Bovine leucocyte adhesion deficiency: a review of a modern disease and its implications

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SUMMARY

Bovine leucocyte adhesion deficiency (BLAD) is a genetic disease of cattle which affects the hematopoietic system. In the last decade BLAD has become a disease of economic importance in the dairy industry. This review describes the chronological developments and thinking that led to the elucidation of BLAD as a disease distinct from previous models in canine and human populations. All species affected show signs of chronic and recurrent infections. Necrotic and/or gangrenous infections of soft tissues are prevalent, in addition to secondary infections with bacteria or fungi. Low birthweight and unthriftiness are key signs in all species affected by leucocyte adhesion deficiency (LAD). Dermatomycoses and impaired pus formation are also common findings. The physiological basis for BLAD is a deficiency in the chemotactic and phagocytic properties of leucocytes and particularly neutrophils. The inhibition of diapedesis in the inflammatory response prevents normal immune reactions to invading pathogens. Chronic infections are a consequence of the faulty immune mechanisms. The biochemical aetiology of BLAD involves cell surface glycoprotein molecules known as integrins. These are responsible for the cell-cell interactions necessary for neutrophils to adhere to vascular endothelium in a normal individual. Experiments with monoclonal antibodies to block LFA-1, Mac-1, and p150,95 (three integrins vital for cell-cell interactions) mimic BLAD symptomatology and have led to the discovery of the reciprocal intercellular adhesion molecule (ICAM). Through pedigree analysis and biochemical detection with restrictive endonucleases, BLAD has been isolated genetically to a single gene locus. The economic significance and prophylaxis of the disease are briefly discussed. In addition, the beneficial aspects of the study of BLAD are considered. There are advantages in producing a BLAD-like state for preventing transplant rejection, ischaemia-reperfusion injury and other problems arising from the deleterious effects of the inflammatory response.

BOVINE leucocyte adhesion deficiency (BLAD) is a relatively recently described haematopoietic disease of cattle which is just one manifestation of a larger group of leucocyte adhesion deficiencies. These deficiencies are defined broadly as ‘a genetic disorder of adhesion dependent leucocyte functions characterised clinically by recurrent soft tissue infections, delayed wound healing and severely impaired pus formation despite striking blood neutrophilia (Williams 1990). The condition was first described some 20 years ago in human patients who were immunodeficient and subject to infections and neutrophil deficiencies in chemotaxis and phagocytosis. Around 1984 its aetiology was determined to be a deficiency in the expression of cell surface glycoproteins known as integrins (Kishimoto et al 1987). It is these integrins which will be the primary focus of this paper. Further investigations have revealed similar syndromes in the Irish setter and the Holstein-Friesian cow, and these were designated canine and bovine granulocytopathy syndrome, respectively.

Leucocytes may be subdivided into granulocytes and agranulocytes. The former synthesise proteins which are stored in cytoplasmic granules. When stimulated these cells can move from the bloodstream into tissues, a process called diapedesis, and release their granular contents by exocytosis. The neutrophil is of particular clinical relevance to BLAD. Neutrophils are phagocytic and contain granules with microbicidal proteins and digestive enzymes. They are involved in cell-mediated cytoxicity and antigen presentation, and they have membrane receptors to recognise and bind the pathogens for phagocytosis (Williams 1990).

The significance of circulating polymorphonuclear cells (PMNs) in host defence mechanisms was recognised as early as the 1880s by Metchnikoff. It is the role of the PMNs’ adherence to vascular endothelium, however, that has been elucidated only recently (Arnaout 1990). From the discovery of LAD in 1974 it was nearly a decade before the biochemical basis of the lack of leucocyte adhesion and migration had been determined.

