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Overview

The Genetic Immunodeficiency Disease, Leukocyte Adhesion Deficiency, in Humans, Dogs, Cattle, and Mice

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This review highlights the genotype-phenotype relationship of the genetic immunodeficiency disease leukocyte adhesion deficiency (LAD) in humans, dogs, cattle, and mice, and provides assessment of the opportunities that each animal species provides in the understanding of leukocyte biology and in developing new therapeutic approaches to LAD in humans. This comparison is important since animal models of genetic diseases in humans provide the opportunity to test new therapeutic approaches in an appropriate, disease-specific model. The success of this approach is dependent on the relationship of the phenotype in the animal to the phenotype of the disease in humans.

Leukocyte adhesion deficiency (LAD) is a genetic immunodeficiency disease in humans that is characterized by defects in the leukocyte adhesion cascade. Currently, three types of LAD have been identified. In LAD type-I disease, the most common type, deficiency of the integrin β2 subunit (CD18) is responsible for the disease phenotype (4, 6). In LAD type-II disease, absence of the selectin ligand, SleX, affects leukocyte rolling (21). Recent findings indicate presence of a third form of LAD (type III), which is caused by defects in G protein-coupled receptor-mediated integrin activation (3, 41). This review will focus on LAD type-I disease, designated LAD, and its corresponding animal models.

Clinically, LAD is characterized by recurrent, life-threatening, bacterial infections (4). The increased susceptibility to bacterial infections associated with LAD results from the inability of leukocytes (especially neutrophils) to adhere to the blood vessel wall and migrate to sites of infection (6). These adhesion defects stem from mutations in the common CD18 subunit of the leukocyte integrin family of molecules that is required for surface expression of the CD11/CD18 leukocyte integrin heterodimers (65).

During the past 20 years, naturally occurring forms of LAD in two other animal species, Irish Setter dogs and Holstein cattle, have been described (36, 59). In addition, a CD18 gene-targeted mouse model has been generated (62, 73). All three animal species with LAD display a disease phenotype similar to the human form of the disease, thus confirming the important contribution of the CD11/CD18 molecules to leukocyte adherence reactions in inflammation and host defense. However, each species offers unique insights into the pathology of LAD. Since animal models of human disease often fail to match the phenotype of the disease in humans, it is essential to characterize the specific manifestations of LAD in each species. This is especially pertinent if these animal models are proposed for use in testing new therapeutic approaches to LAD.

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Leukocyte Integrins

Defects in the leukocyte integrin CD18, which result in the inability to express functional CD11/CD18 leukocyte integrin heterodimers on the cell surface, form the basis for type-I LAD disease in humans and animals. The leukocyte integrins are membrane glycoproteins composed of non-covalently associated CD11 and CD18 subunits that mediate cell-cell and cell-matrix interactions (45). The CD18 molecule heterodimerizes with four distinct CD11 subunits to form CD11a/CD18 (leukocyte function-associated antigen 1 [LFA-1]), CD11b/CD18 (macrophage antigen 1 [Mac-1]), CD11c/CD18 (p150,95) and CD11d/CD18 (αDβ2) (Fig. 1) (26).

Prior to processing, the CD11 and CD18 subunits precursors form a heterodimer intracellularly. In LAD patients, lack of CD18 or presence of a mutant CD18 subunit results in failure of dimerization and transport of the CD11 subunit to the cell surface, despite synthesis of a normal CD11 subunit precursor. Thus, defects in the CD18 molecules result in failure to express all four CD11 subunits on the leukocyte surface (6).

The CD11 subunits of the leukocyte integrin family are selectively expressed on distinct populations of leukocytes where they mediate a variety of adhesion-related functions. The CD11a subunit is expressed at high levels on B and T lymphocytes, and at lower levels on monocytes, macrophages, and neutrophils (61). This pattern of CD11a/CD18 expression is consistent among the

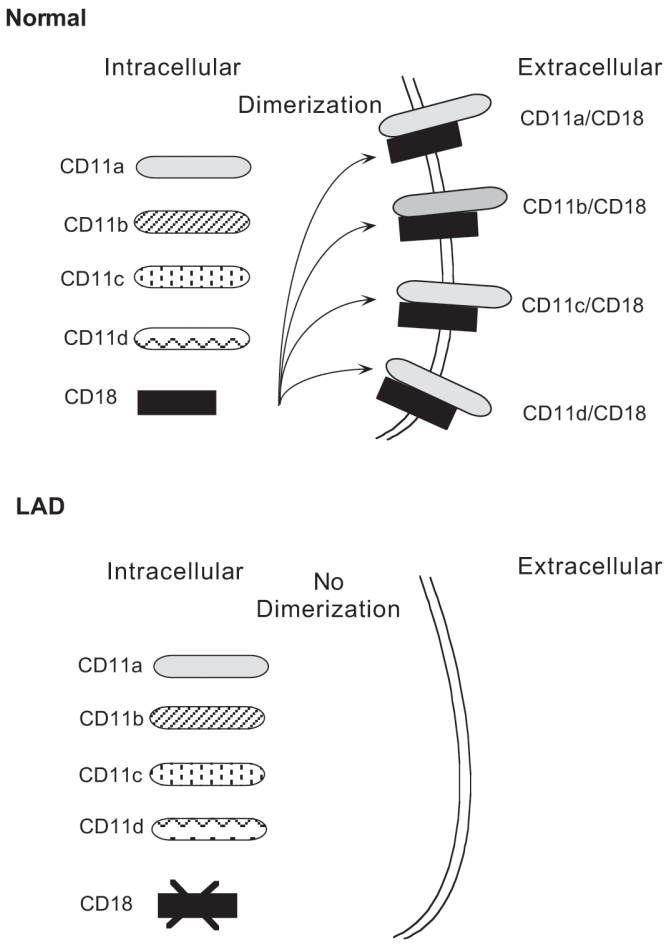

Figure 1. Schematic diagram of normal CD11/CD18 heterodimerization and expression on cell surface. The CD11 and CD18 genes are transcribed, translated, and non-covalently associated before processing and membrane insertion. In leukocytes from leukocyte adhesion deficiency (LAD)-affected patients, defects in the CD18 gene result in the inability to express all four CD11/CD18 heterodimers on the cell surface.

