CLINICAL MANIFESTATIONS OF BOVINE LEUKOCYTE ADHESION DEFICIENCY: A NEWLY-RECOGNIZED, INHERITED DISEASE OF HOLSTEIN-FRIESIAN CATTLE

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Introduction

Bovine granulocytopathy (now recognized as bovine leukocyte adhesion deficiency or BLAD, a disease analogous to the human condition called leukocyte adhesion deficiency) was described in the U.S.A. in 1983 by Hagemoser et al.[1] These authors described a Holstein heifer with a disease complex characterized by a generalized increase in susceptibility to infectious agents over the two year lifespan of the heifer. They documented impaired neutrophil function in this animal, which had persistent circulating neutrophilia, but seemed unable to mount a peripheral inflammatory response. Neutrophils appeared structurally normal when examined by electron microscopy (there were no lysosomal inclusions characteristic of Chediak-Higashi syndrome), but random migration, bacterial ingestion, nitroblue tetrazolium reduction and iodination were depressed relative to normal controls. Antibody-dependent, cell-mediated cytotoxicity was not affected.

A similar disease was subsequently described in Japan, in Holstein-Friesian calves of American descent.[2-4] The condition in these animals was marked by chronic and recurrent infection (pulmonary or gastro-enteric), and persistent circulating neutrophilia. Again, no ultrastructural abnormalities were detectable. The phagocytic ability and nitroblue tetrazolium reduction of neutrophils from affected calves were dramatically reduced compared with controls, as were random and directed migration under agarose. Takahashi et al[2] provided pedigree information for seven affected animals which strongly suggested a hereditary disease, with a simple autosomal recessive mode of inheritance. These authors also described pathologic lesions of the gastro-intestinal tract in which invading micro-organisms could be identified in ulcers, with prominent absence of any local infiltration of neutrophils. Also observed were hyper-gamma-globulinemia, hypocholesterolemia and hypocalcemia which worsened as the animals became terminal. Many of these findings were confirmed in a later report.[3]

In 1990, Kehrli et al[5] identified the basis of the neutrophil dysfunction as a deficiency of the Mac-1 (CD11b/CD18) glycoprotein, a β_2 integrin on the surface of leukocytes. They also identified one of the most prominent bull families in the Holstein breed as potential carriers of the disease. Furthermore, their data supported the presumed autosomal recessive mode of inheritance. The deficiency of the CD18 component of the neutrophil integrin was confirmed by indirect immunofluorescent flow cytometry. In doing so, they identified this disease as a homologue of human LAD.

Leukocyte adhesion deficiency has been described in people. The disease is biochemically identical to the bovine disease. The same, or at least a very similar, condition has been described in dogs.[6-9] A line of related Irish Setters was identified which had a condition characterized by severe neutrophilia and susceptibility to infection. Their leukocytes were deficient in expression of CD11/CD18.

Recently, Shuster et al have sequenced the normal gene for bovine CD18 and identified the defective bovine CD18 gene. Polymerase chain reaction technology is now available to identify homozygous (affected), heterozygous and homozygous (normal) animals by examination of DNA. This diagnostic technology has allowed positive identification of affected and carrier animals. The technique has also been applied to archival, formalin-fixed tissues, allowing retrospective diagnosis of the disease.

Pathogenesis

BLAD is transmitted as a simple autosomal recessive inherited trait. Affected calves express little or no CD11/CD18 on the surface of leukocytes. The function of

neutrophils and monocytes appear to be most severely depressed. Lack of Mac-1 (CD11b/Cd18) expression on the neutrophil surface prevents adhesion of neutrophils to the vascular endothelium in inflammatory conditions. Such adhesion is a necessary prelude to the emigration of neutrophils from the circulation into inflamed tissue. Consequently, neutrophils remain trapped in the vascular lumen, and very high neutrophil counts may result. Infectious loci are conspicuously devoid of invading neutrophils, although blood vessels in the affected tissue may contain high numbers of segmented cells. The CD11b/CD18 dimer is also the binding site for C3b. Consequently, neutrophils of affected animals are also deficient in adhesion to and phagocytosis of opsonized particles. The overall result is a profoundly immunocompromised animal susceptible to common pathogens. Contrary to some earlier reports, neutrophil oxidative metabolism does not appear to be compromised. It is known that in vitro oxidative burst is more sustained and in adherent neutrophils, and this may be the basis for the original observations. Abnormalities in monocyte function are expressed as delayed wound healing and aberrant bone remodelling.

