Bovine Leukocyte Adhesion Deficiency (BLAD): A Review

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ABSTRACT. Bovine leukocyte adhesion deficiency (BLAD) in Holstein cattle is an autosomal recessive congenital disease characterized by recurrent bacterial infections, delayed wound healing and stunted growth, and is also associated with persistent marked neutrophilia. The molecular basis of BLAD is a single point mutation (adenine to guanine) at position 383 of the CD18 gene, which caused an aspartic acid to glycine substitution at amino acid 128 (D128G) in the adhesion molecule CD18. Neutrophils from BLAD cattle have impaired expression of the β2 integrin (CD11a,b,c/CD18) of the leukocyte adhesion molecule. Abnormalities in a wide spectrum of adherence dependent functions of leukocytes have been fully characterized. Cattle affected with BLAD have severe ulcers on oral mucous membranes, severe periodontitis, loss of teeth, chronic pneumonia and recurrent or chronic diarrhea. Affected cattle die at an early age due to the infectious complications. Holstein bulls, including carrier sires that had a mutant BLAD gene in heterozygote were controlled from dairy cattle for a decade. The control of BLAD in Holstein cattle by publishing the genotypes and avoiding the mating between BLAD carriers was found to be successful. This paper provides an overview of the genetic disease BLAD with reference to the disease in Holstein cattle.

KEY WORDS: adhesion deficiency, β2 integrin, BLAD, genetic defect, Holstein.

HISTORY OF BLAD

A case of suspected leukocyte dysfunction, named granulocytopathy, in a Holstein heifer was reported in 1983 [17]. In 1987, several cases of Holstein calves showing similar clinical signs characterized by persistent or recurrent infections associated with marked persistent neutrophilia were reported from Japan [32, 33, 70]. Bovine granulocytopathy in Holstein cattle is characterized by recurrent bacterial infections, progressive periodontitis, ulcers of oral mucosa, and impaired inflammatory responses [21, 32, 33, 70]. These clinical findings were associated with impaired neutrophil functions such as markedly decreased adherence, chemotaxis, and phagocytosis, and bovine granulocytopathy was termed bovine granulocytopathy syndrome because of the heterogeneity of the clinical and leukocyte functional differences [32].

In 1990, a lack of β2 integrin molecules expressed on the leukocytes from affected animals was found in a calf with granulocytopathy syndrome [21], and this disease was termed bovine leukocyte adhesion deficiency, which was considered to be analogous to human LAD [7, 8, 23, 66].

MOLECULAR BASIS OF BLAD

The gene encoding bovine CD18 and its abnormal mutation was sequenced [62, 63]. The molecular basis of BLAD is a single point mutation (adenine to guanine) at position 383 of the CD18 gene, which caused an aspartic acid to glycine substitution at amino acid 128 (D128G) in the glycoprotein. This mutation occurs near the center of 26 consecutive amino acids that are identical in normal bovine, human, and murine CD18 and lies within a large extracellu-
lar region that is highly conserved across integrin \( \beta \) subunits [23, 63]. The other mutation, cytosine to thymine, between the normal and the BLAD allele detected at position 775 in the CD18 gene was silent [63]. Restriction analysis of polymerase chain reaction (PCR) amplified DNA containing position 383 of the CD18 gene allows discrimination between normal (TL), carrier (BL) and affected (BLAD) animals [63]. Modified DNA tests based on the PCR followed by restriction enzyme digestion were developed for this purpose [20, 24, 69, 71].

LEUKOCYTE INTEGRINS

The \( \beta_2 \) integrins include LFA-1, CR3, and p150,95 (CD11c), which consist of unique subunits, CD11a (180 KDa), CD11b (170 KDa), and CD11c (150 KDa), respectively, and a common \( \beta_2 \) subunit, CD18 (95 KDa) [8, 19, 23] (Table 1). LFA-1 is expressed on all leukocytes. CR3 and p150,95 are found on the surfaces of neutrophils, monocytes/macrophages and natural killer cells [8, 23, 67]. They are transmembrane proteins with relatively small cytoplasmic domains. BLAD is homozygous for the D128G allele of the CD18 gene and as a result there is impaired expression of the \( \beta_2 \) integrin (CD11a,b,c/CD18) of leukocyte adhesion molecules [62, 63]. Specific binding of CR3 and LFA-1 on the neutrophil surface with intercellular adhesion molecule 1 expressed on vascular endothelium is required for neutrophil emigration into vascular sites of inflammation [8, 23, 67].

