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Bovine leukocyte adhesion deficiency

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Abstract : A disease of young Holstein calves characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth, periodontitis, delayed wound healing, persistent neutrophilia and death at an early age had been originally described in 1983 and again in 1987. Most of these calves had stunted growth and a persistent, progressive neutrophilia (often exceeding 100,000/ml). By investigation of pedigrees, all of the affected calves have now been traced to a common sire and confirmed by polymerase chain reaction (PCR) diagnostic DNA testing to be homozygous carriers of a defective allele for bovine CD18. Neutrophils from these calves have several functional deficits and, most importantly, fail to adhere in a β_2-integrin dependent manner. The β_2-integrins represent a family of glycoproteins which participate in various leukocyte adhesion reactions during host defense. The presence or absence of β_2-integrin molecules can be demonstrated on the surface of neutrophils, monocytes and lymphocytes from normal or affected calves using specific monoclonal antibodies and flow cytometry, or by colloidal gold immunolabeling and scanning electron microscopy in backscatter mode. Deficiency of the β_2-integrins on all leukocyte types in Holstein calves is analogous to leukocyte adhesion deficiency (LAD) seen in humans. Neutrophils in bovine (BLAD) and human LAD patients are unable to adhere to the endothelial lining of the cardiovascular system thus interrupting egression of neutrophils into infected tissues. Other leukocytes, while still deficient in expression of the β_2-integrins, are still able to efficiently egress from the blood stream due to interactions of other adhesion molecules that are not as highly expressed on neutrophils. Both BLAD cattle and LAD children (who do not receive bone marrow transplants) often die at an early age as a result of the failure of neutrophils to extravasate into infected tissues.

In 1991, Shuster, *et al*[27], identified two point mutations within the alleles encoding bovine CD 18 in a Holstein calf afflicted with leukocyte adhesion deficiency. One mutation causes an aspartic acid to glycine substitution at amino acid 128 (D128G) in an extracellular region of this adhesion glycoprotein that is highly conserved ($>$ 95% identity) between humans, cattle and mice. The other mutation is silent. Numerous calves with clinical symptoms of leukocyte adhesion deficiency have since been tested and all have been found homozygous for the D128G allele. In

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addition, calves homozygous for the D128G allele have been identified during widespread DNA testing in the United States. All cattle with the mutant allele are related to one bull, who through artificial insemination (A.I.), sired many calves in the 1950's and 1960's. The carrier frequency of the D128G CD18 allele among U.S. Holstein cattle had reached approximately 15% among active A.I. bulls and 8% among cows. By 1993, the organization of the dairy industry and the diagnostic test developed to genotype cattle, enabled virtually complete eradication of bovine leukocyte adhesion deficiency among current and future A.I. bulls.

Key words : BLAD, CD18, D128G.

Introduction

Calf mortality on dairy farms represents a major economic loss. Overall calf mortality has been reported as 8% of all live heifer calf births[1]. An etiologic agent is not determined in 36% of dairy calf deaths attributed to diarrhea, a major cause of calf mortality[2]. Although seldom diagnosed, a granulocytopathy syndrome was first reported in 1983 and again in 1987 as a cause of death in young Holstein Friesian cattle[3-5]. This syndrome was described as a disease of young Holstein cattle characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth, periodontitis, delayed wound healing, persistent neutrophilia and death at an early age[3-6]. For some time clinicians in North America had recognized a condition colloquially known as "leukemoid response of calves" that is now thought to have represented calves with the granulocytopathy syndrome. Most of these calves have stunted growth and a persistent, progressive neutrophilia (often exceeding 100,000/ml) and a less dramatic lymphocytosis[3-7]. Histologically, capillaries, sinusoids and blood vessels throughout the body contain numerous neutrophils although a few neutrophils are present subjacent to ulcerated lesions of mucosal surfaces. Large crescents of numerous neutrophils often circumscribe splenic periarteriolar lymphocytic sheaths and increased myeloid/erythroid ratios are present in the bone marrow. Neutrophils have also been noticed in the lung of some calves with the granulocytopathy syndrome. Lymph nodes range from hyperplastic, in some reports, to diffuse, hypocellularity with necrosis of secondary follicles[4,6].

