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REVIEW

Leukocyte Adhesion Deficiency in Children and Irish Setter Dogs

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ABSTRACT

Children with the genetic immunodeficiency disease leukocyte adhesion deficiency, or LAD, develop life-threatening bacterial infections as a result of the inability of their leukocytes to adhere to the vessel wall and migrate to the sites of infection. Recently, the canine counterpart to LAD, known as canine leukocyte adhesion deficiency, or CLAD, has been described in Irish setter dogs. This review describes how the clinical phenotype of dogs with CLAD closely parallels that of children with the severe deficiency phenotype of LAD, thus enabling the CLAD dog to provide a disease-specific, large-animal model for testing novel hematopoietic stem cell and gene therapy strategies before their translation to children with LAD. **(*Pediatr Res* 55: 363–367, 2004)**

Abbreviations
CLAD, canine leukocyte adhesion deficiency
LAD, leukocyte adhesion deficiency
GVHD, graft-*versus*-host-disease

LAD represents one of several genetic diseases of childhood that involve leukocyte functional defects. These disorders have in common the propensity to develop life-threatening bacterial infections. The syndrome that came to be known as LAD was first described nearly 25 y ago in patients with delayed separation of the umbilical cord, neutrophilia, neutrophil defects, and systemic bacterial infections (1–3). Leukocyte functional studies confirmed a neutrophil adhesion defect; flow cytometric and protein analysis indicated the absence of the leukocyte integrins on the cell surface; and molecular analysis demonstrated heterogeneous molecular defects in the leukocyte integrin CD18 subunit (4, 5).

The canine analog of LAD, known as CLAD, was originally described in the mid-1970s in Irish setter dogs by Renshaw and colleagues (6), and was termed canine granulocytopathy syndrome. Although studies in the late 1980s indicated that this syndrome was due to deficiency in surface expression of the CD11/CD18 complex (7), the precise molecular defect in the CD18 molecule responsible for CLAD was determined only recently (8).

CLINICAL PHENOTYPE OF LAD AND CLAD

Recurrent bacterial infections are the hallmark of LAD. A severe infection involving the umbilical stump usually represents the initial presentation. Episodes of severe bacterial infection then ensue throughout the perinatal, childhood, and young-adult years. These infections take the form of severe gingivitis and periodontitis, and recurrent, cutaneous, nonhealing wounds (Fig. 1). These infectious episodes in LAD are accompanied by a leukocytosis ranging from 15,000 to 100,000 cells/μL. Despite the marked leukocytosis, the inability of neutrophils to migrate to the site of infection in LAD results in the absence of pus at the inflammatory or infectious sites (4).

A similar clinical scenario is present in CLAD-affected pups. In CLAD pups, the initial clinical signs manifest shortly after birth with the development of omphalitis. This is followed by severe gingivitis, lymphadenopathy, poor wound healing, low body weight, and episodes of infection manifesting as pyrexia

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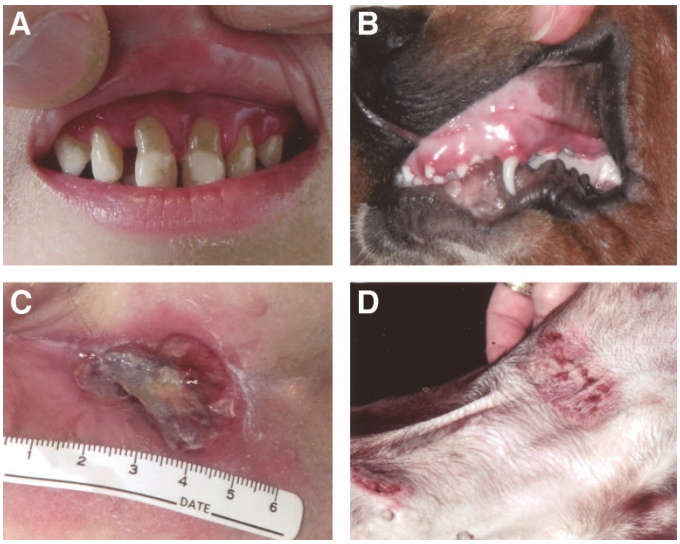

Figure 1. Pathology of LAD in humans and dogs. (*A*) Severe gingivitis in an LAD patient; (*B*) extensive gingivitis in a CLAD-affected dog; (*C*) perianal, nonhealing abscess in a LAD patient; (*D*) nonhealing skin lesions on a CLAD-affected dog.

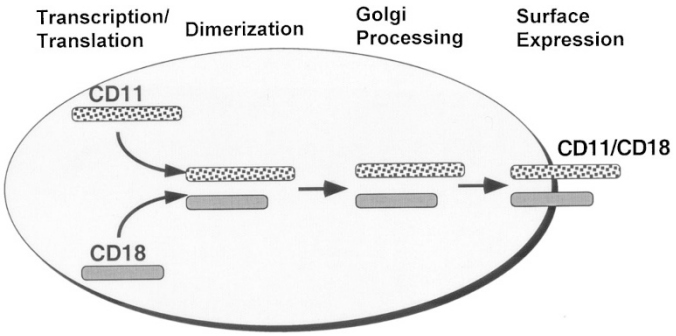

Figure 2. Heterodimer formation and surface expression of the CD11/CD18 adhesion complex. Under normal circumstances, the CD11 and CD18 subunits are synthesized as separate precursors, assembled as a dimer, processed to a higher molecular weight complex in the Golgi, and inserted into the cell membrane. Defects in CD18 in LAD may prevent its transcription or translation, prevent heterodimer formation, or interfere with processing of the CD11/CD18 complex. Point mutations in the CD18 coding region or at splice sites may lead to decreased expression of the CD18 protein. These mutations all result in severe impairments in the surface expression of the CD11/CD18 complex. With certain rare variant CD18 mutations, a CD11/CD18 heterodimer may become membrane inserted, but not be fully functional.

