Acute Phase Proteins: How they portray Mastitis – A review

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ABSTRACT

Acute Phase Proteins are blood proteins primarily synthesized by hepatocytes as part of the acute phase response (APR). APR to disease is accompanied by an increase in the circulating concentration of a number of plasma proteins which are collectively known as the Acute Phase Proteins. According to the concentration, APPs are classified in positive APP, if they increase or negative APP, if they decrease. During mastitis, APPs move from the systemic circulation to mammary gland or there could be de-novo synthesis of the APP within mammary gland tissues. Among the APPs, Serum Amyloid A (SAA), Haptoglobin (Hp), Alpha 1-acid glycoprotein and Lipopolysaccharide binding protein play a major role during mastitis in cattle and their concentration will increase with the severity of the infection, inflammation or trauma. SAA is involved in defense against Gram positive and Gram negative pathogens. SAA acts by modulating innate immune system and by acting as an opsonin. Among the SAA isoforms, mammary associated SAA3 (m-SAA3) is important. The blood concentration of SAA and Hp increases dramatically after LPS infusion, with SAA (acute and middle phase of APR) appearing before Hp (Late phase of APR). The herd level APP might be useful for determining the prevalence of clinical and subclinical infections indicated by the high serum concentration of selected APP and by serving as the prognostic tool. APPs, used as markers of animal health may possibly be influenced by environmental factors, handling procedures and other types of stress in the absence of disease.

Key words: α1-acid glycoprotein, Haptoglobin, Lipopolysaccharide binding protein, Mastitis, Serum amyloid A.

Local inflammation is the first response of the immune system to noxious stimuli. When infections and tissue injuries overcome local defense mechanisms, the organism responds by activating a wide range of systemic response known as acute phase response (Gabay and Kushner, 1999). It is an intricate process where several cell types and a network of proteins initiate, amplify, sustain, control and eventually reduce the inflammatory reaction (Ceciliani et al., 2012). The APR was defined for the first time by Abernethy and Avery in 1941. These systemic responses to disease are accompanied by an increase in the circulating levels of a number of plasma proteins which are collectively known as the Acute phase proteins. (Moshage, 1997). In veterinary medicine, determination of the plasma concentration of these proteins gives valuable clinical information on infection and inflammatory processes (Gruys et al., 1994). APP synthesis occurs in the hepatocytes and extra hepatic sources like mammary gland. Depending on the concentration, it is classified into positive if concentration increases and negative if concentration decreases upon stimulus. Recent evidences suggest that APPs act as modulators of inflammatory response by interacting with foreign and defense cells (Petersen et al., 2004). During infection, majority of APP synthesis occurs in the liver and is released into general circulation and reaches the target tissue such as mammary gland, but de novo synthesis of APP occurs in mammary gland also (Thulasiraman et al., 2013). In India, around Rs. 72 billion per year is lost due to mastitis. Mastitis also alters the milk composition depending upon the type of pathogen (Bansal and Gupta, 2009). So, it is highly recommended to use APP for therapy and control of mastitis due to its immunomodulatory effects (Sunagar et al., 2013).

Cytokines in acute phase protein Synthesis: Lipopolysaccharide (LPS), a component of the Gram negative bacteria cell wall is a potent inducer of inflammation and the acute phase response (Berczi, 1998). Liver is a major contributor of circulating inflammatory cytokines having a local regulatory role in the hepatic APP synthesis. The proinflammatory cytokines like tumor necrosis factor-α (TNF-α), IL-1β and IL-6 activate hepatocytic receptors and initiate the synthesis of APP such as serum amyloid A, lipopolysaccharide binding protein, haptoglobin (Hp) and α1-acid glycoprotein (Murata et al., 2004). Bovine Kupffer cells, the major cytokine-producing cells (Yoshioka et al., 1998) secrete SAA and Hp in a time and dose dependent manner when stimulated with recombinant human

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proinflammatory cytokines (Yoshioka et al., 2002). In dairy cows, LPS administered I/M increased the milk as well as plasma concentrations of TNF-α, SAA and Hp suggesting both a local tissue production in the udder and a release of inflammatory cytokines and APP into the general circulation (Hiss et al., 2004; Lehtolainen et al., 2004).

**Acute phase protein response**: The major stimulatory cytokines during APR from macrophages and monocytes at the inflammatory site are interleukin-1 (IL-1), interleukin-6 (IL-6) and TNF-α (Heinrich et al., 1990). Production of the acute phase proteins by IL-6, following binding to the IL-6 receptor, is through the phosphorylation of the transcription factor, NF-IL6 which is then translocated to the nucleus where it mediates the transcription of acute phase genes. IL1 and TNFα, after binding to their native receptors cause phosphorylation and degradation of IκB, the inhibitor of transcription factor, NF-KB leading to release of NF-KB and subsequent activation of acute phase genes in the nucleus (Jensen and Whitehead, 1998).