By investigation of pedigrees, all of the affected calves have been traced to a common sire[6]. In 1990[6], we reported the molecular definition of the bovine granulocytopathy syndrome as a deficiency of the Mac-1(CD11b/CD18) glycoprotein on the surface of neutrophils. At the time, we predicted the probable cause as a deficiency of the CD18 β-subunit of the β_2-integrin family of leukocyte adhesion glycoproteins on all leukocytes in affected cattle. We based this on the prior knowledge of a similar genetic defect in children called leukocyte adhesion deficiency (LAD)[8,9] and in Irish Setters (termed canine granulocytopathy syndrome)[10,11]. This autosomal recessive trait is clinically characterized by recurrent soft tissue infections, severely impaired pus formation, persistent leukocytosis, and abnormalities of various adhesion-dependent functions of leukocytes *in vitro*. Leukocytes of human patients with LAD have a deficiency or total lack of a family of structurally and functionally related glycoproteins, including Mac-1, LFA-1 and p150,95[12], now termed CD11/CD18 by the World Health Organization[13]. Each of these molecules contains an α and β subunit, noncovalently associated in an a_1b_1 structure. They share an identical β subunit (CD18) and are distinguished by their α subunits designated CD11a, CD11b, and CD11c for LFA-1α, Mac-1α, and p150,95α, respectively. Mac-1 (CD11b/CD18) is also known as CR3 and binds, most importantly, the opsonin C3bi and the endothelial cell adhesion molecule CD54 (ICAM-1). *In vivo*, Mac-1 mediates tight adherence of neutrophils to ac-

tivated postcapillary venule that express ICAM-1, endothelial cells; whereas another adhesion molecule, leukocyte selectin (L-selectin), mediates loose adherence of leukocytes to non-activated endothelial cells[14]. Various distinct mutations of the gene encoding the common β subunit (CD18) have been identified as the basis for disease in all human cases[15-19].

BLAD, the acronym for *B*ovine *L*eukocyte *A*dhesion *D*eficiency, is a lethal autosomal recessive genetic disease that occurs in Holstein cattle. We demonstrated that neutrophils from calves with BLAD fail to adhere in a β_2-integrin dependent manner to protein-coated surfaces[7]. The presence or absence of β_2-integrin molecules has also been demonstrated on the surface of neutrophils, monocytes and lymphocytes from normal or affected calves using specific monoclonal antibodies and flow cytometry, or by colloidal gold immunolabeling and scanning electron microscopy in backscatter mode[20]. β_2-integrin deficiency in Holstein calves is analogous to severe LAD phenotypes seen in humans. Neutrophils in LAD patients are unable to adhere to the endothelial lining of postcapillary venules thus interrupting egression of neutrophils into infected tissues. Calves with BLAD die prematurely as a result of the failure of neutrophils to extravasate into infected tissues as is the case with most untreated human LAD patients. Moreover, severe generalized prepubertal periodontitis, similar to that seen in cattle with LAD, has been reported in children with LAD[7,21]. Often, the infections in LAD patients are the result of otherwise normal flora for immunologically competent individuals.

In 1991 Shuster, *et al*, identified two point mutations within the alleles encoding bovine CD18 in a Holstein calf afflicted with leukocyte adhesion deficiency[22]. One mutation causes an aspartic acid to glycine substitution at amino acid 128 (D128G) in an extracellular region of this adhesion glycoprotein that is highly conserved ($>$95% identity) between humans, cattle and mice. The other mutation is silent. A DNA-PCR-Restriction Fragment Length Polymorphism (RFLP) technique was developed around the patented locus of the mutation that can be utilized to unequivocally identify the genotype of cattle at the CD18 locus. Numerous calves with clinical symptoms of leukocyte adhesion deficiency have since been tested and all have been found homozygous for the D128G allele. All cattle with the mutant allele are related to one bull, who through artificial insemination (A.I.), sired many calves in the 1950's and 1960's.

