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Animal Model of Human Disease

Bovine Leukocyte Adhesion Deficiency

β_2 Integrin Deficiency in Young Holstein Cattle

Marcus E. Kehrli Jr.,* Mark R. Ackermann,* Dale E. Shuster,* Martin J. van der Maaten,* Frank C. Schmalstieg,† Donald C. Anderson,‡ and Bonnie J. Hughes‡

From the Metabolic Diseases and Immunology Research Unit, National Animal Disease Center, USDA—Agricultural Research Service, Ames, Iowa, the Child Health Center,† University of Texas—Medical Branch, Galveston, Texas, and the Departments of Pediatrics and Microbiology and Immunology,‡ Baylor College of Medicine, Houston, Texas and the Speros P. Martel Leukocyte Biology Laboratory, Texas Children's Hospital, Houston, Texas*

Biologic Features

Leukocyte adhesion deficiency (LAD), is a rare, autosomal recessive disorder caused by a lack or partial absence of a family of leukocyte integrins, Mac-1, LFA-1, and p150,95.[1] The leukocyte (β_2) integrins are glycoproteins essential for normal leukocyte-endothelial cell adherence and emigration. Affected children develop recurrent bacterial infections, persistent leukocytosis, severe hypoplasia of lymphoid tissues and isolated neutrophils, eosinophils, macrophages, and lymphocytes have abnormal function when tested *in vitro*.[1,2] Most patients without bone marrow transplants die at an early age.

The β_2 integrins are classified as CD11/CD18 by the World Health Organization according to their α and β subunits. They are composed of identical β subunits (CD18) and α subunits that vary in structure and are designated as CD11a, CD11b, and CD11c for LFA-1, Mac-1, and p150/95, respectively. Mac-1 is the CR3 receptor and binds, most importantly, C3bi and CD54 (ICAM-1). *In vivo,* Mac-1 mediates tight adherence of leukocytes to activated endothelial cells, whereas a leukocyte selectin (L-selectin) mediates loose adherence of leukocytes to nonactivated endothelial cells.[3] In all species, abnormalities have been identified in only the β subunit of the β_2 integrins.

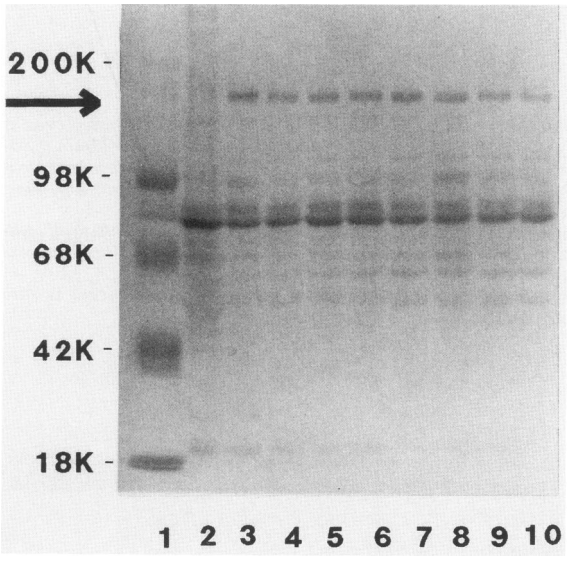

Figure 1. *Lectin (Concanavalin A) blot of neutrophil membrane lysates for Mac-1 glycoprotein. Lane 1: Molecular weight standards (kD); Lane 2: Neutrophils from the affected calf that lacks the Mac-1 α subunit (large arrow); Lane 3–10: Neutrophils from clinically normal cows.*

Animal Model

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 has been described.[4–7] By investigation of pedigrees, all of the affected calves can be traced to a common sire.[5] Neutrophil lysates from one calf were shown to lack the α subunit (CD11b) of Mac-1 by lectin blotting (Figure 1).[5] Immunofluorescent studies