No functional deficits of any sort have been identified in neutrophils or other cells of carrier (heterozygous) animals. They are symptomless. They may be positively identified by DNA amplification and CD18 genotype determination.

Clinical Signs

Typically, signs of the disease are recognized between 2 weeks and 8 months of age. In experimental settings, neutrophilia is identifiable within a few days of birth in affected calves. Organ systems most commonly affected are respiratory and gastrointestinal. Virtually all affected animals have some degree of lower airway disease, although the presenting signs may include diarrhea, inappetence or weight loss. Common clinical findings at presentation include dyspnea, bruxism, laryngitis, ulcers or erosions of the muzzle or oral mucosa, peripheral lymphadenopathy or generalized dermatomycosis. A striking lesion which appears to be a consistent finding in calves in which the disease has progressed for a period of months, is severe periodontitis, progressing to alveolitis and loss of deciduous or permanent teeth. This is usually evident upon diligent examination of the oral cavity, and can be confirmed radiologically.

Routine hematological assessment of calves with BLAD reveals marked and persistent mature neutrophilia. In all published accounts of the disease, neutrophil counts have reached levels of 40,000 to 300,000 cells/$\mu\ell$. This is particularly noteworthy in cattle which are notorious for having a rather sluggish bone marrow response to infection. In most cases, all or nearly all circulating neutrophils are mature, segmented cells. Left shift does occur during periods of acute exacerbation of the clinical condition, but is not a persistent feature of the disease. Monocytosis is also present in most affected calves. Affected calves are consistently hypoalbuminemic and hyperglobulinemic. Blood urea nitrogen and serum creatine concentrations are usually low normal or below reference ranges, and affected calves are usually hypoglycemic.

The hallmarks of this condition are chronicity of disease, and failure to respond to conventional therapy. In most affected animals, treatment with appropriate antimicrobials results in temporary alleviation of symptoms with remission of fever and a decline in leukocytosis and neutrophilia (although neutrophil counts do not return to the normal range). In animals older than about 3 months, impairment of growth is conspicuous. Poor hair coat and poor body condition are typical of affected animals.

Infectious agents isolated from cases of BLAD include common pathogens rather than opportunistic organisms typical of some immunodeficiencies, and include Pasteurella spp, Actinomyces pyogenes and Pseudomonas aeruginosa.

Postmortem findings in cases of BLAD include pneumonia and bronchiectasis, periodontitis and loss of teeth, ulcerative lesions of the muzzle, oral cavity or intestinal tract and generalized lymphadenopathy. In some cases, swelling and edema of the larynx, smaller than expected thymus or osteodystrophy have been reported. Fibrin and excessive fluid are often reported in one or more body cavities (usually the

thorax, but sometimes the abdomen or joints). Histopathological lesions are consistent with macroscopic findings and also include myeloid hyperplasia of bone marrow, lymphoid hyperplasia and histiocytosis of lymph nodes. Intravascular accumulation of neutrophils is usually conspicuous with few neutrophils in the infected or damaged tissue.

Chronic and unresponsive infections in calves that persist despite appropriate therapy mandate laboratory testing including a complete blood count which would alert the clinician to the possibility of BLAD. Although clinical signs are variable, the combination of history, signs and laboratory data are very suggestive of the diagnosis. In cases where this is necessary, the diagnosis can be confirmed by genetic testing (DNA amplification restriction endonuclease analysis) or by flow cytometric demonstration of severely deficient expression of leukocyte integrins {}. Because the disease is inherited as an autosomal inherited condition, the pedigree of the affected animal provides additional supportive evidence for the diagnosis since both parents must be heterozygotes for the disease to occur in the offspring.

Diseases most resembling BLAD in presentation include persistent infection with bovine viral diarrhea virus, internal abscessation, parasitism, chronic salmonellosis, neoplasia of hematopoietic tissue, endocarditis, pleuritis, autoimmune disease and abscessation of the larynx. Exuberant neutrophilia, of the magnitude seen in calves with BLAD, is rare in cattle, but may be seen in cases of bacterial septicemia or septic peritonitis and pleuritis.