The carrier frequency of the D128G CD18 allele among U.S. Holstein cattle had reached approximately 15% among active A.I. bulls and 8% among cows. The organization of the dairy industry and the diagnostic test developed to genotype cattle, has enabled nearly complete eradication of bovine leukocyte adhesion deficiency among future A.I. bulls. BLAD, is the acronym chosen for *B*ovine *L*eukocyte *A*dhesion *D*eficiency which has an inheritance pattern of an autosomal recessive trait[5]. Cattle that have been tested for this genetic condition and found free of the defective allele are designated *TL, carrier animals are designated *BL. To date, tens of thousands of cattle around the world have been genotyped for the CD18 locus. From January 1991 to January 1996, the BLAD carrier rate averaged 12.5% among the semi-annual summary rankings of the top 100 Holstein bulls in the United States. The BLAD carrier rate among these bulls peaked at 17% in 1991 and again during the summer of 1994. However, as a result of the patented diagnostic test, which was licensed to 2 companies for use world-wide, the young bulls entering A.I. service has been virtually zero since 1993[23]. Since 1994, the carrier rate among A.I. bulls has been steadily declining and it is estimated that after 1998 virtually no carriers will be ranked among the top 100 genetic merit bulls.

Although, the heterozygotic condition has not been completely studied with regard to effects on health, at this time it appears there are no detrimental effects on cattle carrying the defective allele. We have found a slight favorable advantage for carrier cows on clinical mastitis incidence in lactating dairy cows[24].

Background History of BLAD

The discovery of BLAD in 1989 was serendipitous and occurred in conjunction with a study on a new method to prevent mastitis in periparturient dairy cows[25,26]. An apparently healthy, Holstein heifer born to a cow randomly assigned to the placebo-injected control group, exhibited a leu-

kocytosis of 34,600 cells/ml at seven days of age after having counts of 14,000 and 17,000/ml at birth and two days of age, respectively. The calf was immediately examined more closely and a mild fever (39.6℃) was detected. Analysis of blood samples obtained over the next 41 days revealed chronic progressive neutrophilia, which peaked at $>$85% neutrophils and exceeded 100,000 leukocytes/ml. *In vitro* assessment of isolated blood neutrophils obtained from the heifer at 38 and 45 days of age revealed selected functional abnormalities. Similar *in vitro* assessment of neutrophils obtained from the calf's dam revealed no functional abnormalities. The calf died at 48 days of age, with persistent fever and chronic diarrhea, despite administration of antibiotics. Histologic examination at necropsy revealed large numbers of intravascular neutrophils in most tissues, including massive neutrophil sequestration in the spleen. However, a striking lack of extravascular neutrophils was evident in inflamed submucosa adjacent to intestinal ulcers heavily contaminated with enteric microorganisms. Bone marrow examination revealed diffuse myeloid hyperplasia, but no other abnormalities. The proband, a Holstein heifer, was the fourth calf born to its dam and was the result of artificial insemination. Health information on previous calves of the dam was unavailable.

Eight months after the death of the proband, we were able to demonstrate a deficiency of the a-subunit of the Mac-1 β_2-integrin heterodimer[6]. Flow cytometric analysis of neutrophils from the sire and dam of the proband using a monoclonal antibody specific for canine CD18 indicated that the parents of the proband expressed 65% to 70% of what was an apparently normal level of CD18 expression on other cattle[6]). In our early studies, it was also apparent that if the reduced expression of CD18 was indicative of a carrier state for an allele producing a defective CD18 protein, that random testing of Holsteins would often find other cattle with similarly reduced levels of CD18 expression.

Based upon the apparent deficiency of the Mac-1 β_2-integrin heterodimer on neutrophils of the proband, we sequenced a normal allele for bovine CD18[27]. The 2833 nucleotide bovine sequence coded for a protein with 769 amino acids. Compared to human and murine sequences, portions of the 5' and 3' non-coding regions of the cDNA sequence were conserved including an AT-rich region believed to regulate mRNA stability and translational efficiency. Overall, the deduced amino acid sequences were $>$80% identical among the three species. Amino acids 96-389 and those in the cytoplasmic domain were very highly conserved. All cysteine residues and potential N-glycosylation sites present in the bovine sequence were also present in the human and murine sequences. Amino acid identity was found in those regions where mutations were found to cause the genetic disease, leukocyte adhesion deficiency. These interspecies conserved regions identify portions of the CD18 molecule which presumably are functionally important for neutrophil egression.