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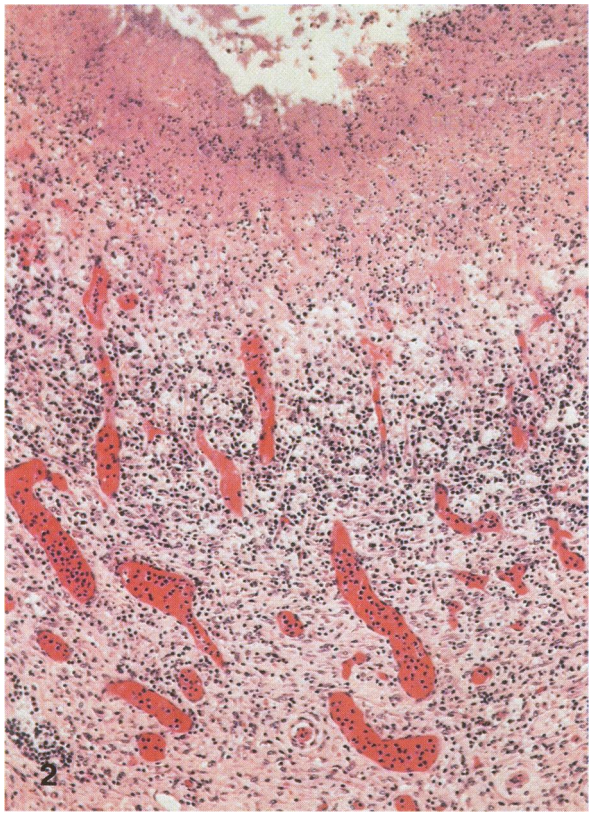
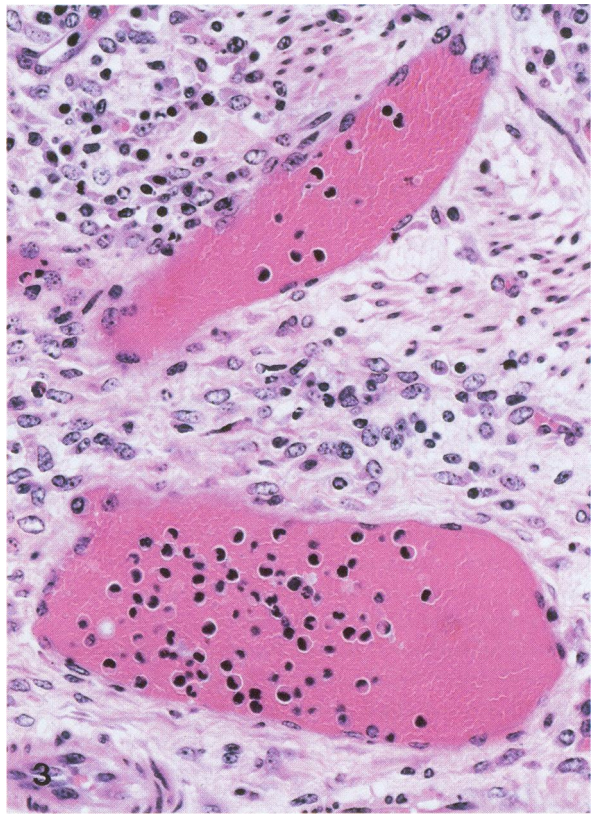
Address reprint requests to Dr. M. E. Kehrli Jr., Metabolic Diseases and Immunology Research Unit, National Animal Disease Center, USDA-Agricultural Research Service, Ames, IA 50010.





