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Review article

Bovine leukocyte adhesion deficiency (BLAD) and its worldwide prevalence

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Abstract. Bovine leukocyte adhesion deficiency (BLAD) is a well characterised lethal autosomal recessive disease that occurs in Holstein cattle. The discovery of this genetic disorder in 1990 by KEHRLI et al. was serendipitous and occurred in conjunction with studies of new methods to prevent mastitis in periparturient dairy cows (KEHRLI et al. 1992a). In this review article we are attempting to summarise the last 6-year research (1990-1995) covering major aspects of BLAD syndrome, its worldwide prevalence with emphasize on current and future development on BLAD research.

Key words: BLAD diagnosis, clinical findings, gene.

Introduction

New breeding strategies in animal production involve intense insemination, embryo transfer and international exchange of semen, embryos or animals. These breeding practices not only increase genetic relationship of individuals within a breed because of the extensive use of selected sire but also supports worldwide spread of inherited diseases and other genetic disorders (WOMACK 1992). Although there are few well characterised primary immunodeficiencies (PID) in domestic animals, these disorders are important because they tend to be severe and are incurable so far. Animals with such disorders do not receive intensive and aggressive care required for survival. Because of the inheritable nature of PID, livestock producers need assistance from veterinarians to identify carriers and to establish sound breeding and control programs (MCVEY, TIZARD 1993).

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MULLER (1994) and SCHWERIN et al. (1994) described the most common autosomal recessive gene defects in cattle which are caused by artificial insemination, namely, BLAD, deficiency of uridine mono-phosphate synthase (DUMPS), the weaver syndrome, syndactylia, citrullinaemia, bovine cardiomyopathy, arachnomelia, porphyria, thyroglobulin deficiency (congenital hypothyroidism) and chondrodysplasia. Among all these genetic disorders, BLAD syndrome is of greatest economical importance. Because of intense research on this genetic defect, we would like to review the current knowledge of BLAD to reveal some future prospects.

Definition of BLAD

BLAD is an autosomal recessive disease characterised clinically by severe persistant and recurrent bacterial infection including ulcerative gingivitis, periodantitis, pneumonia, loss of teeth, ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth associated with mark neutrophilia and early death. Biochemically, it is characterised by a deficiency in the surface expression of the heterodimeric beta-2 integrin adhesion molecules on leukocytes, resulting in multiple defects in leukocyte function. The beta-2 integrins are classified as CD11\CD18 by the world health organisation according to their alfa and beta subunits. They are composed of identical beta subunits (CD18) and alfa subunits that vary in structure and are designated as CD11a, CD11b and CD11c for LFA-1, Mac-1 and p150,95, respectively (SPRINGER, 1990). Mac-1 is the CR3 receptor and binds, most importantly, C3bi and CD54 (ICAM). In vivo, Mac-1 mediates tight adherence of leukocytes to activated endothelial cells, whereas a leukocyte selectin (L-selectin) mediates loose adherence of leukocytes to nonactivated endothelial cells (KISHIMOTO et al. 1987). Because beta-2 integrin expression requires intracellular association of the CD11 and CD18 subunits, defects in CD18 prevent expression of all beta-2 integrins. OLCHOWY et al. (1994) studied the in vitro assessment of neutrophil integrin expression (CD18, CD11a, CD11c) aggregation and transendothelial migration. They observed that neutrophils isolated from the affected BLAD calf had decreased expression of leukocyte integrins on their cell surface, decreased ability to aggregate in response to chemotactic stimuli, and decreased ability to migrate across bovine endothelial cell monolayers.

The BLAD is the homoloque of human genetic disorder leukocyte adhesion deficiency (LAD), which has been extensively described in human patients. The leukocytes of human patients with LAD have a deficiency or total lack of structurally and functionally related beta-2 integrin glycoprotiens called

Mac-1, LFA-1 and P150,95. Heterogenous mutation of the gene encoding the common beta subunit (CD18) have been identified as a primary basis for disease in all human cases (KISHIMOTO et al. 1987).