various species. The CD11a/CD18 complexes enable T lymphocytes to adhere to target cells and mediate cytotoxic T-lymphocyte activity (61). Neutrophils, monocytes, and certain lymphocyte subpopulations express CD11b at high levels (18, 51). The CD11b/CD18 complex mediates diverse adhesion-related functions, including chemotaxis and phagocytosis. The expression of CD11c/CD18 is restricted to leukocytes derived from myeloid lineages, as well as subsets of T and B lymphocytes and natural killer (NK) cells. The CD11c/CD18 integrin mediates the binding of iC3b-opsonized particles, cytotoxic T lymphocyte-mediated killing, and chemotaxis of myeloid cells (15). The expression of CD11d/CD18 is limited to macrophages and some lymphocyte populations (19).

Leukocyte Adhesion Deficiency in Humans

Clinical and histopathologic features of LAD. The severity of neutrophil function abnormalities and clinical infectious complications among patients with LAD are directly related to the degree of glycoprotein deficiency. Leukocyte adhesion deficiency has been categorized as moderate or severe according to quantitative differences in expression of the leukocyte integrins on the leukocyte surface (4). Patients with moderate deficiency express 1 to 10% of normal CD11/CD18 levels, whereas patients with severe deficiency typically express < 1% of normal CD11/CD18 levels (31). Severely affected patients have a profound defect in neutrophil immigration into tissues and suffer from extensive bacterial infections. Historically, approximately 75% of severely affected patients die before the age of 2 years (24). The prognosis appears better for patients with moderate phenotype; however, only 25% of patients with the moderate phenotype of LAD are predicted to survive to age 40 (4, 23).

In children with the severe deficiency phenotype of LAD, the clinical manifestations of LAD typically begin in infancy or early childhood. Delayed umbilical cord detachment and accompanying omphalitis is a frequent finding, and often represents the initial presentation of the disease. During the early years of life of children with the severe type of LAD, these infections take the form of otitis media, perianal abscesses, and non-healing, cutaneous wounds. Recurrent, necrotic, indolent infections of soft tissues involving the skin, mucous membranes, and gastrointestinal and respiratory tracts also are common. The skin lesions are frequently insidious in that they start with a small, erythematous, nonpustular lesion, which then progresses to a large wound with an ulcerative crater (Fig. 2, panel A). These wounds heal slowly despite antibiotic therapy (4, 6). Typically, the histologic features of skin wounds from LAD patients are characterized by ulceration with extensive necrosis, and are accompanied by the presence of neutrophils in the blood vessels but not in the surrounding tissue. Severe chronic gingivitis and rapidly progressive periodontitis are chronic problems in patients who survive infancy (Fig. 2, panel B). Subsequently, these patients develop alveolar bone loss and partial or total loss of deciduous and permanent dentitions (4, 70).

Infections are less frequent and usually less severe in patients with the moderate deficiency phenotype of LAD, although in terms of location and evolution, the infections themselves are similar to those in children with the severe phenotype. Recurrent skin/subcutaneous infections, stomatitis, gingivitis, otitis, sinusitis, and pneumonia have been associated with this moderate phenotype of LAD (4).

Although the leukocyte integrins on lymphocytes, monocytes, and neutrophils are deficient in patients with LAD, the predominant clinical manifestations are those of a phagocytic rather than a lymphocytic disorder. This is likely because neutrophils are principally dependent on the CD18 integrins for firm adhesion and transendothelial migration, whereas lymphocytes have overlapping or redundant adhesion cascades (57). This inability of neutrophils to adhere to the vessel wall and migrate to the site of infection results in the absence of a suppurative inflammatory reaction at sites of infection (Fig. 3A).

Results of in vitro and in vivo neutrophil functional studies are strikingly abnormal in LAD patients. Adhesion and adhesion-related functions such as chemotaxis reflect the most profound impairments. The magnitude of the defective in vitro responses is related to the level of expression of the CD11/CD18 complexes. In vivo skin window tests of leukocyte mobilization have confirmed the presence of a striking defect in leukocyte migration.

A variety of in vitro abnormalities in lymphocyte function also have been documented in LAD patients, although the clini-