Discussion

Retrospective analysis of archival tissue material has confirmed that cases of BLAD have been seen in the northeastern USA since 1977. The first known bull to carry this mutation (Osborndale Ivanhoe) was born in the 1950s and has had a profound influence on the Holstein breed since then, such that by late 1991 approximately 13% of AI sires in the USA were heterozygous for this condition. The North American artificial insemination industry was quick to realize the implications of BLAD, and sponsored much of the research into this condition. Currently, all active sires have been tested. BLAD-status is designated by a suffix after the name of the bull: TL indicates that bulls have been tested and found free of the mutant gene, and BL indicates known carriers of the defect. It is not possible for a bull free of the mutation to sire an affected calf. Young sires are screened for the defect upon admission into progeny testing programs; only bulls free of the mutation are entered into these programs. In areas where the status of sires is not known, avoidance of inbreeding represents the best defense against the condition.

There is compelling evidence that BLAD exists in most countries of the world which have imported American Holstein genetics during the past decades - the disease has been identified in Japan, Germany, The Netherlands, South Africa, and can be expected to occur in many other countries.

For some time, clinicians in North America have recognized a condition colloquially known as the "leukemoid response of calves." Some efforts have been made to characterize this syndrome and various clinicians performed histochemical staining for leukocyte alkaline phosphatase to rule out myeloid leukemia in such cases. It now seems certain that most or all of these cases actually represent BLAD. Although BLAD is not spectacular in its presentation, mimicking common calfhood diseases, it is potentially significant since its gene frequency seems to be well in excess of other currently recognized, lethal, inherited conditions.

Summary

A condition then termed bovine granulocytopathy was first described in 1983. The affected heifer in that report was prone to bacterial infections, exhibited severely impaired wound healing and had a persistent neutrophilia. Several function impairments of neutrophil function were demonstrated in this animal. Subsequently a similar disease was described in Japan in several related calves. Features of the disease

included outspoken mature neutrophilia, with abnormalities of some neutrophil functions but no demonstrable ultrastructural defects, and susceptibility to common respiratory and enteric bacterial pathogens. In 1990, it was recognized that this disease was the result of a deficiency of Mac-1 (CD11b/CD18) - an important adhesion-related molecule expressed by neutrophils. Specifically, the deficit lay in the defective production of the CD18 component, therefore influencing the function of other marrow-derived cells as well as neutrophils. Subsequently, both the normal and mutant genes were sequenced, and a gene probe developed which allowed identification of normal, heterozygous, and homozygous-affected individuals. North American AI bulls have been tested for the presence of the mutant gene; those carrying the gene for BLAD are designated by the suffix "BL" after their names, and those tested and found free of the mutation are designated "TL". The condition probably has a world-wide distribution.

Signs of the disease may be detected within a few days of birth, and affected animals are usually presented before the age of 8 months. Common signs include lower airway disease, erosions or ulceration of the muzzle, oral mucosa, or gastrointestinal tract. Periodontitis with alveolar bone loss and loss of dentition has been observed. All affected calves have extreme leukocytosis characterized by outspoken, mature neutrophilia and mild monocytosis. Hypoalbuminemia, hyperglobulinemia and hypoglycemia are consistent clinical chemistry findings. Physical findings and laboratory data are usually highly suggestive of a diagnosis of BLAD; the disease may be confirmed by demonstration of the presence in homozygotic form, of the mutant gene, or by demonstration of extremely low levels of expression of CD18 on the surface of leukocytes from the patient.

