gene and its Association with Mastitis Resistance in Cattle- a Review

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Abstract

Genes associated with immune responses of mammary gland are potential genetic markers because of their importance in mastitis. Neutrophil functions are potential genetic markers for mastitis, as neutrophil migration from blood to the sites of infection is essential for resolution of most mastitis pathogens. The ability of neutrophils to migrate into infected tissues is dependent upon recognition of inflammatory mediators by neutrophil cytokines, chemokines, and complementary receptor. Among various chemokines, one of the important chemokine associated with leukocyte migration is interleukin-8 (IL-8). Being receiver and transmitter of the signal from IL-8 to downstream, the receptors of IL-8 (CXCR) are the important candidate genes for mastitis tolerance/ susceptibility study in the cattle herd.

1. Introduction

Mastitis is the inflammation of the mammary gland resulting mainly from the invasion of contagious or environmental pathogens into the teat canal. Classically, mastitis pathogens have been classified as either contagious or environmental (Blowey and Edmondson, 1995). Watts (1988) identified 137 different organisms as a cause of mastitis. Based upon the severity of the inflammatory response, mastitis manifests itself in clinical and (or) sub-clinical forms and both leading to substantial economic losses (Owen et al., 2000). The contagious pathogens are generally spread from cow to cow during the milking process (Radostits et al., 1994). Incidence of contagious mastitis depends upon the dose and type of microorganisms to which a cow is exposed, physical and physiological barriers and the innate and acquired immunity. The most common contagious organisms are *Staphylococcus aureus* and *Streptococcus agalactiae*. The *Staphylococcus aureus* emerges as one of the most prevalent contagious mastitis pathogens. *Str. agalactiae* causes higher somatic cell count (SCC) of milk than the other pathogens. It spreads widely within a herd, causing immediate loss due to reduced milk yield. The infections caused by *S. aureus* and *Str. agalactiae* are mainly of a sub-clinical character, with periodic flare up to clinical symptoms. The environmental pathogens are usually found in the housing environment. Most common of this group are *Escherichia coli, Klebsiella sp*. and *Streptococcus uberis* (Larry et al., 1996).

2. Chemokines

Among various chemokines, one of the important chemokine associated with leukocyte migration is interleukin-8 (IL-8). IL-8 is an ELR^+ CXC chemokines, which interacts with specific chemokine receptors viz. CXCR1 and CXCR2 present on the neutrophils surfaces. These chemokine receptors are required for maximum neutrophil function during infection (Murphy and Tiffany, 1991). Recognition of chemokines by CXCR1 and CXCR2 induces neutrophil activation, chemotaxis and eventual phagocytosis of pathogen (Peveri et al., 1988). Being receiver and transmitter of the signal from IL-8 to downstream, the receptors of IL-8 are the important candidate genes for mastitis tolerance/ susceptibility study in the herd. Variations in the inflammatory and immune responses to pathogen are mediated, in part, by variations in the DNA sequences of immune-related genes. Inflammatory and immune response are polygenic traits by nature, and numerous studies have demonstrated statistical and functional associations between inflammatory diseases such as mastitis and polymorphism in various immune-related genes. SNPs in the receptors can

critically alter the role of neutrophils and other immune cells. Thus they can help to determine an animal's susceptibility to disease. Identification of one or more genetic markers associated with mastitis susceptibility, resistance, or both would allow producers to decrease costs associated with mastitis by improving herd health through animal selection.

Chemokines are the family of functionally diverse protein that mediates a variety of inflammatory responses as well as cell trafficking and homing. It is well recognized that chemokines are the master controllers of leukocyte migration from the periphery to the site of infection (Moser and Williams, 2004). Around 50 chemokines have been identified in human and mice, to the date only 18 are known in cow. Chemokines are small proteins (8-10kda) with similar 3-dimentional structures. The chemotactic cytokines belong to the chemokine superfamily, which can be divided into four families (CXC, CX3C, CC, and C) according to the position of the first two closely paired and highly conserved cysteines near the amino terminus of the protein (Zlotnik and Yoshie, 2000). CXC chemokines can be subclassified into ELR^+ and ELR^- molecules based on the presence or absence of a specific amino acid motif of glutamic acid–leucine–arginine immediately before the first cysteine (Hebert et al., 1991). The ELR^+ CXC chemokines induce the migration of neutrophils.

3. Interleukin-8

IL-8 is an inflammatory cytokine that is produced by various cell types including lymphocytes (Gregory et al., 1988), neutrophils, monocytes/macrophages (Schroder et al ., 1987), and epithelial cells (Elner et al., 1990), including bovine mammary epithelial cells (Boudjellab et al., 1998). At the site of inflammation, IL-8 plays an important role in recruiting and activating neutrophils (Baggiolini et al., 1994; Caswell et al., 1999). During the acute phase of coliform mastitis, the concentration of IL-8 is greatly increased in mastitic milk. Interleukin-8 is therefore considered to be involved in the infiltration of neutrophils into mammary secretions during mastitis.

An influx of serum proteins into mammary secretions is one of the features of mastitic milk and is a sign of impairment of the blood-milk barrier (Sordillo et al., 1987). Interleukin-8 may impair the epithelial barrier function by regulating the tight junctions between epithelial cells (Coyne et al., 2002). Decreased concentrations of milk-specific proteins and increased serum protein concentrations have been observed in mastitic milk (Sordillo et al., 1987). Increased concentrations of IL-8 have been detected in mastitic mammary secretions (Barber et al., 1998). An increase in IL-8 was also reported in milk from quarters infused with LPS. IL-8 protein and its mRNA expression have been associated with bovine mastitis (Lee et al., 2006). The cows infused with 25 mg of rbIL-8, the rectal temperature and serum haptoglobin level were transiently elevated after the infusion, showing that intra-mammary infusion of rbIL-8 could elicit systemic inflammation (Atsushi et al., 2008). The chemotactic property of bovine IL-8 in attracting neutrophil was established by using bovine recombinant IL-8 (Caswell et al., 1999).