CLINICAL SIGNS

Humans

The clinical signs of LAD in human beings are recurrent, necrotic or even gangrenous infections of soft tissue (subcutaneous tissue or mucous membranes). The patient with LAD is immunosuppressed and is therefore susceptible to secondary bacterial and fungal pathogens such as Straphylococcus aureus, Pseudomonas species, other Gram-negative enteric rods and Candida species (Williams 1990). In the new-born the severance of the umbilical cord is delayed and wound healing is generally impaired. A key feature is the presence of recurring infections despite a persistent neutrophilia. The neutrophils cannot migrate from the vasculature to where they are most needed, at the sites of tissue infection.

Dogs

A similar condition was originally described by Renshaw in 1975 in a male Irish setter with recurrent bacterial infections, associated pyrexia and persistent neutrophilia. Normal values for canine leucocytes are 8000 to 14,000 μl⁻¹ with 55 to 75 per cent neutrophils. Affected animals have 25,000 to 65,000 μl⁻¹ with 75 to 90 per cent neutrophils, and if the infection is exacerbated they can rise to 120-540,000 μl⁻¹. There is a moderate normocytic, normochronic anaemia. Neutrophilia can also occur in cases of granulocytic leukaemia, malignant lymphoma, chemical and/or metabolite intoxication and, most commonly, from infectious agents (Renshaw and Davis 1979). In another
study of a litter of Irish setters similar clinical signs were observed (Renshaw and Davis 1979). The neutrophils appeared normal ultrastructurally and the lysosomes were normal, suggesting that the defect was more subtle. The in vitro killing of \textit{Escherichia coli} by the affected leucocytes was impaired (Renshaw and Davis 1979). There was a reduction of the overall growth of the affected litter mates with the result that at one month of age they were half the size of their unaffected siblings. Broad-spectrum antibiotics were necessary to keep them alive for more than a few weeks to complete the study.

Other signs in the Irish setter include omphalophlebitis, suppurrative skin lesions, gingivitis, periodontitis, lymphadenopathy, pododermatitis, pneumonia, superficial pyoderma and, in advanced cases, osteomyelitis. Low bodyweight, dull haircoat and multiple bone involvement were recorded in another study of 12 Irish setter pups (Trowald-Wigh et al 1992).

\textbf{Cattle}

In the early 1980s a LAD-like disease began to occur in Holstein-Friesian cattle, most often when they were between two weeks and 15 months old (Muller et al 1984). Hagemoser et al (1983) described the condition in a yearling heifer that was thin (60 per cent of its expected size), with a poor appetite and swollen denuded plaques on its muzzle. Biopsy of the nasal lesions revealed a granulomatous inflammation. In an attempt to isolate cells from the bone marrow the animal’s thorax was injured; it became swollen and the injury took a long time to heal (Hagemoser et al 1983). The submandibular lymph nodes were swollen to three to five times their normal size. There was generalised lymphoid hyperplasia. In addition, splenomegaly was observed and the red pulp was infiltrated with neutrophils and plasma cells. The hepatocytes and intracytoplasmic inclusion bodies were determined to be megamitochondria. The portal triads were mildly infiltrated with neutrophils which also aggregated in sinusoids. The circulating leucocytes appeared normal with no lymphomatos inclusions. This would be a differential diagnosis for Chediak-Higashi syndrome. There was a leucocytosis, sometimes with a left shift shortly after birth, reaching levels of \(100 \times 10^9\) leucocytes litre\(^{-1}\). The myeloid/erythroid ratio was 9:1, as would occur with a marked neutrophilia without concomitant anaemia. In another study of seven Holsteins focal necrotic ulcers of the nose, tongue and mucous membranes were present in all animals. The affected calves also exhibited lymph node enlargement, splenomegally, thickened maxilla and mandibles, pneumonia, fibrotic pleurisy, cachexia and lymphoid hyperplasia. In six of the seven the rumen or small intestine was involved. Bacterial pathogens (\textit{Corynebacterium pyogenes} and \textit{Pasteurella hemolytica}) were isolated from lung lesions in two cases (Takahashi et al 1987).