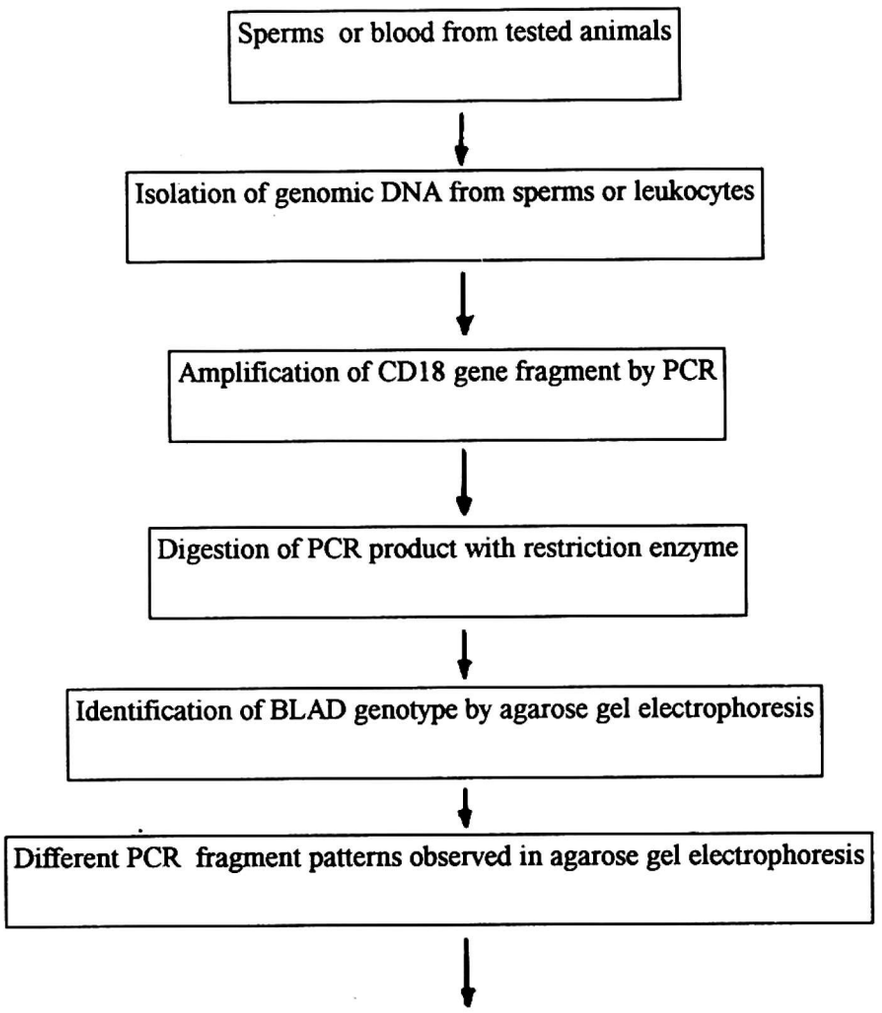
GIGER et al. (1987) first reported leukocyte adhesion deficiency in Irish setter dogs. They reported that the findings of deficient expression of Mac-1, LFA 1, and p150, 95 on leukocyte surface suggest a similar underlying defect of the gene encoding canine CD18. TROWALD-WIGH et al. (1992) reported that the aetiology, pathogenesis and clinical manifestations in Irish setter dogs were similar to those of the LAD in human and investigated leukocyte adhesion protein deficiency with a total lack of CD11b and CD18 in 12 Irish setter puppies from six litters.

Molecular basis of BLAD gene

So far, the genomic sequence of entire gene encoding bovine CD18 is unknown. A comparative study of DNA sequences of bovine cDNA encoding CD18 gene with the human and murine sequences was performed by SHUSTER et al. (1992a). The cDNA for CD18 was sequenced by isolating messenger RNA from leukocyte of affected patients and then subjecting to the reverse transcription. The portions of the 5- and 3- untranslated regions of the nucleotide sequences were conserved among the three species, including a 3 A+T rich region believed to regulate mRNA stability and transitional efficiency. Two point mutations were identified within the gene coding bovine CD18 in a Holstein calf afflicted with leukocyte adhesion deficiency (LAD). One point mutation (adenine to guanine) causes aspartic acid to glycine substitution at amino acid 128 (D128G) in the highly conserved extracellular region of the adhesion glycoprotein. The other mutation was silent. This mutation (first one) was very prevalent among Holstein cattle throughout the world, placing this disorder among the most common genetic diseases known in agricultural animal (SHUSTER et al. 1992b).

Methods for BLAD diagnosis

A combination of clinical signs, pedigree and laboratory findings provides compelling evidence for presumptive diagnosis in BLAD cases. Definitive diagnosis may be made by demonstration of mutant allele in a homozygous form, or by flow cytometric demonstration of severely deficient expression of leukocytes beta-2 integrins. In BLAD affected animals, the isolated neutrophils expressed a very low level of CD18 by flow cytometry analysis (SHUSTER et al. 1992b). Because the disease is inherited as an autosomal recessive condition, the pedigree of the affected animal provides additional suggestive evidence for the diagnosis.