Resume

La granulocytopathic bovine est une condition qui fut reconnue et decrte en 1983. Dans cette etude, la geniose alteiate de la condition avait tendance aux infections bacteriennes, un temps de gelerison prolonge, et une neutrophile persistante. Plusieurs defauts fontionnels neutrophiliques furent demontres chey cet animal. Par la suite, une maladie semblable fut decrite au japon chey plusieurs veaux apparente's. Les caracteristiques de cette maladie comprement une hypersegmentation des neutrophiles avec des anormalites de fonctionnement des neutrophiles mais sans defauts ultrastructuraux demontrables, et une susceptibilite aux bacteries pathogenes (respiratories et enteriquec). En 1990 on s'apercevat que cette maladie etait consequente a un deficit de Mac-1 (CD 11b/CD18), une molecule jouante un role important a l'adhesion du neutrophile. Plus precisement, cette deficience se trouwait dans la production anormale de la partie CD18 une entite qui influence lafonction du mentrophile, mais auni celle d'autres celldules derivees de la moitle. Depuis, la sequence du gene nomral aet du gene mutant fut clonie et on developpa une sonda genetique, ce qui permettait l'identification des individus normaux, heterozygotes, et homozygotes. Les taureaux geniteurs dos centres d'I.A. Nord-Americanis furent examines pour la presence du gene mutant; lest porteurs de gene de BLAD sont designes par le suffixe "BL" apris leurs noms, et ceux qui sont examines et s'averent exempte de la mutation, sont designes "TL". Il est fort probable que cette condition a une prevalance mondiate. Les symptomes de la maladie prevent etre deceles des quelque jours apris la parturition et lesw animaux atteints sont normalement presentes avant l'age de 8 mois. On observe des maladies des voiex resperatoires inferieures, des erosions et/ou ulceration du mufle, de la muqunse buccale, et ou gastes-intesinalis. On observe la periodontite avec lyse osseuse qui peut etre associe ou non a la perte dentaire. Lous les veaux ont une leucocytose avec virage a droite et une monocytose legere. On observe frequemment l'hypolbrinemie, l'hyperglobulidemie, et l'hypoglycimie. L'examen physique et les analyses de laboratoire sont tres souvent suggestifs d'un diagnostic de BLAD; la maladie peut etre confirmie par la presence due gene mutant de type hodmozygote, ou par la mise en evidence de niveaux extremement bas de l'expression de CD18 sur la surface des leucocytes du patient.

Sumario

Una condición conocida entonces como granulocitopatía bovina fué descrita por primera vez en 1983. En aquel reporte, la vaquilla afectada era propensa a infecciones bacterianas, exhibía una habilidad para sanar heridas severamente afectada y tenía una neutrofilia persistente. Varios defectos en función neutrófila fueron demostrados en este animal. Posteriormente en Japón, una enfermedad similar fué descrita en varias vaquillas emparentadas. Rasgos de la enfermedad incluian una neutrofilia madura con anomalías de algunas funciones neutrofilas pero sin defectos ultraestructurales demostrables, y una susceptibilidad a patógenos bacterianos respiratorios y entéricos comunes. En 1990, se reconoció que ésta enfermedad era el resultado de una deficiencia de Mac-1 (CD11b/CD18) - una molécula importante relacionada con adhesion y expresada por neutrófilos. Específicamente, el déficit reside en la producción defectuosa del componente CD18, consecuentemente influenciando la función de otras células derivadas de la médula ósea asi como a los neutrófilos. Subsiguientemente, el gene normal y el mutante fueron secuenciados, y un gene de sondeo fue desarrollado que permitió la identificación de individuos normales, heterocigotos y homocigotos-afectados. Toros norteamericanos utilizados en inseminación artificial han sido probados para la existencia del gene mutante; aquellos que cargan el gene para BLAD son designados por el sufijo "BL" despues de sus nombres, y aquellos probados y encontrados libres de la mutación son designados "TL". La condicion probablemente tiene una distribución mundial. Señas de la enfermedad pueden detectarse a los pocos días de nacer, y los animales afectados generalmente son presentados antes de la edad de 8 meses. Señas comunes incluyen enfermedades de la via respiratoria baja y erosiones o ulceraciones del hocico, mucosa oral, o canal gastrointestinal. Periodontitis con pérdida de hueso alveolar y pérdida de denticion han sido observados. Todas las vaquillas afectadas tienen una leucocitosis extrema caracterizada por una neutrofilia madura y una monocitosis leve. Hipoalbuminemia, hiperglobulinemia e hipoglicemia son resultados químicos consistentes. Los resultados físicos y datos de laboratorio son generalmente altamente sugestivos de un diagnosis de BLAD; la enfermedad puede confirmarse demostrando la presencia del gene mutante en forma homocigotica o por la demostración de niveles de expresión extremadamente bajos de CD18 en la superficie de leucocitos del paciente.

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