Within 2 and 7 weeks after completing sequencing of the apparently normal bovine CD18 allele, two additional Holstein calves (10 and 14 mo of age) with symptoms of LAD were brought to our attention and then purchased from commercial dairy farms. Both heifers had a history of respiratory disease and diarrhea, exhibited periodontal gingivitis with gingival recession and tooth loss, and were only 60% of normal weight. One calf had ulcers in and around the mouth that eventually healed after continued topical application of antibiotics. Both calves presented with a persistent and pronounced mature neutrophilia of $>$47,000 neutrophils/ml blood, compared with a normal level of $<$4,000 neutrophils/ml. Neutrophils from both calves expressed $<$2% of normal level of β_2 integrins by flow cytometry, demonstrating that both calves had LAD. Pedigree information was provided by the owners and the Holstein-Friesian Association of America. Leukocyte RNA was isolated from one of these LAD calves and a Holstein cow that had normal β_2-integrin expression. Northern blot analysis revealed that CD18 transcript was present at normal levels and size in the LAD calf, ruling out genetic defects that block transcription or cause large deletions. Messenger RNA was isolated from this LAD calf and used to generate cDNA for sequencing of the bovine CD18 alleles present in BLAD cattle[22]. The resulting cDNA sequence for CD18 in the BLAD calf was compared to the normal sequence. A point mutation (adenine®guanine) resulting in a substitution of gly-

cine for an aspartic acid at position 128 (referred to as the D128G allele) of the protein sequence in the gene for CD18 was identified in the first heifer and confirmed in the second heifer, and all subsequent cases we have found to date (〉 100 cases world-wide). 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 larger extracellular region (aa 96-389) that is highly conserved (95% identity) across integrin β subunits[18,22,27,28].

Another mutation detected in the sequence from the LAD calf replaced a cytosine at nucleotide 775 with thymine. Both parents, obligate heterozygotes, of the other LAD calf were heterozygous for both mutations. The mutation at nucleotide 775 was silent as it did not alter the deduced amino acid sequence. Retrospective case studies indicated the presence of only one allele in the Holstein cattle population which causes LAD[5,29,30].

Testing for the mutation at nucleotide 383 of the CD18 locus among additional cases of the bovine granulocytopathy syndrome, the Holstein A.I. bulls and sire dams was performed with genomic DNA. DNA was amplified for 35 cycles (94℃ 15 sec, 69℃ 20 sec) in a 20μl reaction containing 1X polymerase chain reaction buffer, 0.2mM dNTPs, 0.5U Amplitaq polymerase, and 4 pmol of sense primer (5'-TCCGGAGGGCCAAGGGCTA) and antisense primer (5'-GAGTAGGAGAGGTCCATCAGGTAGTACAGG). Reaction tubes and contents were kept on ice until placed directly into the hot thermo cycler block. Ten-microliter aliquots of amplification product were subjected to restriction endonuclease digestion separately by direct addition of 4U of *Taq*I or *Hae*III followed by incubation for 1.5hr at 65℃ or 37℃, respectively. Digested product was separated by 4% agarose gel electrophoresis and visualized by ethidium bromide staining and ultraviolet light illumination.

Results of Immunological Assessments of BLAD Calves

Because of recurrent bacterial infections in spite of a striking neutrophilia, defective neutrophil function in bovine granulocytopathy syndrome cattle had been suspected[3-6]. Neutrophils from BLAD patients have several functional deficits consistent with deficient expression of CD11/CD18 glycoproteins. *In vitro* assessments have identified abnormalities of motile, phagocytic, and oxidative functions of neutrophils which appear to mediate inflammatory deficits *in vivo*[3-6,31]. Diminished phagocytosis-associated oxidative and secretory functions during ingestion of C3bi opsonized zymosan by neutrophils of BLAD patients' are consistent with a deficiency of the Mac-1 α subunit that contains the complement receptor type 3 (CR-3) epitope reactive with C3bi deposited on zymosan particles[8,32]. Adherence and surface CD18 levels on neutrophils from normal cattle increased following stimulation with platelet activating factor (PAF)[7]. These responses were not observed with neutrophils from BLAD patients. PAF-enhanced neutrophil adherence from normal cattle can be inhibited by treating control neutrophils with anti-CD18 mAb but this mAb was without effect when incubated with BLAD patient neutrophils. Surface expression of CD18, CD11a, and CD11b was initially evaluated using the following mAb : R15.7 (anti-CD18), R3.1 (anti-CD11a) and Leu 15 (anti-CD11b) with neutrophils of one of our BLAD patients. CD11a and CD11b were detected at 6% and 10% of normal values. Repeated analyses of several BLAD patients that we have cared for, demonstrate CD18 to be expressed at = 2% of normal levels for resting and PAF-stimulated neutrophils. Resting neutrophil L-selectin levels in BLAD patients are markedly reduced compared to controls[7]. The low levels of L-selectin in BLAD patients may be a reflection of chronic subclinical infections.