**Serum amyloid A**: These are produced by liver during the acute phase after induction by proinflammatory cytokines, such as IL6 and TNFα (Uhlar and Whitehead, 1999). The SAA3 is synthesized mainly in extra hepatic tissues. In cattle, a mammary associated SAA3 (M-SAA3) has been isolated and characterized (McDonald et al., 2001).

**Role of SAA**: Bovine mammary gland epithelial cells (MAC-T) have been shown to produce a mammary associated SAA3 (M-SAA3) under normal physiological conditions (McDonald et al., 2001). It modulates innate immune reactions by acting as a chemoattractant and mediates the migration, adhesion and tissue infiltration of monocytes and neutrophils to the infected mammary gland (Badolato et al., 1994). It also acts as an innate immunity opsonin where lipoteichoic acid (LTA), the Gram positive associated PAMP stimulates the expression of M-SAA3 by mammary gland epithelial cells (Weber et al., 2006) and is involved in mammary gland defense against both Gram positive and Gram negative pathogens. Stimulation of MAC-T cells with LPS and prolactin revealed an increased expression of M-SAA3 while other SAA transcripts were not detected (Larson et al., 2005). The M-SAA3 peptide showed antibacterial activity against E. coli, Streptococcus uberis and P. aeruginosa (Molenaar et al., 2009). Experimental infection with S. aureus resulted in region-specific SAA3 (M-SAA3) changes and expression was increased in alveolar, ductal and gland cistern tissues (Whelenah et al., 2011). SAA value in milk (µg/ml) of healthy, subclinical and clinical mastitis infected from Streptococcus and Staphylococcus were observed as 0.06±0.03, 2.37±0.81 and 8.66±4.52, respectively whereas SAA value in serum (µg/ml) were observed as 6.12±5.44, 94.18±24.15 and 221.31±46.69, respectively (Kumar et al., 2014a).

**Haptoglobin**: Bovine Hp is composed of two 20-kDa peptides (α chain) and two 35-kDa peptides (β chain) linked by disulfide bonds (Morimatsu et al., 1991). Purified native Hp has a molecular mass of 1000–2000 kDa. In cattle, plasma haptoglobin exists as polymers in association with albumin (Eckersall and Conner, 1990).

**Role of Hp**: By binding to hemoglobin, Hp acts as an antioxidant for iron stabilization (Lim et al., 1998). It results in a reduction of oxidative damages to Hb itself (Buehler et al., 2009). Free Hb can bind to nitric oxide in a fast and irreversible way scavenging NO and limiting its bioavailability (Rother et al., 2005). The binding of the complex Hp–Hb to CD163 of monocytes/macrophages results in the up-regulation of anti-inflammatory mediators, such as inducible heme oxygenase-1 (HO-1) and IL10 release (Philippidis et al., 2004, Schaer et al., 2006), thus activating an anti-inflammatory response. Neutrophil activity can be down-regulated by inhibiting both lipoxygenase and cyclooxygenase (Saeed et al., 2007). Hp expression in the mammary gland is restricted to pathological conditions (Hiss et al., 2004). The role of Hp is to bind to hemoglobin released from damaged erythrocytes and to help restrict the availability of free iron to invading bacteria. The affected quarter may also produce substances related to the acute phase proteins (Eckersall et al., 2001). Hp in cattle, with acute inflammation was significantly higher than that in cattle with chronic inflammation (Horadagoda et al., 1999). Hp value in milk (µg/ml) of healthy, subclinical and clinical mastitic cows infected from Streptococcus and Staphylococcus were observed as 28.96±28, 168.23±41.13 and 216.00±42.01, respectively whereas Hp value in serum (µg/ml) were 18.60±3.26, 143.46±46.45 and 724.30±307.32, respectively (Kumar et al., 2014b). In Murrah buffaloes, the Hp value in milk (µg/ml) of healthy, subclinical and clinical mastitis infected from Streptococcus and Staphylococcus were observed as 19.27±4.16, 92.00±16.54 and 395.00±170.76, respectively whereasHp value in serum (µg/ml) were 82.36±29.58, 1754.00±336.24 and 2512.50±681.82, respectively (Kumar et al., 2014a).

**Lipopolysaccharide binding protein**: LBP is one of the key members of the innate immune response against bacteria. LBP has been identified as a 50 kDa polypeptide which, after post-translational modification is released as a 60–65 kDa glycoprotein in the blood stream (Horadagoda et al., 1994; Schumann et al., 1990; Khemlani et al., 1994). The main role of LBP is to modulate the innate immune response (Lamping et al., 1996). LBP binding and presenting activity is not limited to LPS, since LBP can also bind LTA, a PAMP
exposed on the surface of Gram positive bacteria such as S. pneumonia and S. aureus (Schröder and Schumann, 2005). As other APPs, LBP also acts as an opsonin by binding to Salmonella and K. pneumoniae and promoting their phagocytosis (Wright et al., 1989, Fan et al., 2002). Mammary tissue from healthy animals exhibits high expression of LBP in the teat cistern and the parenchyma (Rahman et al., 2010). With regard to pathological conditions (E. coli infection), LBP expression is increased in lobulo-alveolar regions but not in the teat cistern or the gland cistern (Rinaldi et al., 2010).

