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The Role of ß-Defensins in the Immune Defense Against Bovine Mastitis

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Abstract: Mammary gland of a cow is highly susceptible to inflammation. Defensins that are recognized as one of the innate defensive response systems against the pathogenic microorganisms can affect the quality of cow milk. One hundred and ten milk and blood samples were collected from cows with infected or apparently normal mammary glands. Microbiological examination of the collected milk samples revealed that 40 out of 110 were infected (36.37%), 13 with *S. aureus*, 11 with *E. coli*, 8 with *Strep. agalactiae* and 8 with coagulase negative staphylococci (CNS). PCR of the genomic DNA extracted from the blood of cows suffering from mastitis showed expression of the defensin gene in 76.92 (10/13), 75 (6/8), 72.73 (8/11) and 50% (4/8) of mastitic cases caused by *S. aureus*, *Strept. agalactiae E. coli* and coagulase negative staphylococci, respectively. PCR of the genomic DNA extracted from the blood of cows with normal udder showed no expression of the defensin gene. So, it was concluded that ß-defensins display a major defensive mechanism against the pathogenic microorganisms that can affect the quality of cow milk. We recommend the introduction of selection markers (defensin peptides or genes) into animal breeding programs that could lower costs arising from treatment of mastitis and help to prevent the transmission of milk borne diseases to humans, especially immunocompromised individuals.

Key words: Mastitis · Cows · ß-defensins · Microorganisms · PCR

INTRODUCTION

Bovine mastitis, caused by bacterial, viral or fungal pathogens is one of the most costly dairy-based diseases worldwide. Contagious pathogens, like *Staphylococcus aureus* or *Streptococcus agalactiae*, tend to result in chronic, sub clinical mastitis, while the environmental coliform bacteria very often cause acute, clinical infections of the gland [1]. The reasons for these pathogen-related differences in the ability of the host's immune defense system to cope with infections of the mammary gland are unknown. They might reside in factors contributing to the innate branch of the immune system, since the Toll-like receptor (TLR) pathogen receptors [2] and the β-defensin-type bactericidal effector molecules [3] of the innate immune system are both known to display some pathogen specificity.

Defensins is a group of antimicrobial peptides with antibiotic and cytotoxic activity against bacteria, viruses and fungi existing in various human and animal organs including the mammary gland, their antibacterial properties refer both to the Gram-positive and Gram-negative bacteria [4,5]. DAS *et al.* [6] reported that testing of four purified defensin peptides against *Escherichia coli*, *S. aureus*, *Strept. pyogenes*, *Candida albicans*, Rinderpest Virus (RPV) and Newcastle Disease Virus (NDV), showed that these peptides possessed antimicrobial and antiviral activities. Minimum inhibitory concentration (MIC) values varied according to the peptide amounts and the exposure time.

In the human genome, the β-defensins are encoded by a group of genes localized to one locus on chromosome 8 [7], while in cattle they have been mapped to chromosome 27 [8]. There are many beta-defensins that are common to all bovines and are likely the result of a duplication event [9]; however, the genomic structure of these genes remains largely unknown. Because beta-defensins have been associated with the bovine immune reaction to mastitis [10, 11], they can be investigated as candidate genes for the development of potential genetic markers for mastitis resistance and susceptibility.

The present study aimed at determining the presence of defensin genes in cows with different udder health status .

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MATERIALS AND METHODS

One hundred and ten milk and blood samples were collected from local Egyptian cows either with infected or apparently normal mammary glands. Blood samples were collected from the jugular vein into tubes containing EDTA. All samples were collected in sterile containers under sterile precautions, kept in an ice box and transferred to the laboratory.

Microbiological Examination of Milk Samples: 10 ml from each of the milk samples was centrifuged for 20 min. at 3000 rpm. Milk sediment of each sample was inoculated onto plates of nutrient agar, blood agar and MacConkey agar. Inoculated plates were incubated at 37°C for 48hr. Suspected colonies appearing on the different media was examined microscopically and isolated in pure culture for further identification according to Holt *et al.* [12].

PCR Technique

DNA Extraction:. The isolation of DNA from whole blood samples was done using blood extraction kit (Biobasic, Canada).

PCR Amplification of Defensin Gene: The following primers were used for the amplification of defensin genes: BBD1S. 5.-GCCAGCATGAGGCTCCAT-3.and BBD2A. 5.-AACAGGTGCCAATCTGT-3.