LAD in Human and Veterinary Medicine

Granulocytopathies in dogs[33], humans[34] and cattle[3,4] characterized by persistent progressive neutrophilia in patients affected with severe recurrent bacterial infections and failure to form pus were reported between 1975 and 1987. In all three species, these conditions were eventually determined to be heritable deficiencies of leukocyte surface glycoproteins associated with diminished cell adherence between 1984 and 1990[5,6,8,10,35]. Although published reports of its diagnosis were few at the time, BLAD has been diagnosed at

Veterinary Schools throughout the world during the past several years. To date, well over 100 Holstein bovine LAD cases have been tested by DNA-PCR-RFLP and confirmed to be homozygous for the D128G mutation[22,29,30,36,37]. Many of these cases were found using formalin-fixed tissues from cattle suspected to have bovine granulocytopathy syndrome in Iowa, New York, Wisconsin, and Germany, dating back to 1977. Thus, all evidences to date indicate that all BLAD patients among Holsteins represent a homozygous genotype for a single mutant allele of CD18 (D128G).

The severe clinicopathologic consequences of CD11/CD18 deficiency in people as well as dogs and cattle reflect the diverse contributions of the β_2 integrins to leukocyte adherence reactions of importance in inflammation and host defense. The severity of neutrophil function abnormalities and clinical complications among recognized human beings with LAD is directly related to the degree of glycoprotein deficiency. Human patients with the severe phenotype (expressing $<$ 1% of normal ab complexes on cell surfaces) are susceptible to life-threatening infectious complications in infancy, whereas patients with moderate deficiency (expressing 3 to 10% of normal amounts) develop less severe complications and generally survive into adulthood[32]. Studies of their neutrophils *in vitro* indicate less-severe functional impairment than has been observed among subjects with the severe phenotype[32].

It is our opinion, that BLAD patients have a moderately severe phenotype since affected cattle express 2% of normal amounts of CD18 protein on neutrophil surfaces. It is clear that BLAD is an eventually lethal genetic condition for homozygous animals and because environmental hygiene conditions for cattle are more challenging than for humans, the likelihood of premature death is greatly increased versus human LAD patients. It is also likely that BLAD calves would be the first die if a disease outbreak due to a recognized pathogen would occur on a dairy farm. It also seems apparent that BLAD calves may die as a result of disease caused by normal flora[38]. Based on our clinical and research experience with BLAD patients, the majority of these calves would die before one year of age. It is possible, however, for some of the animals to live past two years of age but they are severely stunted in growth (50% of expected body weight) and suffer from the various infectious conditions of the skin (including severe ringworm), gastrointestinal and respiratory tracts. Of the older survivors, a striking periodontal gingivitis with marked recession of the gingiva may be the most obvious clinical sign.

Impact of BLAD Testing on the Dairy Industry

The dairy industry relies on the use of artificial insemination. Testing of bulls and cows mated for the purpose of producing future sires used in artificial insemination throughout the world has been in progress since 1991. Results from this testing has provided the necessary information to phase out the use of carrier bulls designated (* BL) by 1998 without any significant loss of the existing gene pool necessary for high quality milk production[23]. BLAD was an especially serious condition within the Holstein breed because some of the most prominent 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. It is certain that a small proportion of calfhood deaths in this breed are, in fact, attributed to infectious complications of BLAD. Assuming random mating and a carrier rate for the D128G allele of 13.5% among bulls and 8% among cows, not more than 1.1% of all random matings would be between carrier animals. Of these matings only 25% would result in homozygous affected BLAD progeny, therefore, only 0.27% of all random matings would result in an affected BLAD calf. With 9.7 million dairy cows in the United States in the early 1990s, this predicted about 26,000 BLAD calves born each year. World-wide this number may have approached 100,000 calves per year. The organization of the dairy industry such that relatively few bulls sire most of the progeny allows rapid elimination of autosomal recessive diseases.