Unthriftiness is a consistent finding in this disease. In one study of eight calves there was extreme stunting of growth (40 per cent of normal bodyweight) and anaemia in some cases, but were of relatively normal stature and had normal oestrous cycles (Muller et al 1984). Calves whose growth is stunted often appear with large heads and hooves in relation to body size (Jorgensen et al 1993b). Dermatomycoses, particularly ventral dermatitis and vasculitis occur. A lack of pus formation is also a prominent feature. In a study of 14 cases between 1977 and 1991 (Gilbert et al 1993), in addition to the signs normally reported, bruxism and wounds associated with dehorning or ear tagging that were still suppurating six months after the procedure were observed. A survey of many studies reveals that gastrointestinal and respiratory processes are mainly involved in the infectious pathogenesis of the condition. The molecular basis of bovine granulocytopenia syndrome was found to be, as in human LAD or canine LAD, a deficiency of integrins. These have been studied extensively in recent years. Bovine granulocytopenia syndrome has since been renamed BLAD (Kehrli et al 1990).

\textbf{BIOCHEMISTRY}

The biochemical basis of all LAD syndromes is an inherited (autosomal recessive) deficiency of the cell-surface glycoproteins known as integrins. The integrins are one of three families of cell-surface molecules which regulate the migration of leucocytes and mediate the immune response. The immunoglobulin superfamily includes receptors for T and B cells. Selectins, the third group, are necessary for the interaction of lymphocytes and neutrophils with vascular endothelium (Springer 1990). The integrins are cellular adhesion/recognition mechanisms which are important not only in diapedesis and inflammation but also in embryological differentiation and development (Kishimoto et al 1992). In evolutionary terms, these molecules are related to other integrins, such as fibronectin receptors and platelet group IIb/IIia, which guide cells during embryogenesis and normal healing processes.

\textbf{Integrin subunits}

The integrins which are important in BLAD are leucocyte function-associated antigen (LFA-1), macrophage antigen (Mac-1) and p150,95. These are heterodimers which each consist of a unique \(\alpha\) subunit (CD11a, CD11b and CD11c, respectively). All three share a common \(\beta\) subunit (CD18) (Springer 1990). As a result, there is a redundancy; all three contribute to the adhesion of neutrophils and monocytes to endothelial cells and substrates. No selective deficiency of just one integrin is observed (Kishimoto et al 1992). The \(\alpha\) and \(\beta\) subunits are non-covalently associated. They are transmembrane proteins with relatively small cytoplasmic domains. There is evidence that they are intrinsically involved in connections with the cytoskeleton (Haynes 1987). It seems that the \(\beta\) unit distributes with extracellular fibronectin and the intracellular components of the cytoskeleton (vinculin, talin, actin, \(\alpha\) actinin and tropomyosin.) The CD11a, CD11b and CD11c molecules are 180, 155 and 150 kD proteins whereas the \(\beta\) subunit is 94 kD. Divalent cations (Ca\(^{2+}\) and Mg\(^{2+}\)) are essential for maintaining the structural integrity and function of these \(\alpha\beta\) complexes (Arnaout 1990). The CD11a/CD18 is expressed on all leucocytes regardless of subtype. CD11b/CD18 and CD11c/CD18 are found on the cell membranes of monocytes, macrophages, PMNs and natural killer (NK) cells. In addition, CD11c is found on some CD5 B cell lines and some activated lymphocytes and is a marker for hairy cell leukaemia (Kishimoto et al 1992). T and B cells normally express CD11a/CD18. Active granulocytes express CD11b/CD18 in a greater proportion than the other cell adhesion molecules. A resting macrophage, for example, expresses CD11a most abundantly, followed by CD11b and then CD11c. An activated tissue macrophage expresses CD11c>CD11a>CD11b (Arnaout 1990). There are synonymous designations for both Mac-1

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and p150,95. Mac-1 is also known as Mo-1, OKM-1 and complement receptor type 3 (CR3); p150,95 can also be referred to as CR4 and LeuM5 (Kishimoto et al 1987).