BLAD affected cattle have deficient expression of the \( \beta_2 \) integrins on leukocytes [2, 11, 12, 34, 60]. CD18 expression of bovine normal neutrophils is increased after zymosan activated serum and phorbol myristate treatment [39]. Chemotactic responses and phagocytosis of normal neutrophils are decreased when cells are pretreated or continuously treated with an anti-CD18 monoclonal antibody [39].

\( \beta_2 \) integrin expression on neutrophils from heterozygous animals is 56 to 90% that of normal cows [12]. Receptor expression for aggregated IgG is greater on neutrophils from BLAD affected calves than on those from normal calves [42, 75]. Leukocytes from homozygous calves seem to upregulate alternative host defense capabilities to partially compensate for the lack of typical adherence dependent host defense functions [65].

FUNCTIONAL CHARACTERISTICS OF \( \beta_2 \) INTEGRIN DEFICIENT LEUKOCYTES

Diapedesis requires the ability of leukocytes to bind vascular endothelial cells, cross the basement membrane, and enter the infected tissues [8, 19, 23, 67]. Adherence of polymorphonuclear phagocytes from cattle with BLAD is markedly impaired, and their chemotactic responses have diminished values, compared with controls [35]. Activities of chemotactic movements and phagocytosis of neutrophils isolated from bone marrow of cattle affected with BLAD are severely impaired [40]. Adhesion independent responses such as cell polarization in response to chemotactic factors appear to be almost normal. The adhesive activities of CR3 deficient neutrophils to collagen I, collagen IV and fibronectin are significantly diminished; however, similar adhesion to laminin is observed in CD18 deficient neutrophils and control neutrophils [44]. Impaired expression of leukocyte CD18 has marked effects on the adhering activity of mononuclear phagocytes, and significantly decreases chemiluminescent response of mononuclear phagocytes mediated by inactivated complement 3b-dependent functions [38, 43]. Transendothelial migration of neutrophils from normal calves is reduced to levels comparable to the BLAD neutrophils by treatment with an anti- CD18 monoclonal antibody [56]. The CD18 stimulated N-acetyl-\( \beta \)-D-glucosaminidase release from CD18 deficient neutrophils has been found to be severely decreased [44].

Biosynthesis of \( \beta_2 \) integrins in bovine leukocytes, intrac-
cellular $\text{Ca}^{2+}$($\text{Ca}^{2+}$) signaling, chemiluminescent responses and electron spin resonance (ESR) combined with a spin trapping of neutrophils from control heifers and a heifer with BLAD were evaluated to elucidate the relationship between CR3, Fc receptor expression and their functional responses [25, 43, 45]. The synthesis of $\beta_{2}$ integrin complex was clearly detected in leukocytes from a normal heifer, but not in a BLAD affected heifer [45]. The transient phase of increased $[\text{Ca}^{2+}]_i$ was clearly detected in neutrophils from a heifer with BLAD stimulated with opsonized zymosan and aggregated bovine IgG, whereas the sustained phase was deficient compared with control heifers [45]. The $[\text{Ca}^{2+}]_i$ aggregation bovine IgG, whereas the sustained phase was deficient compared with control heifers [45].

The $[\text{Ca}^{2+}]_i$ concentration and chemiluminescent responses of neutrophils from a control heifer were clearly decreased by treatment with anti-CD18 and anti-IgG antibodies [45, 56]. No difference in membrane fluidity was detected between neutrophils derived from control and BLAD cattle [45]. The characteristic changes of $[\text{Ca}^{2+}]_i$, signaling and functional responses of $\beta_{2}$ integrin deficient neutrophils have been demonstrated [45, 47].

The antigen specific immune responses in vivo were studied and $\beta_{2}$ integrin deficiency was found to lead to delayed and severely impaired immune responsiveness in vivo [31].

The expression of L-selectin on isolated neutrophils from blood from normal calves and calves with BLAD has been compared [51]. Expression of L-selectin on neutrophils from BLAD calves was significantly decreased compared to that of controls. The amount of tyrosine phosphorylated $100 \text{kDa}$ protein in neutrophils from BLAD calves stimulated with sulphatides was $57\%$ of that in control calves [51].

The concentrations of interleukin (IL) -1$\beta$ and IL-6 in serum from a BLAD heifer from 6 months to 4 years old were evaluated, and serum IL-6 levels in the BLAD heifer were 1.7 to 2.7 fold higher than those of normal controls [52].