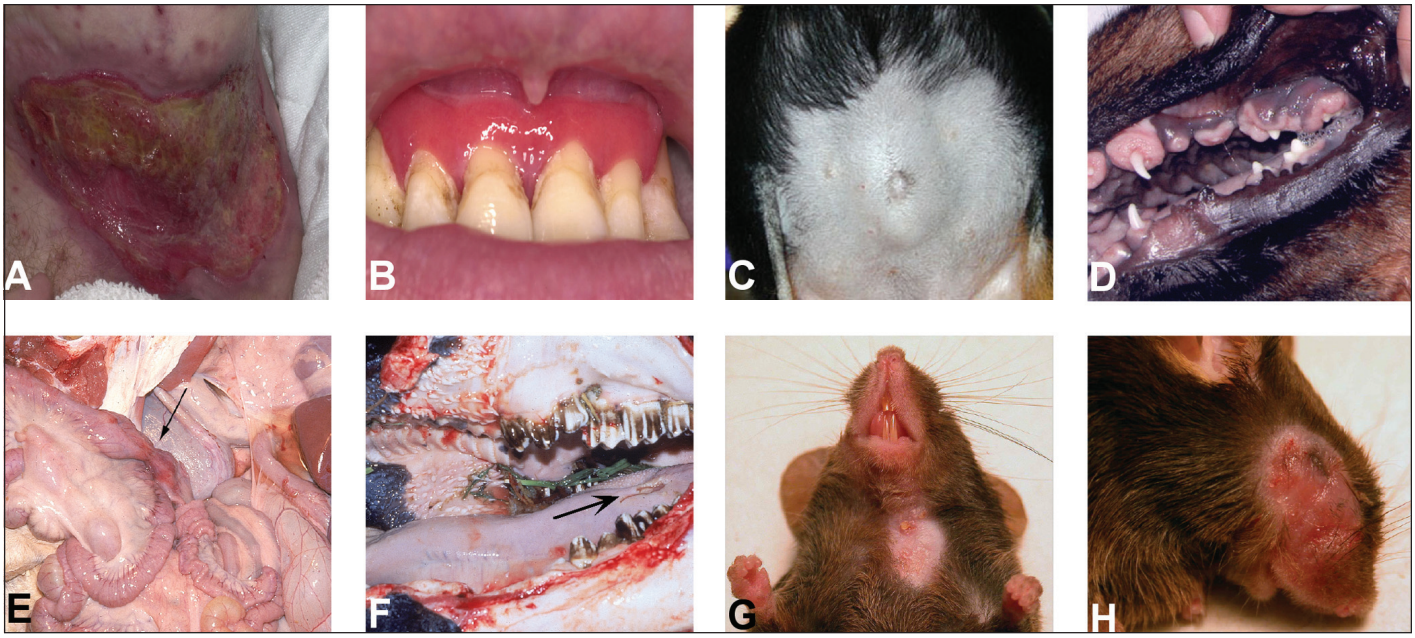

Figure 2. Pathology of LAD in affected species. (A) Severe skin wound and (B) gingivitis in LAD patients. (C) Omphalitis and (D) gingivitis in canine LAD-affected dogs. (E) Intestinal subserosal fibrosis (arrow) and (F) an ulcer on the tongue (arrow) in bovine LAD-affected cattle. (G) A *Cd18* $^{-/-}$ mouse showing crusting dermatitis abscess in the neck and (H) peri-orbital ulcerative dermatitis.