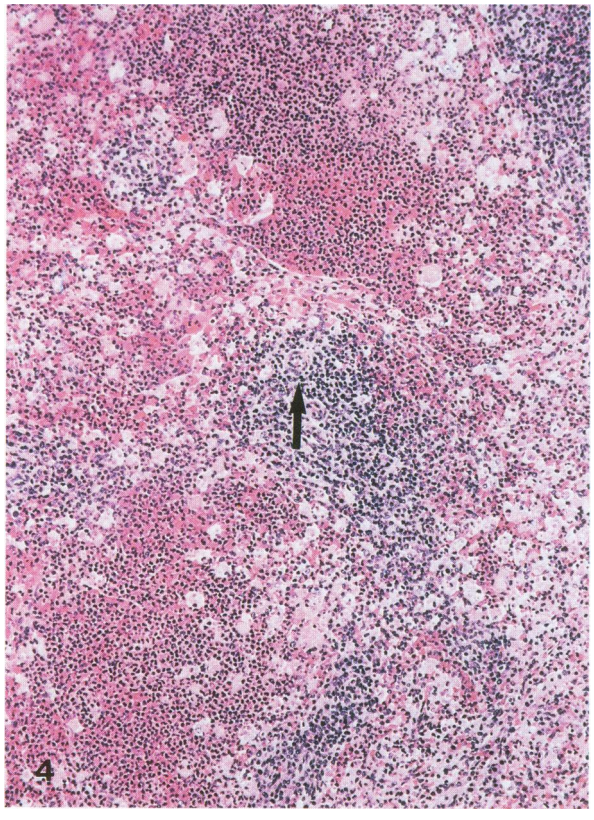


Figure 2. *Section of ileum that is ulcerated. The ulcerated area is covered by a thick mat of cell debris and bacteria. The lamina propria contains many lymphocytes and plasma cells but few neutrophils. Blood vessels are dilated, congested, and contain many neutrophils (H&E ×250).*
Figure 3. *High magnification of blood vessels of the intestinal lamina propria with numerous intravascular neutrophils and rare extravascular neutrophils (H&E ×400).*
Figure 4. *Spleen. Ensheathed splenic arteriole (arrow) is surrounded by numerous neutrophils (H&E ×250).*

of neutrophils from the calf's dam, sire, and 8 of 15 half-siblings demonstrated decreased levels of the β subunit of the β_2 integrins on the surface of leukocytes by flow cytometric analysis.[5] Recently, three additional calves, which have neutrophils expressing the α and β-subunits of the β_2-integrins at levels ~2% of normal cattle leukocytes, have been identified by flow cytometric analysis.[8] Neutrophils from these calves do not adhere in a β_2-integrin dependent manner to protein-coated surfaces.[8]

Neutrophil counts in affected calves are persistently elevated (often exceeding 100,000/μl) and lymphocytes are also increased.[4–8] Most calves have stunted growth, oral ulcers, and diarrhea. Histologically, capillaries, sinusoids, and blood vessels throughout the body contain numerous neutrophils although few neutrophils are present subjacent to ulcerated lesions of mucosal surfaces (Figures 2, 3). Large crescents of numerous neutrophils often circumscribe splenic periarteriolar lymphocytic sheaths and increased myeloid/erythroid ratios are present in the bone marrow (Figure 4). Lymph nodes range from hyperplastic, in some reports, to diffusely hypocellular with necrosis of secondary follicles.[5,6] No abnormalities are present in neutrophils examined by transmission electron microscopy; however, large hepatocellular mitochondria with numerous closely associated cristae were reported in one case.[4] The presence or absence of Mac-1 molecules can be demonstrated on the surface of neutrophils from normal or LAD calves using colloidal gold immunolabeling and scanning electron microscopy in backscatter mode (Figure 5). In addition to Holstein calves, Mac-1 deficiency has also been described in Irish Setter dogs.[9,10]

Comparison with Human Disease

Mac-1 deficiency in Holstein calves is analagous to LAD seen in humans. Neutrophils in LAD patients are unable to adhere to the endothelial lining of the cardiovascular system, thus interrupting egression of neutrophils into infected tissues. Calves die at an early age as a result of the failure of neutrophil to extravasate into infected tissues, as is the case with most human LAD patients who do not receive bone marrow transplants. Severe generalized prepubertal periodontitis, similar to that seen in cattle with LAD, has been reported in children with LAD.[8,11]