Problems with DNA-PCR Testing

Due to the existence in dizygotic bovine twins of the leukochimeric condition, it is important to identify even slight aberrations in DNA polymorphism assays. The potential risk of false genotyping of cattle, and the expense to breeders, emphasizes the importance of this issue. Reports of leukochimerism, warrant against the exclusive use of blood samples for DNA typing when twinning is properly documented[39]. Breed associations have determined it is important to eliminate the presence of genetic lethal conditions from populations of animals. The dairy cattle industry affords the most striking example of how rapidly the use of DNA-PCR technologies can be applied with minimal negative impact on the available finite gene pool. Previous efforts to screen for a genetic defect in Australian Holstein cattle also used DNA-PCR technology (typically DNA extracted from blood)[40]. Testing of animals for citrullinemia using blood leukocyte DNA led to the false genotyping of a few animals which were not originally known to be twins. Apparently, a certain percentage of live births (1 in 1500 animals) involve a calf with twins that died in uterus and was reabsorbed during gestation or was never observed in the placental membranes (personal communication, Dr. Peter Healy, 1992). These unidentified twins pose a potential delay to breed associations who desire to quickly eliminate genetic defects through DNA testing methods. A similar lack of a known co-twin has recently been reported in a parentage dispute involving a mare who was excluded as a parent based upon blood-derived DNA[41].

Limitations of applying DNA-PCR for genotype identification include financial as well as technical errors. If 1499 out of 1500 births are accurately recorded as twin or single births, then a test performed on blood-derived DNA would be 99.93% accurate. This level of accuracy should be acceptable provided twinning is documented where possible and it offers the cattle industry the ability to test the DNA of calves shortly after birth, thus saving considerable expense associated with performing skin biopsies or waiting until semen is available from bulls which need to be tested. For cows, only an alternative source of DNA (such as skin) is a satisfactory solution to the potential leukochimerism condition causing a false genotype. We recommend testing the most influential Holstein sires and any sires descendent from a known carrier through the use of semen. Matings between known carriers should be avoided unless a deliberate effort to identify homozygotically normal embryos or fetuses is made. This type of mating might result in valuable progeny but would be achieved only through considerable expense and effort. We urge that this condition be removed from the bovine gene pool through the screening of potential A.I. sires as has been taking place since 1991.

Several commercial laboratories conduct the DNA-PCR-RFLP test for BLAD. This test has been licensed to Immgen Inc., College Station, TX and The Bovine Blood Typing Laboratory of Canada, Saskatoon, Saskatchewan, S7N 2X8. All testing in the United States is coordinated through and recorded with the Holstein Association of America, (802) 254-4551.

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References

1. James RE, McGilliard ML, Hartman DA. Calf mortality in Virginia Dairy Herd Improvement herds. *J Dairy Sci*, 67:908-911, 1984.
2. Morin M, Larivière S, Lallier R. Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. *Can J Com Med*, 40: 228-240, 1976.
3. Hagemoser WA, Roth JA, Löfstedt J, Fagerland JA. Granulocytopathy in a Holstein heifer. *J Am Vet Med Assoc*, 183:1093-1094, 1983.
4. Nagahata H, Noda H, Takahashi K, Kurosawa T, Sonoda M. Bovine granulocytopathy syndrome: Neutrophil dysfunction in Holstein Friesian calves. *J Vet Med Ser A*, 34:445-451, 1987.
5. Takahashi K, Miyagawa K, Abe S, Kurosawa T, Sonoda M, Nakade T, Nagahata H, Noda H, Chihaya Y, Isogai E. Bovine granulocytopathy syndrome of Holstein-Friesian calves and heifers. *Jpn J Vet Sci*, 49: 733-736, 1987.
6. Kehrli ME Jr, Schmalstieg FC, Anderson DC, Van Der

Maaten MJ, Hughes BJ, Ackermann MR, Wilhelm-sen CL, Brown GB, Stevens MG, Whetstone CA. Molecular definition of the bovine granulocytopathy syndrome : Identification of deficiency of the Mac-1 (CD 11b/CD18) glycoprotein. *Am J Vet Res*, 51:1826-1836, 1990.