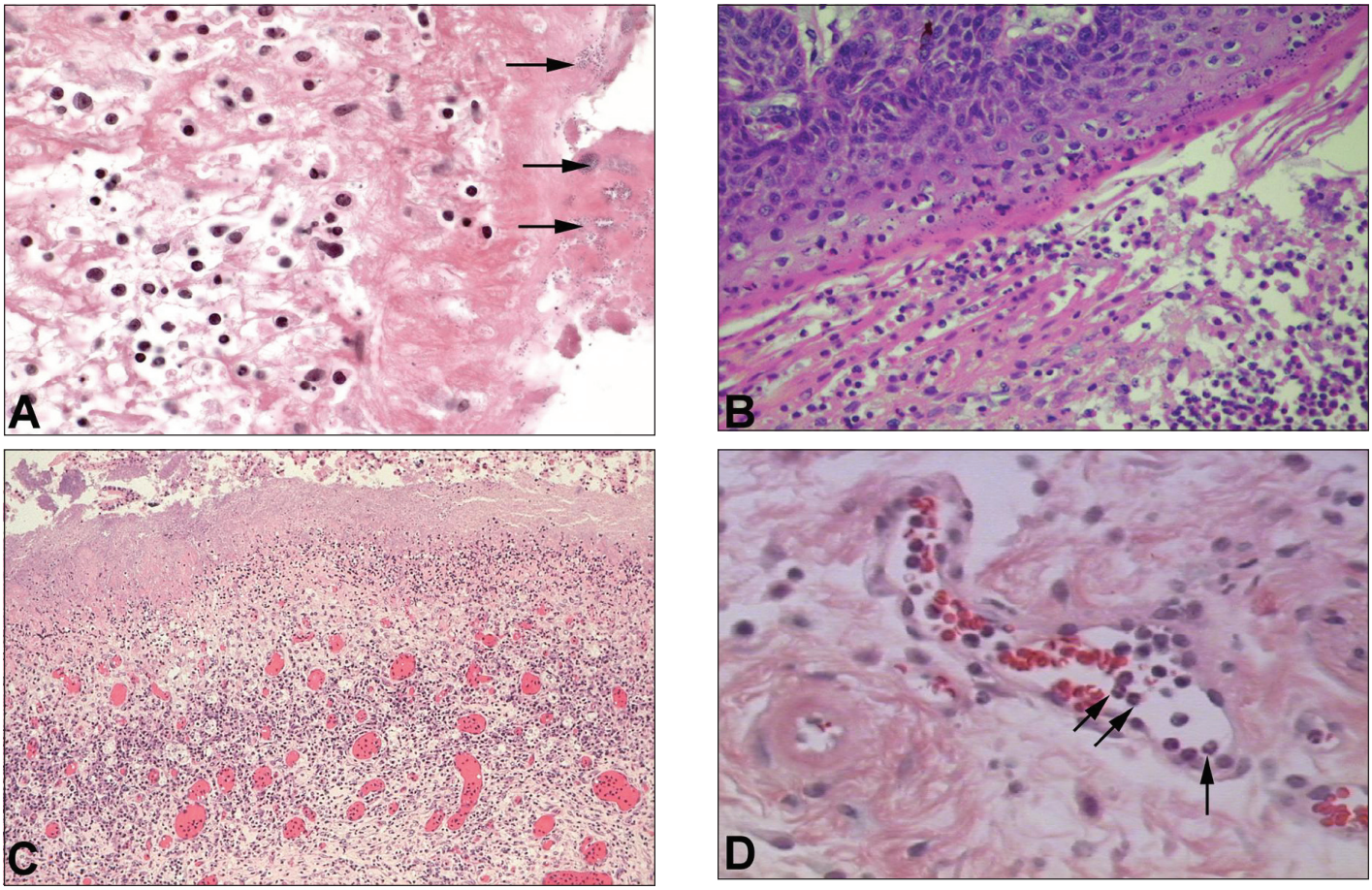

Figure 3. Histologic findings of LAD in affected species (H&E staining). (A) Photomicrograph of a section of skin from a LAD-affected patient showing bacterial colonies (arrows) and lack of neutrophils in the ulcerated area (400×). (B) Photomicrograph of a section from the anal gland of a dog with canine LAD; notice neutrophils in hyperplastic mucosal epithelium (400×). (C) Photomicrograph of a section of ulcerated intestinal mucosa with numerous congested vessels containing neutrophils from a cow with bovine LAD (100×). (D) Photomicrograph of a section of skin from a *Cd18*$^{-/-}$ mouse. Notice venule at the site of inflammation showing neutrophils (arrows) in the vessel but none in the tissue (~400×).

cal relevance of these abnormalities is less clear. The T lymphocytes from LAD patients respond poorly to low concentrations of lectins and anti-CD3 monoclonal antibodies, and antigen-induced T-cell proliferation to low concentrations of antigens also is diminished (12, 44).

Molecular defects in LAD patients. Leukocyte adhesion deficiency was first recognized in the early 1980s when a number of children who suffered from acute infections and whose neutrophils lacked a protein variably reported as 95 to 138 kD in size were reported (11, reviewed in 5). Subsequent studies using monoclonal antibodies identified the missing protein as the leukocyte integrin CD18 molecule. Springer (65) had previously proposed that the primary defect in the patients resided in the CD18 subunit, which is shared by all CD11 subunits, and that CD18 was required for CD11/CD18 heterodimer formation and surface expression. After the cloning of the CD18 cDNA (43, 46), mutations in the CD18 gene in a number of patients with LAD were reported (7, 42, 64). Since that time, more than 40 mutations have been identified in LAD patients, including missense, nonsense, splice site, insertion, and deletion mutations (5, 42). In addition to the nonsense mutations that preclude translation of a functional CD18 protein, most mutations arise in locations important for CD18 heterodimerization with CD11, or in regions important for CD18 function. Since the CD11 proteins contain several domains important for function, the lack of a functional CD11/CD18 heterodimer on the surface of the leukocyte precludes CD11, as well as CD18 function. If consanguinity is excluded, patients with LAD are typically compound heterozygotes with a different CD18 mutation in each allele (5, 32).

Diagnosis of LAD. Although a presumptive diagnosis of LAD can be suspected on the basis of delayed umbilical cord separation, non-healing skin wounds, and an extremely high white blood cell (WBC) count consisting mainly of mature neutrophils, the diagnosis of LAD is typically confirmed by use of flow cytometric analysis of CD18 expression on the patient's leukocytes (Fig. 4, panel A). As described earlier, patients with LAD are categorized as having moderate (1 to 10%) or severe (< 1%) disease, depending on the level of CD18 expression on the leukocyte surface. Although use of flow cytometric analysis detects most CD18 mutations, variant cases have been described in which the DNA mutation allows expression of the CD18 protein; however, the CD11/CD18 dimer is not fully functional (49). In these particular cases, demonstration of leukocyte dysfunction is carried out using in vitro tests such as chemotaxis or adherence assays. Additionally, DNA sequencing of the patient's CD18 gene typically confirms the presence of mutations in the CD18 subunit.

Treatment of LAD. Treatment of LAD depends on the severity of symptoms. Long-term antibiotic prophylaxis appears to decrease the frequency of infections; however, this therapy does not prevent episodes of severe infection. During episodes of severe infection, infusions of donor granulocytes have been used with some success. However, antibiotic treatments and granulocyte infusions represent only supportive care. Curative therapy for LAD requires hematopoietic stem cell transplantation.

Allogeneic hematopoietic stem cell transplantation is recommended for all patients with severe deficiency LAD who have matched sibling donors. Transplantation has also been proposed for patients with the moderate phenotype of LAD, since these patients also have decreased life-span (67). However,

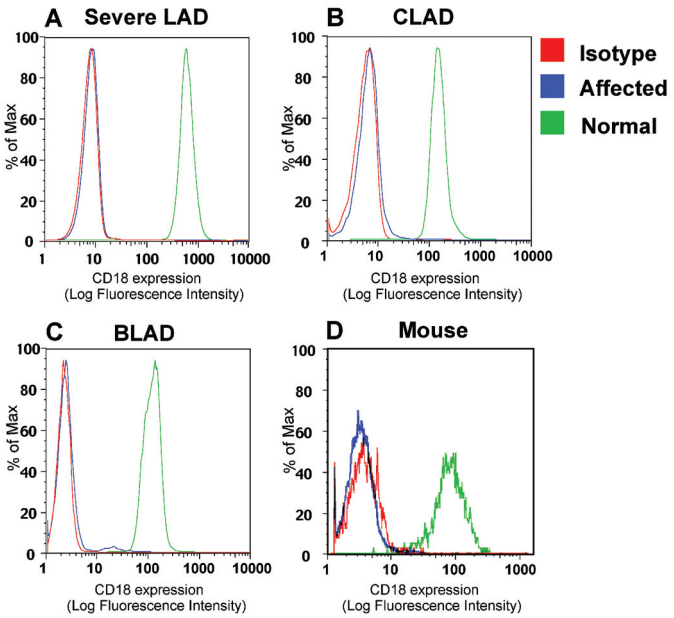

Figure 4. Comparison of LAD disease by use of flow cytometric analysis in the severe phenotype of a humans (severe LAD), Irish Setter dogs (CLAD), Holstein cattle (BLAD), and *Cd18*$^{-/-}$ mice. Neutrophils were isolated from the peripheral blood of all four species and were stained with fluorescently labeled control or anti-CD18 monoclonal antibodies and were analyzed by use of flow cytometry.