PATTERN-1 (SHUSTER ET AL. 1992b)

PCR product	BLAD			Base pairs
	+/+	+/-	-/-	
==				58
		==	==	49
	==	==		30
	==	==		19
	==	==	==	9

PATTERN-2 (MULLER, 1994)

PCR product	BLAD			Base pairs
	+/+	+/-	-/-	
==				88
		==	==	65
	==	==	==	46
	==	==	==	23
	==	==	==	19

+/+ : normal; +/- : heterozygote mutant; -/- : homozygote mutant

Fig. 1. General procedures of different PCR fragment patterns for genotyping BLAD using PCR and allele specific RFLP analysis

The basic protocol for the identification of mutation in bovine CD18 gene was first described by SHUSTER et al. (1992b). Later, modified protocols have been developed by GROBET et al. (1993), TAJIMA et al. (1993), TAMMEN et al.

(1994), BATT et al. (1994), NAGAHATA et al. (1995a) and KAMIŃSKI et al. (1995). All these protocols are based on PCR amplification of CD18 gene fragment followed by restriction enzyme digestion. TAMMEN et al. (1994) developed a rapid and efficient method which allows a restricted use of carrier bulls with high breeding values in commercial embryo transfer programs. They developed a multiplex PCR with Y-chromosome-specific primers from the Bov97M sequence and primers surrounding the BLAD mutation in the CD18 gene which is followed by two specific PCRs, for sex and BLAD separately. This procedure (biopsy, DNA preparation, PCRs, restriction enzyme digestion and gel electrophoresis) gave 90% definite results in 50 male embryos from BLAD unsuspicious parents. BATT et al. (1994) developed a nonisotopic ligase chain reaction (LCR) assay to detect the D128G mutation for BLAD. They synthesized two PCR primers and six LCR primers based upon the published sequence for CD18 and the mutant BLAD allele.

Prevalence of BLAD

BLAD is an especially serious condition within the Holstein breed because some of the most prominant sires of the breed are heterozygous for the D128G allele. Osborndale Ivanhoe, Penstate Ivanhoe Star and Carlin-m Ivanhoe Bell are some of the elite sires of the breed diagnosed as carriers of this allele on the basis of DNA testing. The carrier frequency for the D128G allele among Holstein cattle in the USA was observed to be approximately 15% among bulls and 6% among cows by KEHRLI et al. 1990.

NIELSEN et al. (1992) reported that BLAD was introduced to Danish cattle by semen imported from American and Canadian bulls. A screening program was instituted after the first identification of BLAD in 1992. They further described the occurrence, inheritance, clinical signs, pathological and histopathological findings, and diagnosis of BLAD. JORGENSEN et al. (1993) reported that under the screening program for BLAD in Danish Holstein Friesian cattle, 1611 animals were tested by a PCR based assay. Out of these animals 1256, 346 and eight were assigned normal, BLAD carrier, and BLAD affected, respectively. Estimation of the BLAD allele frequency based on PCR test results showed that around 450 Danish calves born in 1991 might have been affected with this recessive disorder. While genotyping 783 bulls of unknown breed of Rhineland AI Association DUESMANN (1994) reported that 13% were positive to the PCR for BLAD between August 1992 and March 1993, while 3.5% were positive between April and December 1993.

GILBERT et al. (1993) tested 18 cattle that had been admitted to the Veterinary Medical Teaching Hospital in between 1975 and 1991 and that had

persistent and severe neutrophilia. After testing, 14 cattle were confirmed to have been homozygous for the BLAD gene. GROBET et al. (1993) reported the Holstein bulls of known BLAD status (five homozygous and five heterozygous). Among 15 Holstein bulls of unknown status, three were diagnosed as heterozygous which belonged to the Holstein line above mentioned. No carrier was found among 49 Belgian Blue and 14 Red and White AI sires.

KUCZKA et al. (1993) reported that out of 50 affected animals examined, 4% were aged less than four weeks and 20%, 36%, 38%, and 2% were aged 1-2, 2-6, 6-12 and more than 12 months, respectively. MULLER et al. (1993) reported 10 cases of BLAD which they diagnosed in Netherlands since after 1991. BERNADINA et al. (1993) reported the occurrence of four Dutch BLAD cases with families clusturing. They further suggested that both subnormal expression and lack of polymorphonuclear granulocytes (PMN) CD11 expression are inheritable factors in cattle.

GRZYBOWSKI et al. (1994) reported 7 BLAD carriers bulls while testing 205 AI bulls from the Bydgoszcz breeding station. Almost all of them were grandsons of Puget-Sound-Sheik A-327279. Semen of this bull was imported to Poland from Canada in 1978. This bull seems to be the most important for spreading the D128G mutation in Polish cattle population.