The PCR reactions were performed in a Primus thermal cycler (MWG Biotech, Germany) (DNA denaturation. 94°C; annealing. 63.5°C, elongation. 72°C; 34 cycles) [13]. The quality of the polymerase chain reaction (PCR) product was electrophoretically tested [14] in 2% agarose gel with ethidium bromide.

RESULTS

Microbiological examination of the collected milk samples revealed that 40 out of 110 were infected (36.37%), 13 with *S. aureus*, 11 with *E. coli*, 8 with *Strep. agalactiae* and 8 with coagulase negative staphylococci (CNS). Amplification products of DNA of the cows infected with *S. aureus* (10/13, 76.92%), *E. coli* (8/11, 72.73%), *Strept. agalactiae* (6/8, 75%) and CNS (4/8, 50%) showed a band of 350 KDa representing defensin gene (Figs. 1, 2, 3and4). While those of the DNA of cows with normal udder showed no bands.

DISCUSSION

Mastitis continues to be one of the most detrimental diseases to profitable dairy farming; it not only reduces animal health and productivity, but also poses threats to milk quality and safety. Consequently, it has received considerable attention with regard to the development of informative genetic markers that will allow identification of cows and sires more resistant to disease [15].

Investigations are carried out aiming at identifying genetic markers related to the health status of the udder and particularly to the resistance to mastitis [4, 16]. Localized putative quantitative trait loci (QTLs) affecting milk production and health of dairy cattle were mapped in a very large Holstein granddaughter design [16]. Klungland *et al.* [17] using veterinary records and SCC of Norwegian dairy cattle localized QTLs for clinical mastitis to chromosomes 3, 4, 14 and 27.

It was shown that tissues of an infected human mammary gland contain ß-defensin gene transcripts [18] and that mammary epithelial cells secrete ß-defensins. Defensins are present not only in the mammary gland, but also in milk [19], as well as in leukocyte granules [20] and in macrophages [16], which constitute a part of milk cell population. Both cell types are responsible for phagocytosis of microbes [21]. Moreover, defensins are produced on all epithelial surfaces of the mammary gland [19].

PCR of the genomic DNA extracted from the blood of cows suffering from mastitis showed expression of the defensin gene in 76.92 (10/13), 75 (6/8), 72.73 (8/11) and 50% (4/8) of cases of mastitis caused by *S. aureus*, *Strept. agalactiae E. coli* and coagulase negative staphylococci, respectively. PCR of the genomic DNA extracted from the blood of cows with normal udder didn't show expression of the defensin gene. On the other hand, Petzl *et al.* [22] stated that the outcome of an udder infection is influenced by the pathogen species. They established a strictly defined infection model to better analyze the unknown molecular causes for these pathogen-specific effects, using *E. coli* and *S. aureus* strains previously asseverated from field cases of mastitis. Inoculation of quarters with 500 CFU of *E. coli* (n = 4) was performed 6 h, 12 h and 24 h before culling. All animals showed signs of acute clinical mastitis 12 h after challenge: increased somatic cell count (SCC), decreased milk yield, leucopenia, fever and udder swelling. Animals inoculated with 10 000 CFU of *S. aureus* for 24 h (n = 4) showed no or only

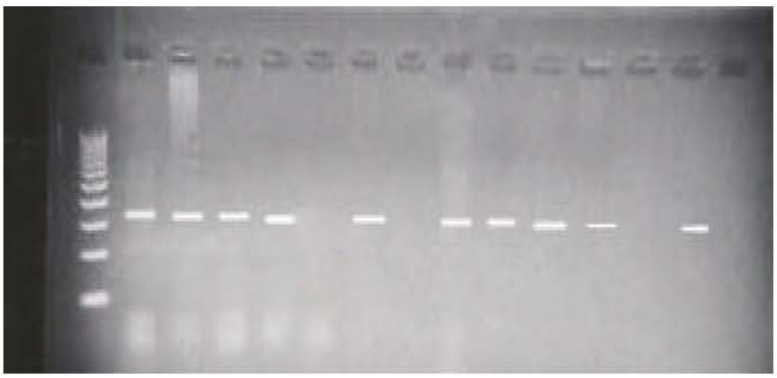

Fig. 1: Amplification of defensin gene (350 bp) of cows infected with *S. aureus*: (1) marker, (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and14): tested samples and (15): negative control