since only 25% of LAD patients will have a matched sibling donor, human leukocyte antigen (HLA) non-identical donors such as parents or other siblings have been explored as a source of donor cells for LAD patients (47, 67). Transplants in this setting are accompanied by a considerably higher incidence of graft rejection and graft-versus-host disease compared with those of matched sibling donor transplants (67). In addition, alternative donor stem cell sources for transplantation, including cord blood (66), matched related, matched unrelated, and one-haplotype mismatched related donor hematopoietic stem cells, have been used (22).

In 1999, two patients with the severe deficiency form of LAD were treated in a human gene therapy clinical trial (10). Mobilized, peripheral blood stem cells (PBSC) were collected from the LAD patients. The $CD34^+$ hematopoietic stem cell fraction was selected and incubated with a retroviral vector harboring the CD18 cDNA. The $CD34^+$ gene-corrected hematopoietic stem cells were re-infused into the patients in the form of an autologous transplant. In both patients, correction of up to 0.04% of the patients' myeloid cells was apparent two weeks after the infusion of the gene-corrected cells. However, $CD18^+$ neutrophils were undetectable in the peripheral blood after 63 days post-infusion of transduced cells. To achieve sufficient numbers of $CD18^+$ neutrophils for this treatment to represent a therapeutic option, further advances in vector design and transduction conditions, and the possible use of myeloablation are likely to be required.

Leukocyte Adhesion Deficiency in Irish Setter Dogs

Clinical and histopathologic features. In 1975, an 8-week-old male Irish Setter dog with a clinical history of recurrent, life-threatening bacterial infections was described (58, 60). Assessment of leukocyte function in the affected dog revealed

significant decrease in neutrophil adherence to plastic, glass, and nylon wool (59). Random migration, chemotaxis, and aggregation of neutrophils in response to phorbol myristate acetate also were impaired (60). These in vitro results led to the designation of the disease as "canine granulocytopathy syndrome" (59).

In 1987, Giger and colleagues (27) described an Irish Setter-type (mixed-breed) dog with a disease phenotype similar to that of the dog with canine granulocytopathy syndrome. Flow cytometric analysis revealed severe deficiency of the CD11/CD18 leukocyte surface expression, similar to the situation described in humans, and the disease was given the designation of canine leukocyte adhesion deficiency or CLAD (27).

Canine LAD has subsequently been described in Irish Red and White Setters (20, 25). In all setter breeds, clinical evidence of disease in CLAD-affected dogs is manifested shortly after birth, with development of omphalitis or umbilical abscess, followed by frequent, severe bacterial infections (Fig. 2, panel C). These episodes are typically accompanied by fever. The subsequent clinical course in dogs with CLAD is quite uniform, with marked neutrophilic leukocytosis, severe gingivitis, lymphadenopathy, poor wound healing, and episodes of infection manifesting as pyrexia and anorexia (Fig. 2, panel D). In dogs with CLAD, development of hypertrophic osteodystrophy with physeal swelling also has been described (16, 68). Skin lesions are a particularly common finding in CLAD-affected dogs. Microscopically, there is overlying epidermal ulceration with abundant necrosis and evidence of bacterial infection. Multiple large abscesses are frequently present in the dermis (68). In contrast to the histologic findings in LAD patients, neutrophil infiltration into the infected site is present in dogs with CLAD (Fig. 3B). In general, the disease manifestations in CLAD-affected dogs are consistent with the reported symptoms in children with the severe deficiency phenotype of LAD (68, 69).

Hematologic studies in dogs with CLAD reveal persistent leukocytosis beginning as early as one week of age, with neutrophils constituting up to 90% of the leukocytes in circulation (68). The bone marrow is hypercellular, with increased numbers of granulocytic myeloid precursors.

Molecular defect in CLAD dogs. The clinical manifestations of CLAD are caused by severe deficiency in expression of the integrin CD18 molecule on the leukocyte surface, similar to the flow cytometric findings in humans with the severe deficiency form of LAD (Fig. 4, panel B). The CD18 cDNA sequences from CLAD-affected dogs indicate a single nucleotide G-to-C transversion at position 107, which leads to a replacement of cysteine by serine at residue 36 (C36S) in the N-terminal extracellular portion of the CD18 protein (39). Since this cysteine residue is highly conserved among the human, dog, pig, and mouse CD18 subunits, the substitution of serine for cysteine likely results in the disruption of a disulfide bond and aberrant tertiary structure culminating in severe defects in CD11/CD18 heterodimer formation. All affected dogs have been documented to be homozygous for the C36S mutation (20, 39). Canine LAD is transmitted in the same autosomal recessive manner as is human LAD.

Diagnosis of CLAD. Canine LAD has only been reported in the highly related Irish Setter (68) and Irish Red and White Setter breeds (20, 25). Dogs of these breeds presenting with recurrent infections should be tested for CLAD. Leukocytes from dogs suspected of having CLAD can be assessed by use of flow cytometric analysis for CD18 expression (Fig. 4, panel B). Initial screening of CLAD carriers can also be done by use of flow cytometry; however, confirmation of carrier status requires DNA testing using an oligonucleotide ligation assay (39), pyrosequencing (40), or DNA sequencing (16). Commercial laboratory testing for CLAD carrier status is now available (Optigen LLC, New York, N.Y.).