HRADIL (1994) genotyped 438 animals which had the BLAD positive progenitor in the three generations before. Out of 377 bulls and 61 cows tested for BLAD, 65 bulls and four cows were BLAD positive. High frequency of BLAD was observed in red variants of Holstein Friesian cattle, out of 64 bulls 34 animals were BLAD positive. SCHWERIN et al. (1994) reported that the genotypic frequency of BLAD in 112 (96.6%) bovine embryo was observed as 82 (73.2%) normal genotypes and 30 (26.8%) heterozygote genotypes.

Clinical findings

Clinical features of BLAD are so variable that simple categorization of clinical signs is difficult. However, involvement of respiratory and gastrointestinal tracts predominates. KEHRLI et al. (1992b) observed recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth, periodontitis, delayed wound healing, persistent neutrophilia and early death in BLAD affected cattle. Consistent findings in BLAD affected cattle included signs of broncho-pneumonia, gingivitis, periodontitis and peripheral lymphadenopathy. Severe neutrophillia, usually without a left shift, was a hallmark of the disease. Consistent clinical biochemical findings include hypoalbuminemia, hyperglobulinemia and hypoglycemia. This disease is important because it mimics common calfhood diseases such as pneumonia and diarrhoea which is ultimately consistently fatal before adulthood (GILBERT et al. 1993). STAD-

LER et al. (1993) reported that the most significant clinical signs in BLAD affected cattle were fever, depression, weakness, emaciation, diarrhoea, pseudomembranous gingivitis, loose teeth, respiratory infection and occult blood in the faeces. MULLER et al. (1994) reported clinical and laboratory findings in eight BLAD affected calves, aged between three weeks and one year. They had gingivitis, periodontitis, alveolar periostitis, diarrhoea, respiratory disease, chronic dermatitis and impaired healing of skin wounds. All had leukocytosis, due to an increase in neutrophils, during periods free from infections.

Histo-pathological findings

The most commonly recorded histopathological lesions were necrotic enteritis, lymphoid hyperplasia and histiocytosis of lymphnodes. In most of the cases, the macroscopic examination revealed gross lesions of pneumonia or pulmonary abscess, larygitis, tracheitis, myeloid hyperplasia of bone marrow, thymic atrophy, ulcerative or necrotic lesions of the nostrils, muzzle, palate, oral cavity or tongue, periodontitis, loss of teeth, esophegeal and ruminal ulcers, swelling and necrosis of the larynx. Characterstic skin lesions revealed lymphoplasmacytic and histiocytic inflammation with vasculitis and thrombosis in a calf with BLAD (ACKERMANN et al. 1992, ACKERMANN et al. 1993).

STADLER et al. (1993) examined five Holstein-Freisian calves at post-mortem which showed emaciated carcasses, granulomatous to necrotising gingivitis, pseudomembranous to necrotising enteritis with perforations, broncopneumonia, splenic atrophy and hypoplasia of the thymus. Histopathological examination supported the macroscopic findings. AGERHOLM et al. (1993) observed fibro-granulomatous, perilienitis, calcification of splenic stroma and pulmonic arteries. These patho-anatomical findings were reported to be different from those described in previously published cases.

NAGAHATA et al. (1994a) reported that pathological findings indicated possible increased susceptibility to the bacterial infection. The physio-pathological mechanisms of BLAD in Polish Black and White cattle was illustrated by GRZYBOWSKI, LUBIENIECKI (1994). GARDEREN et al. (1994) reported that histological examination of necrotic lesions of the digestive and respiratory tract revealed a lack of extravascularly located polymorphonuclear granulocytes despite vascular leukocytosis. However, polymorphs were abundant in extensive catarrhal bronco-pneumonia lesions. They concluded that PMN adhesion to the vascular wall of fibro-angioblastic tissue probably requires normal CD11\CD18 function.

New developments in BLAD research

NAGAHATA et al. (1993) evaluated the neutrophil function, CD18 expression on neutrophils of two BLAD affected Holstein heifers genotyped by PCR test. Results showed that when neutrophils from BLAD affected heifers stimulated with opsonized zymosa (OZ), it decreases chemiluminescent (CL) response. In contrast, neutrophils stimulated with latex beads and phorbol 12-myristate 13-acetate (PMA) increase CL response as compared with control animals.