The BLAD carrier does not cause detectable functional differences in leukocytes compared with healthy calves [65]. In contrast, selected immunological properties of neutrophils were examined in cows that were BLAD carriers, and there was a $17\%$ reduction in CD18 expression on neutrophils, and adherence and superoxide producing ability were significantly lowered [64].

CLINICAL FINDINGS

The clinical hallmarks of BLAD are recurrent necrotic and indolent infections of soft tissues such as mucous membranes and the intestinal tract [1, 5, 15, 35, 37]. Pyrexia, anorexia, chronic pneumonia, and recurrent or chronic diarrhea are commonly observed in BLAD calves [9, 11, 16, 35, 68]. Affected cattle have severe ulcers on oral mucous membranes, ulcerative stomatitis, gingivitis, severe periodontitis, loss of teeth, chronic pneumonia, and stunted growth [1, 5, 10, 15, 29, 35, 57]. Impaired wound healing, chronic dermatitis, and multiple recurrent infections are also found [29, 35] (Fig. 1).

Marked and persistent neutrophilia is a major characteristic finding of BLAD affected cattle [5, 11, 15, 21, 35]. Morphologic abnormalities of blood leukocytes from affected calves are not observed by light and electron microscopy [2, 35]. Consistent findings on serum biochemical analysis are hypoalbuminemia, hyperglobulinemia, and hypoglycemia [15,36]. Serum total protein content increases with time and is associated with an elevated $\gamma$-globulin level [11, 15, 29, 52].

PATHOBIOLOGICAL FINDINGS

Gross postmortem examination revealed severe and extensive necrotizing processes mainly located in the respiratory and digestive tract [3, 5, 15, 35]. Extensive catarrhal bronchopneumonia was noticed with infiltration of the alveoli and bronchioli by neutrophils [3]. Respiratory tract lesions consisted of dense infiltrates of neutrophils in bronchi, bronchioles, and alveoli [3, 4]. Pathologic findings indicated increased susceptibility to infection associated with BLAD [3, 5, 15, 22, 35]. Systemic kAL amyloidosis was detected in a bone marrow transplanted heifer which survived for 5 years [72].

The emigration of leukocytes from calves with BLAD into bronchoalveolar spaces and scraped tissues was compared to that of normal calves [4, 48]. Polymorphonuclear neutrophils were found in bronchoalveolar lavage fluid from BLAD affected calves showing chronic pneumonia [3, 4, 73]. The chemiluminescent response mediated by CR3 in neutrophils isolated from bronchoalveolar lavage fluid from BLAD calves showed findings similar to those obtained from CD18 deficient neutrophils [48]. Neutrophils from normal calves migrated into scraped tissue which was prepared in the upper glutal surface area, whereas few leukocytes from calves with BLAD migrated to the scraped tissue, evaluated by the Reubuck skin window method [48]. These findings confirmed the extravasation of $\beta_{2}$ integrin deficient leukocytes into the bronchoalveolar lumen in BLAD calves, and demonstrated in vivo characteristics of the extravasating property of normal and CD18 deficient neutrophils into scraped tissues.

Decreased apoptosis was found in CD18 deficient neutrophils and this appeared to be associated with a decreased rate of signaling via a $[\text{Ca}^{2+}]_i$ rise and annexin V expression on the cell surface [53].

Granulocyte transfusion (GT) was performed in an 8-month-old BLAD heifer to monitor the changes in transfused CD18 positive neutrophils and associated neutrophil response in a CD18 deficient host [50]. CD18 positive neutrophils in blood and an associated increased chemiluminescence response were found in peripheral blood during the first 3 hr after GT ($2.6 \times 10^9$ cells) in the BLAD heifer.

BM transplantation with successful engraftment of donor cells completely restores leukocyte function in human LAD patients [14]. Clinical and pathological findings of a BM transplanted heifer with BLAD were evaluated for 1.6 years, from 27 months after BM transplantation [49]. The animal’s
clinical condition appeared to improve and stabilize over the period of 28 months following BM transplantation. Cellular immunity in animals with BLAD was studied by means of skin transplantation, and prolonged allograft survival was also observed in cattle with BLAD; however, skin allografts were ultimately rejected [30]. It is assumed that graft-versus-host (GVH) reaction occurred associated with other factors such as natural killer cell activity and antibody dependent cell cytotoxicity of the immune system.