1-Acid glycoprotein: Bovine AGP is a 219 amino acid residue glycoprotein with a molecular weight of 20.5 kDa. It is a lipocalin that can bind and transport small hydrophobic molecules (Flower et al., 2000). AGP may act as plasma transport protein and could modulate the inflammatory response. They further protect the organism against bacteria, and acts as chaperone.

Role of AGP: AGP can bind more than 300 different molecules and drugs (Israili and Dayton, 2001). The binding and delivery function of AGP is remarkable and may markedly increase its concentration during acute phase, thus becoming one of the most abundant proteins in serum (Sheldon et al., 2001, Eckersall et al., 2001). Bovine AGP down-regulates PMN extracellular release of ROS while still maintaining the capability to kill Gram positive bacteria such as S. aureus (Rinaldi et al., 2008). AGP being stored in secondary granules is released from the cells after activation. An acute phase concentration of AGP reduces the apoptosis of bovine monocytes (Ceciliani et al., 2007). Expression of AGP has been demonstrated in mammary glands from healthy animals (Ceciliani et al., 2005, Ceciliani et al., 2007). In ductal and gland cistern tissue, AGP mRNA is increased whereas in alveolar tissue, it decreased post S. aureus challenge (Whelehan et al., 2011).

Acute phase proteins as biomarkers of mastitis in cattle: Mastitic milk contains mammary isoform of SAA (M-SAA3) which should be differentiated from the other isoforms of SAA in serum (Molenaar et al., 2009, Hiss et al., 2004). Acute and chronic mastitis caused by S. aureus lead to an increase in Hp and M-SAA3 concentration as compared to non-mastitic subjects whereas, increased concentration was found only in M-SAA3 in chronic cases (Gronlund et al., 2003). Staphylococcus aureus infection upregulates the expression of Hp and M-SAA3 by 133 fold, 27 fold for defensins and 15 fold for cytokines (Whelehan et al., 2011). Inoculation of 1500 cfu E. coli in cows caused mastitis more rapidly in the first inoculation than in the subsequent inoculation after 14 days. The pattern of acute phase proteins also followed the same trend (Suojala et al., 2008) which would indicate that the APP response is related to the severity of the disease and was also demonstrated by SCC and N-acetyl-b-D-glucosaminidase (NAGase), another recognized biomarker for mastitis. Extra mammary inflammatory disease caused large increases in serum SAA concentrations but did not affect the M-SAA3 or Hp concentration in milk. Like Hp and SAA, LBP concentration increased in mastitic milk as well as serum in response to LPS from E. coli (Schroedl et al., 2001, Bannerman et al., 2003) whereas inoculation with P. aeruginosa caused increase in milk LBP and increased LBP and SAA in serum. These changes were related to elevated levels of bovine cytokines including IL-1β, IFN-γ and TNF-α. Monitoring the changes in serum LBP and SAA concentrations have also been used to characterize the innate immune response to experimental induction of mastitis with Mycoplasma bovis infection (Kauf et al., 2007). There is a significant correlation between SCC and M-SAA3, which could be used as a potential marker for subclinical mastitis (Gerardi et al., 2009). The Holstein and Jersey cows inoculated with E. Coli developed mastitis and showed same innate immune response as well as Acute phase Protein (SAA and LBP) expression (Bannerman et al., 2008). Naturally affected mastitic cows showed increased milk and blood concentration of LBP whereas, no difference was found between the subclinical mastitic and non-affected cows (Zeng et al., 2009). Hp has the characteristics of a major APP in ruminants where it acts as an indicator of inflammation (Skinner et al., 1991, Cole et al., 1997). Application of Hp analysis in disease investigation in cattle has shown that it has a major diagnostic contribution in making investigations of mastitis (Hirvonen et al., 1996, Salonen et al., 1996). α1-Acid glycoprotein, which is present in higher concentration in healthy animal blood indicates that AGP exerts its action in the early phase of APR. On the contrary, SAA and Hp (not detectable in healthy state) increased drastically after LPS infusion with SAA appearing before Hp. This indicates that SAA plays a role in early and middle phase of APR and Hp in later phase (Vels et al., 2009).

CONCLUSION

It could be concluded that inflammation in the mammary gland stimulates the Acute Phase response in serum and milk but extra mammary inflammation stimulates the systemic acute phase reaction with no effect on APP in milk. APP and its assays can be used for experimental study of infectious, inflammatory diseases, their diagnosis, prognosis and their management. It is established that the cytokines play a vital role in mediating APR. A scanty information is available regarding the genomic regulation of these proteins. A thorough knowledge of APP will help to diagnose the subclinical mastitis at an earlier stage.
REFERENCES