The CL experiment was also carried out to generate oxygen redicals by using electron spin resonance spectrometry (ESR) combined with a spin-trapping technic and luminol-dependent chemiluminescence spectrometry (KUWABARA et al. 1993). Results revealed that when neutrophils were stimulated with PMA, an ESR spectrum confirming the generation of superoxide anions was clearly observed in both healthy and diseased calves. However, when the neutrophils were stimulated by OZ, the appearance of the ESR spectrum was recognised in the healthy calves but not in the diseased calf.

NAGAHATA et al. (1994a) reported that the neutrophil dysfunction is well characterized by severely decreased adherence, chemotactic movements, phagocytosis, luminol-dependent CL response, and O^{-}_{2}-producing activities. NAGAHATA et al. (1994b) analysed the lymphocyte function and mononucleal phagocyte functions in cattle affected with LAD. The lymphocyte functions were evaluated by lymphocyte markers, blastogenic response and immunoglobulin concentrations. The mononuclear phagocyte functions were assessed by chemotactic and luminol dependent CL response to determine the effects of impaired expression of leukocyte CD18 on mononuclear cell functions. They (NAGAHATA et al. 1994c) further evaluated serum biochemical profile and whole blood CL responses in 8 BLAD affected Holstein cattle. The characterstic changes in serum proteins were hypo-albuminemia and hyper-globulinemia. The concentrations of albumin and gammaglobulin in serum from normal cattle and cattle affected with LAD were highly significant. This procedure appeared to be practical and useful for routine evaluation on blood samples from cattle affected with LAD.

Studies on chromosome and sperm size of Holsteins with and without BLAD was carried out by STEINHOLT et al. (1994). They evaluated the relationship between spermatozoal head area and chromosomal area of lymphocytes in Holstein bulls. The video enhanced contrast microscopy (VECM) technology has been used to identify abnormalities in head shapes and size of spermatozoa by measurement of head areas. Area measures of spermatozoal heads showed that bulls with BLAD syndrome had significantly larger head areas than normal bulls.

WORKU et al. (1995) investigated the effect of LAD on ligand binding and receptor expression. Results revealed the increased binding and expression of Fc receptors for IgG and decreased binding and expression for C3b and IgM on neutrophils from calves with BLAD. They suggested that BLAD may be compounded by added defects in the expression and binding of receptors for opsonins, such as C3b and IgM.

In continuation with CL experiments, NAGAHATA et al. (1995a) demonstrated that the expression of CD18 on bovine neutrophils increases after stimulation with zymosan activated serum (ZAS) and phorbol myristate acetate (PMA) and the adhesion molecule CD18 plays an important role in adhesion-related functions. NAGAHATA et al. (1995b) further reported the bone marrow cellularity and CD18 expresssion on neutrophils from bone marrow and their function for characterization of neutrophils isolated from bone marrow of BLAD animals. Mark differences in the bone marrow cellularity were observed as the number of nucleated cells in bone marrow was 2.9 to 8.8 times higher in BLAD animals, in comparison to the controls. MULLER et al. (1995) investigated cell-mediated immunity in animals with BLAD by means of skin transplantation experiments. They observed that allograft survival time was prolonged in three BLAD cattle (28, 30, and 72 days) compared to six healthy controls (12-14 days). They further concluded that, although prolonged allgraft survival is observed in cattle with BLAD, skin allografts are ultimately rejected.

Concluding remarks

For the future research prospectives on BLAD, the following some features can be suggested:

1. As this genetic disorder is prevalent among prominent bulls of Holstein breed with very high economic merit and 15% frequency in male and 6% in female, it is suggested that the possibility of any linked quantitative trait loci (e.g. milk production) can be closely examined in the next phases on BLAD research. Further more research is required to examine beneficial effects of heterozygote conditions with regard to effect on health and pathological changes. Therefore, to remove this genetic disorder from bovine gene pool, effective screening program with well advanced breeding strategy should be evaluated.

2. LAD syndrome affecting the immune system of human, and canine reflects the importance of this syndrome in comparative studies. It is possible to use such comparison for investigations leading to the development of gene replacement therapy for canine and human beings with LAD. For the treatment strategies in LAD patients, gene therapy regimens using genetically engineered

donor cells can make the bovine model useful (KEHRLI et al. 1992c). The occurrence of the mutation in a highly interspecies conserved region of the CD18 protein further indicates the use of the bovine model for integrin protein studies of structural and functional relationships affecting alfa and beta subunit association and adherence functions.

3. Although most of the research on BLAD concerns gene expression role of CD18 or beta-2 integrins, it may be recommended that more attention should be given to a physiopathological role of CD deficient gene on various vital organs viz., liver, kidney, lungs, heart etc. This genetic disorder is an ideal candidate for attempting somatic cell reconstitution by expression of CD11/CD18 proteins in hematopoietic cells.

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