**PHYSIOLOGICAL CONSEQUENCES**

The experimental blocking of LFA-1, Mac-1 and p150,95 with monoclonal antibodies (mAbs) reveals the physiological consequences of deficiencies of these molecules. A mAb to LFA-1 blocks leucocyte adhesion to endothelial cells and inhibits the conjugation required for antigen presentation, cytotoxic T cell killing, T cell proliferation and NK cell activity. mAbs to Mac-1 block binding to IC3b (a fragment from the cleavage of C3b, the third component of complement)-coated particles, myeloid cell adhesion to endothelium, and neutrophil homotypic aggregation and chemotaxis. mAbs to p150,95 block monocyte adhesion to endothelial cells and the formation of cytotoxic T cell conjugates with target cells. These mAb studies led to the discovery of intercellular adhesion molecules (ICAMS) as counter receptors to the integrins (Diamond et al 1990). ICAMS are also transmembrane proteins, but of the immunoglobulin superfamily. They have five immunoglobulin-like domains in the extracellular portion of the molecule. ICAM-1, LB2 (CD54) and ICAM-2 bind to CD11a/CD18. ICAM-1 and ICAM-2 are counter receptors for LFA-1. ICAM-2 is expressed constitutively on endothelial cells. ICAM-1 will bind to Mac-1. These binding interactions facilitate the adhesion of neutrophils to endothelial cells (Springer 1990.)

**Adhesion receptor interactions**

The exact mechanisms that allow diapedesis to occur in normal leucocytes during inflammatory events are complex and poorly understood. However, studies of BLAD have opened up new ideas and speculation in these matters. There seem to be two classes of adhesion receptor interactions which are differentiated by the distance between the plasma membranes of the two closely apposed cells. T cell receptor (TcR) and major histocompatibility complex (MHC) interactions and CD2-LFA3 interactions are close (about 13 nm or less) whereas the ‘distant’ interactions of LFA-1 with ICAM-2 or ICAM-1 occur at 27 and 36 nm respectively. The ‘close’ interactions require extensive interdigitation of the membrane glycoacylcosy of the two adhering cells and are thought to hinder the lateral mobility of surface glycoproteins, which would exclude the major glycoacylcom mutant from the cleavage region of chromosome 16. Kishimoto (1987) recognised five phenotypes by using Southern blot analysis. Some patients had undetectable β subunit mRNA and protein precursors. The gene was present and there was thus a mutation. Other patients had low levels, synthesising some 30 per cent of the normal amounts of α and β complexes. In addition, aberrantly large or small β subunit precursors are observed. The last group had normal sized proteins. The defects affect either the synthesis of β subunits or the ability of α and β subunits to associate with each other.

**AETIOLOGY**

Human LAD, canine LAD, and BLAD are all the result of a mutation in the β subunit (CD180).

**Humans**

The heterogeneity of the human condition is associated with different mutations within the same gene locus. These defects have been isolated to chromosome 21. The α subunits of LFA-1, Mac-1, and p150,95 are within bands p11-p13.1 of chromosome 16. Kishimoto (1987) recognised five phenotypes by using Southern blot analysis. Some patients had undetectable β subunit mRNA and protein precursors. The gene was present and there was thus a mutation. Other patients had low levels, synthesising some 10 per cent of the normal amounts of α and β complexes. In addition, aberrantly large or small β subunit precursors are observed. The last group had normal sized proteins. The defects affect either the synthesis of β subunits or the ability of α and β subunits to associate with each other.

**Dogs**

Pedigree analysis in the Irish setter (Renshaw 1970) also revealed autosomal recessive inheritance. Backcrossing an affected individual with its dam produced litters with both affected and normal puppies (Renshaw and Davis 1979). A normal unrelated female mated with the propositus (the individual in the study) resulted in normal offspring. Subsequent breedings, however, produced affected males and females. The observed ratios were close to the expected 1:3 when heterozygotes mate. Canine granulocytopenia syndrome (CGS) was the first LAD in which no lysosomal aberrations were observed, with the exception of human beings (Giger et al 1987). The significance of LEU-M5 (p150,94) to CGS was discovered by Giger et al (1987). They described a mating with an 18-month-old Irish setter female cross-breed which was one of five offspring of an accidental breeding between a mother and son. This indicates the importance of educating breeders about this disease to avoid matings between carriers.