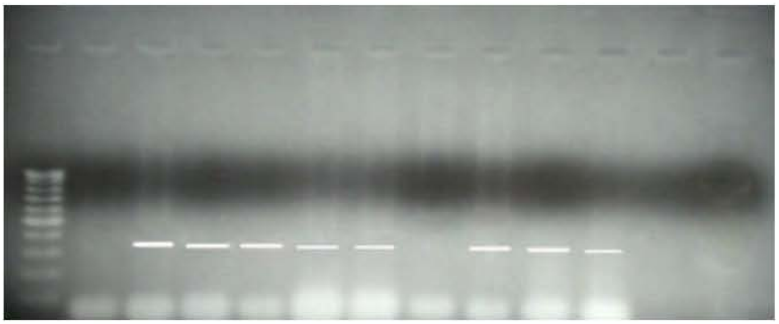

Fig. 2: Amplification of defensin gene (350 bp) of cows infected with *E. coli*: (1) marker, (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and12): tested samples and (13) negative control

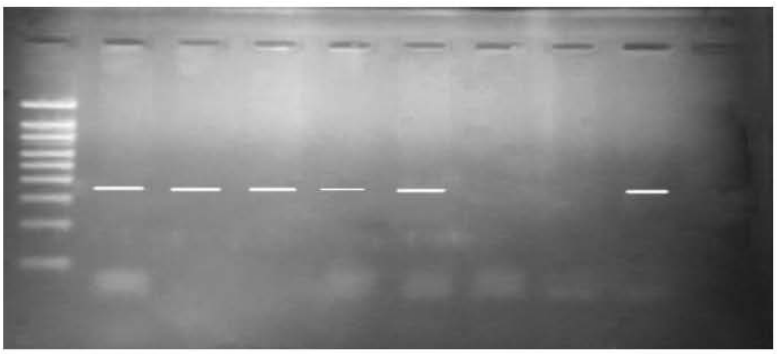

Fig. 3: Amplification of defensin gene (350 bp) of cows infected with *Strept. agalactiae*: (1) marker, (2, 3, 4, 5, 6, 7, 8and 9): tested samples and (10) negative control

Fig. 4: Amplification of defensin gene (350 bp) of cows infected with coagulase negative staphylococci: (1) marker, (2, 3, 4, 5, 6, 7, 8and 9): tested samples and (10) negative control

modest clinical signs of mastitis. However, *S. aureus* caused clinical signs in animals, inoculated for 72 h-84 h. Real-time PCR proved that *E. coli* inoculation strongly and significantly up regulated the expression of beta-defensins, TLR2 and TLR4 in the pathogen inoculated udder quarters as well as in mammary lymph nodes. *S. aureus*, in contrast, did not significantly regulate the expression of any of these genes during the first 24 h after pathogen inoculation. Only 84 h after inoculation, the expression of beta-defensins, but not of TLRs was significantly (> 20 fold) upregulated in five out of six pathogen inoculated quarters. Using the established mastitis model, the data clearly demonstrate a pathogen-dependent difference in the time kinetics of induced pathogen receptors and defense molecules.

In the light of these findings, it was supposed that defensins secreted into milk during lactation may protect the udder tissue from bacterial colonization. So, it can be concluded that ß-defensins display a major defensive mechanism against the pathogenic microorganisms affecting the udder. We recommend the introduction of defensin genes into animal breeding programs as a selection marker that could help to prevent the transmission of milk borne diseases to humans.