The underlying genetic defect of bovine LAD has been identified as an amino acid substitution (D128G) in a 26 amino acid sequence that shares 100% homology with human and murine CD18 protein sequences.[12] The bovine mutation occurs in a 250 amino acid region of CD18, where several mutations causing human LAD have been found.[12]

Usefulness of the Model

Bovine Mac-1 deficiency is a useful model for studying genetic inheritance, genomic transcription and translation, and function of integrin-deficient leukocytes. The occurrence of the mutation in a highly interspecies conserved region of the CD18 protein suggests the use of the bovine model for integrin protein studies of structure/function relationships affecting α/β subunit association and adherence functions. Gene therapy regimens using genetically engineered donor cells make the model useful for development of treatment strategies for LAD patients.

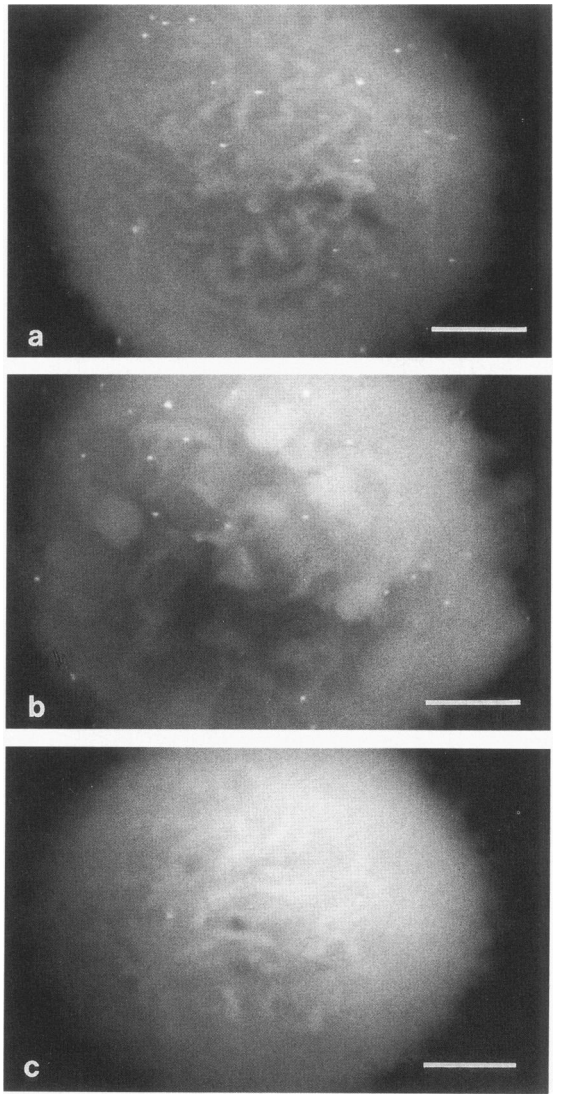


Figure 5. *Scanning electron micrographs of neutrophils labeled with anti-CD18 monoclonal antibody (R15.7) and colloidal gold (30 nm) using backscattering imagery.* **a,b:** *Neutrophils from cows with normal CD18 genotype and normal expression of CD18.* **c:** *Neutrophil from a Holstein cow with homozygous D128G (defective) genotype and expression of CD18 <2% of normal.*

Availability

Genotypic carriers have been used in superovulation and embryo-transfer experiments as part of a program at the National Animal Disease Center to produce additional calves deficient in the β_2 integrins. Calves can be derived by Caesarean section and kept in gnotobiotic isolators until 2 to 3 months of age. Many naturally occurring cases of bovine LAD on farms can now be identified phenotypically by flow cytometric analysis and genotyped by a DNA-PCR test.[12]

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