PREVALENCE OF BLAD-RELATED GENE IN HOLSTEINS

BLAD carriers were among the most prominent bulls of the Holstein breed such as Osborndale Ivanhoe, Penstate Ivanhoe Star, and Carlin-M Ivanhoe Bell [59, 63]. Affected cattle with BLAD were linked to common ancestral sires that had been documented to be carriers [20, 63]. Several Holstein Friesian bulls were identified as BLAD carriers,
and the gene encoding impaired CD18 spread to many countries [5, 9, 11, 13, 18, 46, 54, 55, 61, 64, 71]. Cattle with BLAD were reported from Australia [61], England [9], Denmark [5, 55], France [16], Germany [68], Japan [32, 70], the Netherlands [11], Switzerland [28], and the United States [15, 21, 57].

A formalin fixed tissue sample showed that the BLAD CD18 allele existed in cattle as far back as 1977 [15]. The carrier frequency for the D128G allele among Holstein cattle in the United States was reported to be approximately 15% among bulls and 6% among cows in 1992 [63]. The occurrence rate of BLAD affected Holstein dairy cattle was estimated to be 0.2% at birth in the United States in 1992. In Denmark, 1611 animals were tested by a PCR test in 1993. Of these animals 1256, 346, and 8 were assigned as normal, BLAD carriers (21.5%), and BLAD affected animals (0.5%) [20]. In Japan, BLAD carrier prevalence in 10 herds (363 cows) in which the occurrence of BLAD was not detected ranged from 0 to 12.5% with a mean of 5.4% in 1995 [46]. The BLAD carrier prevalence in 10 herds (433 cows) in which the occurrence of BLAD was confirmed by DNA PCR analysis ranged from 2.6 to 23.5% with a mean of 10.8%, and these values were significantly higher than those of dairy herds in which the occurrence of BLAD was not detected in 1995.

BLAD carrier prevalence in 792 Holstein bulls reared by the Livestock Improvement Association of Japan from 1992 to 1994 ranged from 9.3 to 19.8%, and the mean BLAD carrier prevalence was 13.4% [46]. This value appeared to be similar to the value of 14.1% in the United States [63]. The occurrence rate of BLAD affected Holstein dairy cattle was estimated to be 0.16 to 0.31% in Japan in 1996 [46]. BLAD carrier prevalence in 801 Holstein bulls in same institution from 1995 to 1999 ranged from 0.7 to 3.3% with a mean of 2% (unpublished data). The frequency of BLAD carriers in Taiwanese cows was 5.8 (40/693)% [18]. In Poland, 4.8% (81/1680) of bulls tested were BLAD carriers in 2000 [54]. BLAD in the Holstein breed has been successfully controlled by DNA testing and publishing their genotypes.

CONTROL OF GENETIC DISEASES OF THE HOLSTEIN BREED WITH A SINGLE AUTOSOMAL RECESSIVE MODE OF INHERITANCE

No differences were observed between carriers of BLAD and non-carriers for intramammary infection from coliform, coagulase negative staphylococci, environmental streptococci, or all bacterial species combined [74]. Associations of the allele with yield, productive life, and somatic cell score were tested [13, 18, 59]. A significant negative relation was found with protein yield when the effect of sires was ignored [59]. In contrast, no significant associations were found between carriers and non-carriers with respect to ages at 1st to 3rd calvings, calving interval, lactation duration, milk yield or fat and protein yields [13]. The overall calf growth performance was not suppressed in calves heterozygous at the CD18 locus [10].

A program for the decreasing of BLAD carrier bulls was enacted, and BLAD carrier rates were estimated to be markedly lowered because no BLAD carriers were used for artificial insemination. Common ancestors for complex vertebral malformation (CVM) in the Holstein breed, a newly reported genetic disease described in 2001, were former sires of US Holsteins, which were also carriers for BLAD [6]. Their family lines were used worldwide as sires [6]. Several programs for the elimination of the gene encoding genetic diseases have been enacted in dairy countries [6]. BLAD in Holstein cattle is considered to be a good model for efficient disease control of a genetic disorder mediated by a single recessive mode of inheritance.

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REFERENCES


44. Nagahata, H., Higuchi, H., Noda, H., Tamoto, K. and Kuba-


63. Shuster, D. E., Kehrl, M. E. Jr., Ackermann, M. R. and Gil-