7. Kehrli ME Jr, Shuster DE, Ackermann M, Smith CW, Anderson DC, Dore M, Hughes BJ. Clinical and immunological features associated with bovine leukocyte adhesion deficiency, p.314-327, 1993. *In* Lipsky PE, Rothlein R, Kishimoto TK, Faanes RB, Smith CW (ed.), Structure, Function, and Regulation of Molecules Involved in Leukocyte Adhesion. Springer-Verlag, New York.
8. Anderson DC, Schmalstieg FC, Kohl S, Arnaout MA, Hughes BJ, Hollers MF, Smith CW. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (GP138) : Common relationship to diminished cell adherence. *J Clin Invest*, 74:536-551, 1984.
9. Springer TA, Thompson TS, Miller LJ, Schmalstieg FC, Anderson DC. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J Exp Med*, 160:1901-1918, 1984.
10. Giger U, Boxer LA, Simpson PJ, Lucchesi BR, RFT III. Deficiency of leukocyte surface glycoproteins Mo 1, LFA-1, and Leu M5 in a dog with recurrent bacterial infections : an animal model. *Blood*, 69:1622-1630, 1987.
11. Renshaw HW, Davis WC. Canine granulocytopathy syndrome : An inherited disorder of leukocyte function. *Am J Path*, 95:731-744, 1979.
12. Sanchez-Madrid F, Nagy J, Robbins E, Simon P, Springer TA. A human leukocyte differentiation antigen family with distinct α-subunits and a common β-subunit : The lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J Exp Med*, 158:1785-1803, 1983.
13. Reinherz EL. Human myeloid and hematopoietic cells, p.124-129, 1986. *In* Reinherz EL, Haynes BF, Nadler LM, and Bernstein ID(ed.), Leukocyte Typing II. Springer-Verlag, New York, NY.
14. Kishimoto TK, Jutila MA, Berg EL, Butcher EC. Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science*, 245:1238-1241, 1989.
15. Dana N, Clayton LK, Tennen DG, Pierce MW, Lachmann PJ, Law SA, Arnaout MA. Leukocytes from four patients with completE or partial Leu-CAM deficiency contain the common β-subunit precursor and β-subunit messenger RNA. *J Clin Invest*, 79:1010-1015, 1987.
16. Hibbs ML, Wardlaw AJ, Stacker SA, Anderson DC, Lee A, Roberts TM, Springer TA. Transfection of cells from patients with leukocyte adhesion deficiency with an integrin β subunit (CD18) restore lymphocyte function-associated antigen-1 expression and function. *J Clin Invest*, 85:674-681, 1990.
17. Kishimoto TK, Hollander N, Roberts TM, Anderson DC, Springer TA. Heterogenous mutations in the β subunit common to the LFA-1, Mac-1, and p150,95 glycoproteins cause leukocyte adhesion deficiency. *Cell*. 50: 193-202, 1987.
18. Kishimoto TK, O'Connor K, Springer TA. Leukocyte adhesion deficiency : Aberrant splicing of a conserved integrin sequence causes a moderate deficiency phenotype. *J Biol Chem*, 264:3588-3595, 1989.
19. Marlin SD, Morton CC, Anderson DC, Springer TA. LFA-1 immunodeficiency disease : Definition of the genetic defect and chromosomal mapping of α and β subunits by complementation in hybrid cells. *J Exp Med*, 164:855-867, 1986.
20. Ackermann MR, Kehrli ME, Hawkins HK, Amenson JL, Gallagher JE. Identification of β_2 integrins in bovine neutrophils by scanning electron microscopy in the backscatter mode and transmission electron microscopy. *Vet Pathol*, 30:296-298, 1993.
21. Waldrop TC, Anderson DC, Hallmon WW, Schmalstieg FC, Jacobs RL. Peridontal manifestations of the heritable Mac-1, LFA-1, deficiency syndrome. Clinical,

histopathologic and molecular characteristics. *J Periodontol*, 58:400-416, 1987.