REFERENCES

1. Erskine, R.J., R.J. Eberhart, L.J. Hutchinson, S.B. Spencer and M.A. Campbell, 1988. Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. J. Am. Vet. Med. Assoc., 192: 761-765.
2. Aderem, A. and R.J. Ulevitch, 2000. Toll-like receptors in the induction of the innate immune response. Nature, 406: 782-787.
3. Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature, 415: 389.
4. Tunzi, C.R., P.A. Harper, B. Bar-Oz, E. Valore, J.L. Semple, J. Watson- MacDonell, T. Ganz and S. Ito, 2000. β-defensin expression in human mammary gland epithelia. Ped. Res., 48: 30-35.
5. Swanson, K., S. Gorodetsky, L. Good, S. Davis, D. Musgrave, K. Stelwagen, V. Farr and A. Molenaar, 2004. Expression of a beta-defensin mRNA, lingual antimicrobial peptide, in bovine mammary epithelial tissue is induced by mastitis. Infect Immun., 72: 7311-4.
6. Das, H., N. Swamy, G. Sahoo, S.U. Ahmed and T. More, 2008. Beta-defensin antibiotic peptides in the innate immunity of the buffalo: in vivo and in vitro studies. Altern. Lab. Anim., 36: 429-440.
7. Bevins, C.L. and G. Diamoud, 1997. Molecular biological strategies in the analysis of antibiotic peptide gene Families: The use of oligonucleotides as hybridization probes. Meth. In Mol. Biol., 78: 151-166.
8. Gallagher, D.S., J.A.M. Ryan, G. Diamoud, C.L. Bevins and J.E. Womack, 1995. Somatic cell mapping of beta-defensin genes to cattle synthetic group U25 and fluorescence in situ localization to chromosome 27. Mamm. Gen., 6: 554-556.
9. Luenser, K. and A. Ludwig, 2005. Variability and evolution of bovine beta-defensin genes. Genes Immun., 6: 115-22.
10. Goldammer, T., H. Zerbe, A. Molenaar, H.J. Schuberth, R.M. Brunner, S.R. Kata and H.M. Seyfert, 2004. Mastitis increases mammary mRNA abundance of beta-defensin 5, toll-like-receptor 2 (TLR2) and TLR4 but not TLR9 in cattle. Clin. Diagn. Lab. Immunol., 11: 174-185.
11. Strandberg, Y., C. Gray, T. Vuocolo, L. Donaldson, M. Broadway and R. Tellam, 2005. Lipopolysaccharide and lipoteichoic acid induce different innate immune responses in bovine mammary epithelial cells. Cytokine, 31: 72-86.
12. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology, 9th Ed. Williams and Wilkins, Baltimore.
13. Ryniewicz, Z., L. Zwierzchowski,, E. Bagnicka, J. Krzyz`wski and N. Strzalkowska, 2002. .Preliminary investigations on the polymorphism of defensin genes in cattle - relation with milk somatic cell count. Ani. Sci. Papers and Reports, 20: 125-131.
14. Sambrook, J., D. Russell and J. Gola, 2001. Molecular cloning, third Ed, A laboratory Manual., 1: 32-34.
15. Wall, R.J., A.M. Powell, M.J. Paape, D.E. Kerr, D.D. Bannerman, V.G. Pursel, K.D. Wells, N.C. Talbot and H.W. Hawk, 2005. Genetically Enhanced Cows Resist Intramammary Staphylococcus aureus Infection. Nature Biotechnol., 23: 445-451.
16. Zhang Q., D. Boichard, I. Hoeschel, C. Ernst, A. Eggen, B. Murkve, M. PWfistergensko, L.A. Witte, F.E. Vgrignola, P. Uimarl, G. Thaller and M.D. Bishop, 1998. Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. Genetics, 149: 1959-1973.

17. Klungland, H., A. Sabry, B. Heringstad, H.G. Olsen, H. Gomez-Rays, D.I. Vage, I. Osaker, J. Odegard, G. Klemetsdal, N. Schulman, J. Vikki, J. Ruane, M. Aasland, K. Ronningen and S. Lien, 2001. Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. Genome, 12: 837-842.
18. Exner, K., P.D. Thomsen, S. Paul, E. Kalm and C. Looft, 2000. Characterization of β-defensins- a family of peptide antibiotics also expressed in the epithelium, of the bovine mammary gland. ISAG Conference, Minnesota, July 22-26, Book of abstracts, P: 60, C006.
19. Jia, H.P., T. Starner, M. Ackermann, P. Kirby, B. Tack and B. Mc Cray, 2001. Abundant human b-defensin-1 expression in milk and mammary gland epithelium. J. Pediatr. 138: 109-112.
20. Diamond, G., D. Legarda and L.K. Ryan, 2000. The innate immune response of the respiratory epithelium. Immunological Rev., 173: 27-38.
21. Fehlbaum P., M. Rao, M. Zasloff and M. Anderson, 2000. An essential amino acid induces epithelial b-defensin expression. Proceedings of the National Academy of Sciences, USA., 97(23): 12723-12728.
22. Petzl, W., H. Zerbe, W. Yang, H.M. Seyfert and H.J. Schuberth, 2008. *Escherichia* coli, but not *Staphylococcus aureus* triggers an early increased expression of factors contributing to the innate immune defense in the udder of the cow. Vet. Res., 39: 18-22.