The Detection of a Mutation of CD18 Gene in Bovine Leukocyte Adhesion Deficiency (BLAD)

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(Received 15 June 1992/Accepted 5 October 1992)

ABSTRACT. Two calves (5 and 9 months old) affected with pneumonia and gingivitis were also diagnosed as having bovine leukocyte adhesion deficiency (BLAD). The gene of leukocyte adhesion molecule CD18 in these BLAD calves and their dams (carrier) were examined by means of polymerase chain reaction (PCR) and digestion of restriction endonuclease. The splicing in mRNA coded CD18 reported in human LAD was not recognized in BLAD on the basis of the results of PCR amplification. The region including the portion of point mutation, which corresponded to the region reported in the human patient, was amplified by PCR, and the PCR product was then digested with *TaqI*. An obvious difference was recognized in the pattern of digestion among healthy calves, BLAD calves and their dams. In BLAD, therefore, the point mutation reported in human patients was strongly suggested. Moreover it may be a method able to be used in detecting the carrier.—**KEY WORDS:** bovine leukocyte adhesion deficiency (BLAD), CD18 gene, mutation.

J. Vet. Med. Sci. 55(1): 145–146, 1993

Leukocyte adhesion deficiency (LAD) is reported as an autosomal recessive disorder in human [1]. This disease is characterized by severe, recurrent bacterial infections such as pneumonia, gingivitis, impaired pus formation, and delayed wound healing. The number of leukocytes in peripheral blood is over 100,000/ μ l and almost all leukocytes are neutrophil. The adhesion-related functions of neutrophil, including phagocytosis and chemotaxis, are deficient. Takahashi *et al.* [10] reported LAD-like disease in 7 Holstein calves and called it granulocytopathy syndrome. Up to the present time, 10 cases have been reported [3, 4, 9, 10], and their pedigrees included a common sire. The disease seems to be closely related to genetic disorder.

Adhesive interaction of a cell with other cells or with the extracellular matrix is mediated mainly by three families of adhesion proteins: the immunoglobulin family, the integrin family, and the selectin family [8]. The integrin family includes LFA-1, Mac-1 and p150, 95 [8]. These three adhesion molecules are heterodimers, each consisting of a unique α subunit (CD11a, CD11b and CD11c, respectively) and a common β subunit (CD18) [5]. In human LAD, the heterogeneous mutations of CD18 are closely involved in disorder in adhesion [6]. Also in cattle affected with granulocytopathy syndrome, Kehrli *et al.* [4] showed a deficiency of the Mac-1 glycoprotein (CD11b/CD18) in leukocytes and they called this disease bovine LAD (BLAD). In this study, we aimed to detect the genomic mutation of CD18 in BLAD and its carrier in order to estimate the potential risk of BLAD in breeding cattle by polymerase chain reaction (PCR).

Five and 9 months old calves were hospitalized for treatment of chronic pneumonia in the Veterinary Teaching Hospital, Rakuno Gakuen University. The numbers of leukocytes in the patients were 130,000 and 100,000/ μ l, respectively. Granulocytopathy and neutrophil disfunction were recognized in the patients. These two calves had a common sire, and the sire was of the pedigree of previously reported patients [10]. Western blotting analysis with concanavalin A (ConA) and anti-ConA antibody was carried out according to the method reported by Kehrli *et al.* [4], and revealed that a 160 kD

protein corresponding to Mac-1 glycoprotein (CD11b/CD18) defected in the membrane of leukocytes from each patient. These two calves were, therefore, diagnosed as having BLAD.

Using the leukocytes from these BLAD calves and their dams, genomic analysis of bovine CD18 was performed following the method for the detection of human probands [7]. As normal controls, healthy Holstein cows and Japanese Black cattle were used. These healthy cattle had a 160 kD protein (CD11b/CD18) in the membrane of the leukocytes, and the numbers of leukocytes were within the normal range. Total RNA including the mRNA of CD18 was extracted from peripheral blood leukocytes, and cDNA was synthesized by means of Molony murine leukemia virus reverse transcriptase (Bethesda Research Lab., Gaithersburg) and used as a template for PCR. Four primers for PCR were selected from the cDNA sequence of bovine CD18 (Genebank access number M81233) and synthesized using a DNA synthesizer (Model 380B; Applied Biosystems, Foster City). The nucleotide sequences of the primers were: AF, 5'-AAGCACCAGCCTG GTGAAGA-3' (location and direction: 18–37>); AR, 5'-ACTCGGTGATGCCATTGAGG-3' (<573–592); CF, 5'-CCTGTACAAAAGCAGCAACG-3' (764–783>); and BR, 5'-TTCTGCCAGTTTGTGTGCCA-3' (<1025–1044). In the case of human LAD, the mRNAs of patients and their parents were longer than the normal mRNA based on the analysis of reverse transcription-PCR with primers CF and BR [7]. These abnormal mRNA resulted from an abortive splicing of the intron [7]. In both bovine patients, their dams and healthy cattle, however, the length of the region corresponding to the abortive splicing site in human was the same (data not shown).

Some point mutations of CD18 gene were reported in human patients [2, 6, 7]. On the basis of these reports, in order to estimate the mutation of CD18 gene in BLAD calves and their dams, restriction endonuclease digestion of CD18 DNA amplified with primers AF and AR was performed with various restriction endonucleases. The results are shown in Fig. 1. Approximately a 600 bp long monoband was detected by PCR amplification. The size of

Fig. 1. Electrophoretic profiles of amplified CD18 cDNAs from healthy Holstein (N), 5-month-old calf of BLAD (P) and its dam (carrier) (C). Amplified DNAs (Lane 1) and their digests with *TaqI* (Lane 2) were electrophoresed through 2% agarose gel.

all the amplified DNAs was the same (Fig. 1, Lanes 1). On the other hand, after digestion with *TaqI*, obvious differences were recognized among healthy calf, BLAD calf and its dam. After digestion with *TaqI* in healthy calf, approximately 100, 200 and 300 bp fragments were recognized. In BLAD calf, approximately 200 and 400 bp fragments were detected. The 400 bp fragment may result from vanishing the restriction site between the 100 bp and 300 bp fragments. This 400 bp fragment may include the mutation of a single or a few nucleotides. In the dam, all fragments recognized in healthy and BLAD calf were detected. This suggested that the proband carrier had both normal and abnormal chromosome. In another patient and its dam, the same results were obtained. Also in the

sires of these calves, the same results as in the dams were obtained. These results indicate that it is possible to detect carrier cattle with a heterozygote of an abnormal gene of CD18 by PCR. In this study, it was not made clear where the mutation point was and how substitution of nucleotide occurs in the gene. We are continuing a detailed study of CD18 genomic mutation.

ACKNOWLEDGEMENTS. We thank to Prof. Dr. K. Kobayashi, Department of Laboratory Medicine, Hokkaido University School of Medicine, for helpful discussion and suggestions, and the Hokkaido Livestock Improvement Association for providing the blood of sires.

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