**Cattle**

Point mutations of the gene coding for CD18 are implicated in the aetiology of BLAD. DNA studies done with a restriction endonuclease (Taq I) revealed differences between healthy and affected calves. Normal animals have fragments of 100, 200 and 300 base pairs whereas the BLAD calves have 200 and 400 bp bands. The donor was healthy and had all the fragment lengths and was, therefore, a carrier for the defect. The carrier had both normal and abnormal chromosomes. The sire was identical in this respect to the dam. Using polymerase chain reaction (PCR) techniques carriers can be detected which are heterozygous for the abnormal CD18 gene (Kehrl et al 1990). The deficiency observed in Holstein-Friesian cattle is a deficiency in
Mac-1. There is a single point mutation, a substitution of guanine for adenine at position 383 in the cDNA of the CD18 gene (Shuster et al 1992) which results in the substitution of guanine for aspartic acid at amino acid #128 in the protein. There is another mutation, the substitution of a cytosine for thymine, which has no effect. A bull named NJY Hubert sired 17 per cent of the registered calves born in 1991 and was involved in 820 of 1183 carrier and carrier matings observed. It was mainly responsible for the spread of BLAD in Denmark (Jorgensen et al 1993a). Imported semen of American Holstein bulls has had an enormous impact on the Netherlands in the past decade. The sire of both affected animals in another study were linked to an ancestral sire carrier of the bovine D128G CD18 allele. In another study of 20 calves the incidence was found to be 15 per cent in bulls and 6 per cent in cows, with an overall incidence of 0-2 per cent of Holsteins at birth in the USA (Shustet al 1992). BLAD is a common disease throughout the world, one of the most significant diseases in animal agriculture today. In Japan and Europe many cases can be traced back to ‘Osborndale Ivanhoe’, designated the father of the Holstein breed owing to his prolific nature.

BLAD calves usually die when they are less than a year old. The survivors grow poorly and have low reproductive ability. In the USA 80 per cent of the 10,000,000 dairy cows are Holsteins, and about 16,000 calves with BLAD are born each year. The average economic loss is $300 per calf, so that the total loss due to BLAD in the USA alone is about $5,000,000 per year.

TREATMENT

Treatment consists of supportive measures and prophylaxis with antibiotics (trimethoprim-sulpha) for bacterial infections. Mild phenotypes may be maintained for some time by this method, but the disease usually results in rapid fatality. The only cure is somatic cell reconstitution and bone marrow transplants (Arnaout 1990). LFA-1 function has been restored in lymphoblastic cell lines from human LAD patients by using CD18 cDNA in an Epstein-Barr viral vector or a retroviral vector (Kehrl et al 1990).

FURTHER WORK

The study of BLAD offers many opportunities for further investigation and beneficial applications. The carrier state is helpful to isolate genetic traits (other than LAD) on CD18. Selection against weaver syndrome, a heritable neurological disease in brown Swiss cattle, is now possible. The disease seems to have beneficial effects on ischaemia-reperfusion injury. Acquired deficiencies can be induced by administering mAbs to CD11/CD18, and the antibodies attenuate the tissue destruction associated with myocardial infarctions in dogs, intestinal ischaemia in cats, and haemorrhagic shock syndrome. It reduces tissue injury, microvascular permeability and acidosis. Another positive application is in the use of mAbs to integrins to prevent transplant rejection reactions (Arnaout 1990).

Because the dairy industry breeds for high milk yields there is much genetic relatedness in the Holstein, where only a few sires fertilise many cows. This, however, makes the eradication of the disease easier. Current screening programs and the elimination of affected sires from the breeding pool should greatly reduce the incidence of BLAD (Shuster et al 1992). With strict adherence to these protocols it is certain that the prevalence of BLAD will diminish and the syndrome will become a relic of the past.

REFERENCES


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