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Gene structure analysis of caprine and ovine Mannose Binding Lectin (MBL)

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ABSTRACT

The Mannose Binding Lectin (MBL) is an important factor of natural immunity which acts as a primordial antibody, capable to activate the complement and to link to some sugars (such as mannose) present on the surface of some bacteria as Brucella. In the present study, we report the partial structure of the gene coding for the MBL in sheep and goat as well as the analysis of their promoters. By using as template genomic DNA from leukocytes obtained from individual blood samples we sequenced from -961 nt of the promoter to the 397th nt of the 4th exon of four sheep (EMBL Acc. no. AM933378) and from -978 nt to the 471th nt of the 4th exon of four goats (EMBL Acc. no. AM933377), for a total of 4461 bp and 4515 bp, respectively. By comparing the homologous sequence of the bovine MBL gene (EMBL Acc. no. NC007327), it was possible to determine the size of the exons, similar in both investigated species: 200 bp (exon 1), 117 bp (exon 2), and 69 bp (exon 3). The comparison of the sequences between goat and sheep shows an homology of 92.2%. The differences are due essentially to insertions/deletions at intronic level: the sheep presents with respect to the goat a deletion at the 1st intron of 11 nt, two insertions at the 2nd intron, respectively of 29 and 41 nt, and at the 3rd intron a stretch of eight Guanine in the goat *vs.* eight Thymine in the sheep. The length of the three introns was 395 bp *vs.* 385 (intron 1), 1278 bp *vs.* 1347 (intron 2), and 1028 bp *vs.* 1019 (intron 3) respectively in goat and sheep. For both species, all the splice junctions follow the common role 5'GT/ 3'AG. The ORF (Open Reading Frame) encodes for 249 amino acid residues, whereas the leader peptide is composed by 19 amino acids. The starting codon (ATG, Methionine) is located between the 10th and the 12th nt of the 1st exon, while the stop codon (TGA) between the 372th and the 374th nt of the 4th exon. While from the comparison of the exonic sequences, 23 SNPs were identified, 10 of which give rise to an amino acidic change. The sequence analysis of the promoter region in sheep shows the presence of the following putative binding sites for transcription factors: TATA-box (ATAAA) and CCAAT-box (CCAAAT) in position -49 and -140 nt, respectively. Furthermore, two glucocorticoid responsive elements (GRE) were identified: in position -765 (AGATCAGA) and -971 (ACTGATCT). The promoter analysis for goat was found to be analogous to that of sheep but it shows the disappearance of the regulatory site GRE (ACTGATCT). Further studies need to be performed in order to verify whether the markers identified in the present study can be associated to the resistance to pathogens such as *Brucella melitensis* by carrying out suitable test for antimicrobial activity.