22. Shuster DE, Kehrli ME Jr, Ackermann MR, Gilbert RO. Identification and prevalence of a genetic defect that causes leukocyte adhesion deficiency in Holstein cattle. *Proc Natl Acad Sci USA*, 89:9225-9229, 1992.
23. Powell RL, Norman HD, Cowan CM. Relation-ship of bovine leukocyte adhesion deficiency with genetic merit for performance traits. *J Dairy Sci*, 79:895-899, 1996.
24. Kelm SC, Detilleux JC, Freeman AE, Kehrli ME Jr, Dietz AB, Fox LK, Butler JE, Kasckovics I, Kelley DH. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. *J Dairy Sci*, 80:1767-1775, 1997.
25. Kehrli ME Jr, Goff JP, Stevens MG, Boone TC. Effects of granulocyte colony-stimulating factor administration to periparturient cows on neutrophils and bacterial shedding. *J Dairy Sci*, 74:2448-2458, 1991.
26. Stabel JR, Kehrli ME Jr, Thurston JR, Goff JP, Boone TC. Granulocyte colony-stimulating factor effects on lymphocytes and immunoglobulin concentrations in periparturient cows. *J Dairy Sci*, 74:3755-3762, 1991.
27. Shuster, Brad DE, Bosworth T, Kehrli ME Jr. Sequence of the bovine CD18-encoding cDNA: comparison with the human and murine glycoproteins. *Gene*, 114:267-271, 1992.
28. Wilson RW, O'Brien WE, Beaudet AL. Nucleotide sequence of the cDNA from the mouse leukocyte adhesion protein CD18. *Nucleic Acids Res*, 17:5397, 1989.
29. Gilbert RO, Rebhun WC, Kim CA, Kehrli ME Jr, Shuster DE, Ackermann MR. Clinical manifestations of leukocyte adhesion deficiency in cattle: 14 cases (1977-1991). *J Am Vet Med Assoc*, 202:445-449, 1993.
30. Stöber M, Kuczka A, Pohlenz J. Bovine leukocyte adhesion deficiency(BLAD=Hagemoser-Takahashi-Syndrome): Clinical, patho-anatomical and -histological findings. Dtsch. Tierärztl. *Wochenschr*, 98:443-448, 1991.
31. Nagahata H, Kehrli ME Jr, Murata H, Sanada Y, O-kada H, Noda H, Kuwabara M, Takahashi K, Fujinaga T, Kociba GJ. Bovine leukocyte adhesion deficiency: neutrophil function and pathological analysis. *Am J Vet Res*, 55:40-48, 1994.
32. Anderson DC, Schmalsteig FC, Finegold MJ, Hughes BJ, Rothlein R, Miller LJ, Kohl S, Tosi MF, Jacobs RL, Waldrop TC, Goldman AS, Shearer WT, Springer TA. The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: Their quantitative definition and relation to leukocyte dysfunction and clinical features. *J Infect Dis*, 152:668-689, 1985.
33. Renshaw HW, Chatburn C, Bruan GM, Bartsch RC, Davis WC. Canine granulocytopathy syndrome: neutrophil dysfunction in a dog with recurrent infections. *J Am Vet Med Assoc*, 166:443-447, 1975.
34. Crowley CA, Curnutte JT, Rosin RE, Andre-Schwartz J, Gallin JI, Klempner M, Snyderman R, Southwick FS, Stossel TP, Babior BM. An inherited abnormality of neutrophil adhesion: its genetic transmission and its association with a missing protein. *N Eng J Med*, 302:1163-1168, 1980.
35. Dana N, Todd IRF, Pitt J, Springer TA, Arnaout MA. Deficiencies of a surface membrane glycoprotein (Mo 1) in man. *J Clin Invest*, 73:153-159, 1984.
36. Agerholm JS, Houe H, Jorgensen CB, Basse A. Bovine leukocyte adhesion deficiency in Danish Holstein-Friesian cattle. II. Patho-anatomical description of affected calves. *Acta Vet Scand*, 34:237-243, 1993.
37. Lienau A, Stober M, Kehrli ME, Tammen I, Schwenger B, Kuczka A, Pohlenz J. Bovine leukocyte adhesion deficiency: clinical picture and differential diagnosis. Dtsch. Tierärztl. *Wochenschr*. 101:405-406, 1994.
38. Ackermann MR, Kehrli ME Jr, Laufer JA, Nusz LT. Alimentary and respiratory tract lesions in eight medically fragile Holstein cattle with Bovine Leukocyte Adhesion Deficiency (BLAD). *Vet Pathol*, 33:273-281, 1996.
39. Ryncarz RE, Dietz AB, Kehrli ME Jr. Recognition of leukochimerism during genotyping for Bovine Leukocyte Adhesion Deficiency (BLAD) by polymerase chain reaction amplified DNA extracted from blood. *J Vet Diag Invest*, 7:569-572, 1995.

40. Dennis JA, Healy PL, Beaudet PL, O'Brien WE. Molecular definition of bovine argininosuccinate synthetase deficiency. *Proc Natl Acad Sci USA*, 86:7947-7951, 1989.

41. Bowling AT, Stott ML, Bicke L. Silent blood chimaerism in a mare confirmed by DNA marker analysis of hair bulbs. *Anim Genet*, 24:323-324, 1993.