Treatment of CLAD. Treatment options for dogs with a diagnosis of CLAD are similar to those for humans with LAD. Antibiotic prophylaxis at the onset of clinical signs of CLAD appears to decrease the frequency of infections; however, most CLAD-affected dogs typically die from infection complications by six months of age (68). Given the rapid presentation and severity of CLAD and the limited treatment options available in the community, most dogs presenting with the disease are ultimately euthanized.

Recently, matched related donor bone marrow transplantation in CLAD-affected dogs has been described (17). In that study, recipient CLAD-affected pups received a minimal myeloablative conditioning regimen prior to bone marrow transplantation, and a short (two-month) posttransplantation immunosuppressive regimen. A total of 12 CLAD-affected dogs have been followed for one year, and 11 display stable donor:host chimerism, in which 5 to 90% of the leukocytes are $CD18^+$, and thus, are donor derived (9). These dogs have remained free of all signs of CLAD since the time of engraftment. Thus, the mixed chimerism state is sufficient to protect the dogs from clinical disease, and results in reversal of the disease phenotype (17).

Leukocyte Adhesion Deficiency in Holstein Cattle

Clinical and histopathologic features. In 1983, a 1-year-old Holstein heifer with a syndrome consisting of fever, diarrhea, and marked neutrophilia was described. The disease was referred to as "bovine granulocytopathy syndrome"(29). Calves with the disease displayed clinical signs that were almost identical to those of dogs with canine granulocytopathy syndrome and of children with the severe deficiency phenotype of LAD. Neutrophil functional activities associated with migration and complement receptor type-3 function were notably impaired (29). Immunoblot analysis of neutrophil lysates from an affected calf revealed total deficiency of CD11b (36). Subsequent studies confirmed that bovine granulocytopathy syndrome was the bovine analog of human LAD, and the disease was re-named bovine leukocyte adhesion deficiency (BLAD [56]).

Major clinical features in BLAD-affected cattle are recurrent pneumonia, ulcerative stomatitis, enteritis with bacterial overgrowth, periodontitis, loss of teeth, delayed wound healing, and failure of suppuration (Fig. 2, panels E and F) (37, 54). Mucosal surfaces of the oral cavity and respiratory and gastrointestinal tracts are most commonly affected, presumably due to the intense bacterial colonization on these surfaces. Microscopically, neutrophils are present in numerous congested vessels and the splenic red pulp, but not in surrounding tissues of ulcerated intestinal mucosa and Peyer's patch areas (Fig. 3C). Such affected calves are typically unremarkable at birth; however, with exposure to microbes in their environment, they develop persistent, progressive neutrophilia, and eventually become unthrifty. Untreated BLAD-affected calves frequently die in the first year of life, as is the case in dogs with the severe phenotype of CLAD.

Analysis of blood samples from BLAD-affected calves revealed chronic progressive neutrophilia (> 80,000 cells/μl); normal cattle

have a total WBC count of approximately 4,000 cells/µl (48). Reports of neutrophil counts in excess of 200,000 cells/µl of blood are not uncommon in cattle with BLAD (48). Bone marrow examination of BLAD-affected cattle revealed myeloid hyperplasia with increased myeloid-to-erythroid ratio (28).

Similar to that for human LAD and CLAD, the physiologic basis for BLAD is a deficiency in the functional chemotactic and phagocytic neutrophils. The impaired adhesion and failure of migration of neutrophils into inflammatory sites prevents normal immune reactions to invading pathogens. One exception to this impairment is associated with bovine pneumonia where neutrophils enter the airways and alveolar spaces in response to inflammatory stimuli (2, 28, 36). In BLAD-affected cattle with acute bacterial infection, CD18-dependent infiltration occurs in the bronchi, whereas CD18-independent infiltration occurs in the alveoli (1, 2).

Molecular defects in BLAD cattle. Cloning and sequencing of cDNA from normal and BLAD-affected cattle identified a point mutation at position 383 in the cDNA of the CD18 gene, resulting in the substitution of glycine for aspartic acid at amino acid 128 (D128G) in the CD18 molecule (63). This mutation lies within a highly conserved region in the extracellular domain where at least 125 consecutive amino acids are similar in normal human, canine, bovine, porcine, rat, and murine CD18 (63). To date, all cattle in which BLAD was diagnosed that have been genetically tested have been homozygous for the D128G allele. Interestingly, a mutation at amino acid 128 was also described in a child with the severe deficiency phenotype of LAD. This mutation resulted from a D128N mutation (50).

Diagnosis of BLAD. The diagnosis of BLAD in cattle is usually suspected from the characteristic clinical features, and is confirmed by use of flow cytometric analysis that indicates severe deficiency in CD18 expression on the leukocytes (Fig. 4, panel C). For carrier typing, the D128G allele can be diagnosed by use of a polymerase chain reaction (PCR)-based assay that is performed by several commercial vendors (Immgen, Inc., College Station, Tex.; GenMARK, De Forest, Wis.; VITA-TECH Laboratories, Buffalo, N.Y.). In the original diagnostic studies of BLAD, all cattle with the mutant allele were related to one bull which, through use of artificial insemination, sired many calves in the 1950s and 1960s (35). In 1990, the carrier frequency of BLAD was estimated to be 15% among Holstein bulls and 6% among Holstein cows in the United States. The carrier frequency in Japan was estimated to be 12.6% and about 0.31%, respectively. It is likely the carrier frequency has decreased substantially over the past decade due to extensive carrier testing and selective breeding of non-BLAD carriers.