4. Chemokine Receptors

Chemokines and their receptors are likely to contribute to leukocyte trafficking to the mammary gland (Nishimura, 2003) and play an important role in the host immune response during acute and chronic intra-mammary infections. Presently, the only known mammalian receptors for ELR^+ CXC chemokines are CXCR1 and CXCR2, which belong to the seven transmembrane G-protein-coupled receptors (GPCR). CXCR1 and CXCR2 receptors are the major chemokine receptors expressed on neutrophils. They are prototypical receptors for inflammatory/inducible chemokines. Human neutrophils express both receptors viz, CXCR1 and CXCR2, which respond by chemotaxis to ELR^+ CXC chemokines (Wuyts et al., 1997). The amino acid sequence contains a G-protein-coupled receptors family 1 signature at the junction between the third transmembrane domain and the second intra-cellular loop. The DRY residues are shared by almost all G-protein-coupled seven transmembrane domain receptors (GPCRs), while the full motif, DRYLAIV, is the signature of the chemokine receptors (Horuk, 1994). This motif has been shown to be necessary for efficient coupling to G-protein and its mutation could impair the chemotaxis mediated by either CXCR1, CXCR2, CXCR3, CXCR4 or CCR5 (Burger et al., 1999). The N-terminal region and the three extra-cellular loops contain one cysteine residue. These four cysteine residues are highly conserved in almost all chemokine receptors and are potentially implicated in the formation of two disulfide bonds, one between the N-terminal and the third extra-cellular loop, and one between the first and the second extra-cellular loop. The C-terminal contains nine serine/threonine residues that are potential phosphorylation sites and one leucine-rich motif (LLKIMAIHGLI). The presence of these elements in the C-terminal region is important for the IL-8R-mediated chemotaxis (Fan et al., 2002; Richardson et al., 2003; Sai et al., 2004).

Alignment analysis showed that the C-terminal region of bovine CXCR1 and CXCR 2 are very similar as against the differences observed between human CXCR1 and CXCR2. In human, the C-terminal region determines partially some functional differences between CXCR1 and CXCR2, such as the rate of internalization of the receptor and the chemotaxis capabilities (Richardson et al., 2003). In bovine species, there is no difference in the C-terminal region that consequently does not determine functional differences between CXCR1 and CXCR2 (Lahouassa et al., 2008).

5. Association Studies of CXC Receptors with Mastitis Resistance

Youngerman (2004) have identified five SNPs in the CXCR1 chemokine receptor gene in Holstein cattle. However, of these five SNPs, only IL8RA1c. 777 G>C, that causes an amino acid change from histidine to glutamine at position 245 within the third intracellular loop of the receptor, was included in the mastitis association study. Cattle with CC genotype showed a greater incidence of subclinical mastitis (37%) than those with GC or GG genotype at this position (21 and 22% respectively).

Rambeaud and Pighetti (2005) reported that the neutrophils from cows with a CC genotype exhibited impaired neutrophil migration and CD11b/CD18 upregulation in vitro. Later Rambeaud and Pighetti (2006) also reported that neutrophils from Holstein cows with different CXCR1 genotypes vary in their ability to generate Reactive oxygen species (ROS) and suppress apoptosis. Rambeaud and Pighetti (2007) observed that the cows with the CC genotype had lower neutrophil binding affinity for IL-8 and significantly less Ca^{2+} release following IL-8 stimulation in vitro, suggesting that CXCR1 signaling may be different between these genotypes (CC and GC/GG).

The annotation of CXCR1 was corrected by Pighetti and Rambeaud (2006). They reported that the sequence for CXCR2 with GenBank reference number NM_174360.2 actually corresponds to CXCR1. Although this correction had no impact on the previous association and functional studies performed by Youngerman (2004) and Rambeaud and Pighetti (2005) the investigated polymorphisms now fit in CXCR1 rather than CXCR2.

Leyva-Baca (2008) identified four novel single nucleotide polymorphisms (SNP) in the 5' upstream region of, CXCR1c.–344T>C, CXCR1c.–1768T>A, and CXCR1c.–1830A>G, and a previously identified SNP in the coding region, CXCR1c.777G>C. The four SNP were genotyped in Canadian Holstein bulls (n = 338) and among them SNP CXCR1c.–1768-T>A have been associated with SCS during the first and second lactations. Although Leyva-Baca (2008) did not demonstrate an association between SNP CXCR1c.777G>C and SCS. However this SNP has been previously associated with subclinical mastitis, SCS, milk yield, and neutrophil function in Holstein dairy cattle by others (Youngerman et al., 2004; Rambeaud and Pighetti, 2005). Reasons for the discrepancy might be because of different sample sizes. Leyva-Baca (2008) used a sample of 338 Holstein bulls, whereas Youngerman et al. (2004) reported an association in 37 Holstein cows. Single nucleotide polymorphisms (SNPs) were also identified in CXCR2 receptor gene of Vrindavani cattle (Dige, 2009).

6. Conclusion

Significant associations of genotypes with clinical mastitis found may be used as genetic markers for selection of animals and to include these parameters in cattle breeding programme. Attempts also needed to explore some more candidate genes associated with mastitis.

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