Treatment of BLAD. Treatment of BLAD consists of antibiotic prophylaxis; however, death usually ensues from severe infection of the alimentary tract. In one report (55), bone marrow transplantation was attempted. In that study, 2.2×10^9 bone marrow cells were aspirated from a clinically normal 4-year-old Holstein cow and were infused into a nine-month-old Holstein heifer with BLAD (55). Twelve months after bone marrow transplantation, a small fluorescent region in the $CD18^+$ area (estimated to represent 0.3 to 0.5% of the neutrophils) was detected using flow cytometry. The recipient heifer developed a transient skin rash and diarrhea, then improved clinically over the ensuing 28 months. The investigators concluded that the newly expressed donor CD18 cells caused mild graft-versus-host disease; however, the $CD18^+$ donor leukocytes led to improvement in the clinical signs of BLAD in this heifer.

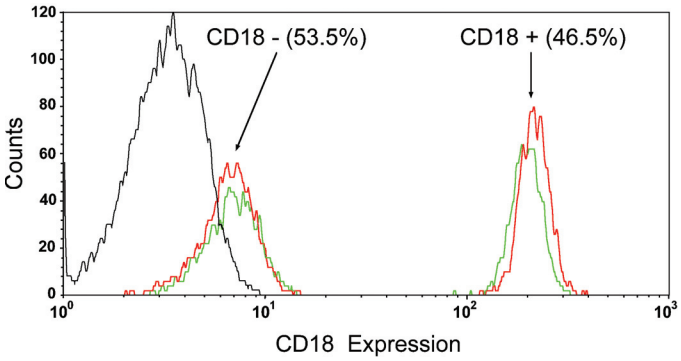

Figure 5. Flow cytometric analysis of CD18-positive and CD18-negative neutrophil proportions in circulation from non-identical leukochimeric bovine twins. At one day after birth, each twin had roughly equal leukocyte proportions of their own true genotype and that of their twin's genotype. One calf's true genotype (determined by use of polymerase chain reaction (PCR) analysis of a skin biopsy specimen) was that of a homozygous BLAD-affected animal ($CD18^{-/-}$); the other twin was a heterozygote ($CD18^{+/-}$) for the D128G CD18 allele. These twins maintained normal health status throughout their lives.

A "natural" cord blood transplant in dizygotic twins with BLAD as a result of anastomosis of the chorioallantioic membranes between the twins was described. Kehrli and colleagues (34) found that each twin was born with two distinct populations of $CD18^-$ and $CD18^+$ leukocytes in circulation in approximately equal proportions, presumably as a result of one twin having BLAD and the other twin being a heterozygote for CD18 expression, with transplacental blood exchange through a placental anastomosis (Fig. 5). On the basis of absence of recurrent infections, as few as 10% of $CD18^+$ cells appeared to correct the BLAD disease in the affected twin.

Leukocyte Adhesion Deficiency in (CD18 gene-targeted) Mice

Clinical and histopathologic features. Gene targeting using homologous recombination in embryonic stem cells has been done to generate mice with a homozygous knockout of the murine CD18 gene. In the first attempt, an insertion mutation in the murine CD18 gene was generated and resulted in a hypomorphic rather than a null allele mutation (73). These hypomorphic mice, designated *Itgb2*^{tm1Bay}, were created on a mixed background of C57BL/6J and 129/Sv mice. The homozygous mutants were viable and expressed 2 to 16% of normal CD18 levels on the leukocyte surface (73). Hypomorphic mice had mild neutrophilia, minimal to mild hyperplasia in spleen and bone marrow, and impaired inflammatory response. The percentage of CD18 expression on the hypomorphic mouse leukocytes was similar to that associated with the moderate phenotype of humans with LAD (4, 23). When backcrossed on a PL/J strain, homozygous mice developed a novel chronic inflammatory skin disease, with hyperplasia of the epidermis. This dermatitis, similar to human psoriasis, was observed only in the PL/J strain, and not in any C57BL/6 or 129/Sv backcrosses (14). This dermatitis has not been observed in humans, cattle, or dogs with LAD. Subsequent analysis has revealed the inflammatory effect to be dependent on low-level CD18 expression, not dependent on microbial flora, and requiring two PL/J-specific loci (8). Recent work in these hypomorphic mice has focused on the role of $CD4^+$ and CD18 T lym-

Table 1. Neutrophil demands of various species

Species	Neutrophils per milliliter	Body weight (kg)	Blood volume (ml)	Neutrophil life span (days)	Neutrophil production per day	Life expectancy (years)	Neutrophil production in life time
Human	5×10^6	70	4,900	0.33	7.4×10^{10}	80	2.2×10^{15}
Cattle	2.3×10^6	636	47,700	0.33	3.3×10^{11}	17	2.1×10^{15}
Dog	7.4×10^6	18	1,550	0.33	3.5×10^{10}	13	3.8×10^{13}
Mouse	1.4×10^6	0.025	1.8	0.33	7.6×10^6	2	5.5×10^9

phocytes in the inflammatory process of the dermatitis (8, 38).

Mice with complete deficiency in CD18 (CD18 null mice, *Cd18*$^{-/-}$ or *Itgb2*$^{-/-}$) were subsequently generated (62). These mice, designated *Itgb2*^{tm2Bay} and produced on a mixed C57BL/6J and 129/Sv background, were less viable than hypomorphic mice, with 10 to 40% of the CD18 null newborns dying during the perinatal period (62). The phenotype of the CD18 null mice more closely resembles that of the severe deficiency type of LAD in humans. The phenotype of these mice included chronic dermatitis due to extensive bacterial infiltration, neutrophilia, increased immunoglobulin concentrations, lymphadenopathy, and splenomegaly (Fig. 2, G and H) (62). Few neutrophils were observed in the skin of CD18 null mice, and the mice had a severe defect in T-cell proliferation. The increased susceptibility to infection in CD18 mutant mice again emphasizes the importance of neutrophils as an antigen-independent first line of host defense.

The peripheral blood of *Cd18*$^{-/-}$ mice contained 6- to 11-fold more neutrophils than those in normal littermates (53, 62). During acute inflammation, the CD18 null mouse is able to recruit neutrophils into the lung and peritoneal cavities via a CD18-independent manner (53); however, recruitment of neutrophils into the peritoneal cavity appears to be markedly reduced compared with that into the lung (71). In comparison, CD18-independent neutrophil emigration into the lung, but not the peritoneum, was observed during autopsy of a human patient with the severe form of LAD who died from infection (30). Similar to that in all species with LAD, myeloid hyperplasia is present in the bone marrow of the *Cd18*$^{-/-}$ mice (62).

Molecular defect in CD18 gene-targeted mice. A hypomorphic and a null mutation in the CD18 gene have been generated in mice by use of gene targeting, resulting in either partial or complete inactivation of the endogenous alleles of the mouse, respectively (62, 73). In the hypomorphic gene-targeted mice, a cryptic promoter was present in the targeting construct that resulted in low-level CD18 transcription, leading to a proportional deficiency rather than an absence of CD18 (73). A subsequent gene replacement strategy was used to generate the null mutation of CD18 gene. This construct disrupts the 5' boundary of exon 3, thereby preventing the synthesis of the CD18 protein (62).

Diagnosis of murine LAD. Unlike the situation with humans, dogs, or cattle, mice with naturally occurring mutations in CD18 have not been described to our knowledge. The aforementioned CD18 gene-targeted mice are available from Jackson Laboratories (Bar Harbor, Maine), or from the original production laboratories, as either the CD18 gene-targeted hypomorphic form (TBASE: TG-000-01-274) or the CD18 null form (TBASE: TG-000-030344). The particular mutation can be identified through DNA analysis by use of Southern blot or PCR analysis. Both models, as previously stated, have distinct pathologies, some of which are dependent on the mouse strain background, the PL/J strain in particular for the hypomorphic mouse. The CD18 null form more closely resembles classical LAD.

Treatment of the murine LAD model. Although CD18 mutant mice have been invaluable in the exploration of the pathology of LAD disease, to our knowledge, treatment modalities such as transplantation and gene therapy for reversal of the disease phenotype of CD18 mutant mice have not been explored, in contrast to studies in man, cattle, and dogs. Rather, experiments in CD18 mutant mice have typically involved agents designed to examine the role of CD18 in the pathology of LAD disease or for CD18 function. For example, CD18 null mice have been infected with *Streptococcus pneumoniae* or *Escherichia coli* to study neutrophil emigration (52, 53). The role of CD18 in the immune response was assessed in CD18 null mice after infection with *Listeria monocytogenes* and the tick *Anaplasma phagocytophila* (13, 74). The important role of the CD18 molecule in neutrophil extravasation, attachment, and production, especially in the exaggerated case of leukocytosis in LAD patients, was also explored in the CD18 null mouse by experimental transplantation of bone marrow cells from these mice into wild-type mice (33, 72). Extrapolation from these studies has provided insights into the pathophysiology and mechanism of LAD disease.

Discussion

The animal models of LAD presented here highlight the important contribution of each species to the study of LAD and leukocyte biology in general. Although it is an artificial model, the development of the CD18 mutant mouse allows faster generation times and larger numbers to study the development and progress of LAD disease. The CD18 null mouse model in particular may be advantageous for the study aspects of the clinical disease that are more costly, or more cumbersome in the dog or cow model, due to their size and difficulty in handling. For the study of organ-specific neutrophil infiltration, *Cd18*$^{-/-}$ mice and BLAD-affected cattle have been used in studies of acute pneumonia. Both models support CD18-dependent and -independent mechanisms of infiltration in regions of lung, which are similar to findings in human LAD patients with pneumonia.

Despite the similarities of these models to human LAD, there are some limitations when extrapolating these results to humans. For example, mice differ from humans and large animals in several aspects, including their small size, short life-span, and the limited proliferative demand on hematopoietic stem cell and progenitor compartments. During its lifetime, a mouse makes as many neutrophils as a human makes in 2 h, a cow in 24 min, or a dog in 4 h (Table 1). The neutrophil demands of humans are more similar compared with those of dogs or cattle, than are those of mice.

The CLAD model is particularly valuable for studying new forms of hematopoietic stem cell transplantation and gene therapy. Dogs have a long history of use in hematopoietic stem cell transplantation, and the dog model has performed a critical role in extension to human transplantation. In transplantation studies, it is easy to cross histocompatibility barriers in mice

and, and in general, there are fewer problems with graft rejection or fatal graft-versus-host disease. This is partially due to less genetic variability in inbred strains of mice. Purpose-bred dogs are more outbred than are mice, and therefore, offer a more relevant model for the studies of the corresponding diseases in humans in the field of transplantation biology.

In conclusion, we present three animal models used in the study of LAD disease. Each of the animal models of LAD provides unique insights into the study of the pathology and molecular basis of LAD. The choice of a particular animal model depends on the nature of the investigation.

Acknowledgments

Animal study protocols were approved by the Institutional Animal Care and Use Committees of the respective institutions. These studies were performed in accordance with the principles outlined in the *Guide for Laboratory Animal Facilities and Care* of the National Academy of Sciences, US Department of Agriculture, and National Institutes of Health guidelines and regulations.

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