and anorexia. A severe leukocytosis of up to 500,000 neutrophils/μL is a characteristic feature of CLAD. Despite antibiotic therapy, CLAD-affected pups in the community usually die or are euthanized within the first few months of life (9). The functional abnormality in LAD and CLAD responsible for the predominant clinical picture is that of neutrophil adhesion defects. However, lymphocyte function is also affected. Studies in both LAD and CLAD show a decreased *in vitro* lymphocyte blastogenesis and proliferation (4, 7). The clinical relevance of these findings is unclear inasmuch as neither CLAD dogs nor LAD individuals are particularly prone to viral infections.

CHARACTERIZATION OF THE MOLECULAR DEFECT IN LAD AND CLAD

LAD is inherited in an autosomal recessive manner, and children with LAD are usually compound heterozygotes with a different mutation on each allele of the CD18 gene (also known as the β2 subunit of the leukocyte integrin family) (10). Because the CD11 subunits, or alpha subunits, of this adhesion receptor family require the CD18 subunit for heterodimer formation and surface expression, deficiency of CD18 results in failed, decreased, or aberrant surface expression of the CD11/CD18 complex in LAD (Fig. 2) (11). The majority of LAD cases involve single point or missense mutations in the CD18 subunit, leading to an altered CD18 precursor that is unable to dimerize with the CD11 subunits.

LAD can be categorized as severe or moderate according to quantitative differences in expression of the leukocyte integrins on the cell surface (4). Children with severe deficiency typically display less than 1% of normal levels of CD11/CD18, and children with moderate deficiency typically express 5–10% of normal levels of CD11/CD18. The severity of clinical complications among children with LAD appears to be directly related to the degree of CD18 deficiency. In one historical study, approximately 75% of children with the severe deficiency phenotype of LAD died within the first 2 y of life as a result of extensive bacterial infection (12). Although the prognosis was better for patients in whom the CD18 expression is at least 5–10% of normal (moderate phenotype), only 25% will typically survive to age 40.

In CLAD-affected dogs, the defect in CD18 has been shown to be due to a single point mutation that results in an amino acid substitution (C36S) in a highly conserved cysteine residue in the extracellular domain of CD18 (8). This structural defect in CD18 results in the failure to express the CD11/CD18 complex on the leukocyte surface. All CLAD-affected dogs identified to date, including Irish red and white setters, carry the identical CD18 mutation (13). Flow cytometric analysis of leukocytes from CLAD-affected puppies demonstrates the complete absence of CD18 expression, analogous to the picture seen in children with the severe deficiency phenotype of LAD (Fig. 3). This finding helps to explain the severity of the clinical phenotype in CLAD.

CONVENTIONAL THERAPY FOR LAD AND CLAD

Despite the use of prophylactic antibiotic therapy, infections invariably develop in LAD and tend to progress in the face of appropriate antibiotic therapy due to the absence of functional neutrophils. Treatment of infections in LAD depends upon the severity of the clinical condition. Wound infections are a particularly common and vexing problem in LAD patients, and frequently require repeated surgical debridement in addition to a prolonged course of parenteral antibiotic therapy. Infusions of allogeneic granulocytes have been used with limited success in patients with LAD who develop refractory infections.

Antibiotics also represent the first line of therapy in CLAD. However, in the study of Trowald-Wigh *et al.* (9), all dogs with CLAD succumbed to infection, or were euthanized due to progression of disease, by the age of 6 mo. Recently, we have described a similar clinical picture and outcome in a mixed-breed CLAD colony (14).

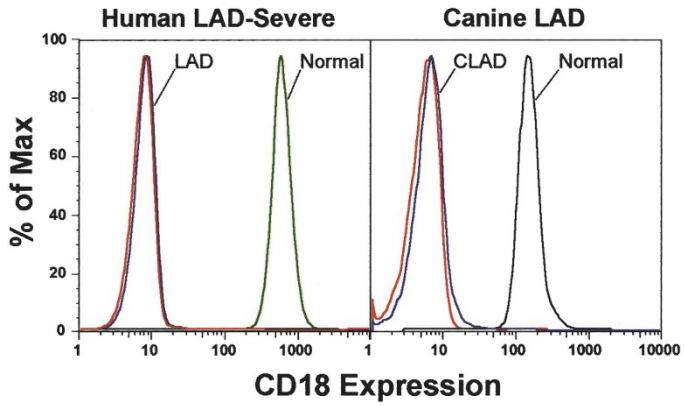

Figure 3. Flow cytometric analysis of neutrophil CD18 expression from a patient with the severe deficiency phenotype of LAD and a CLAD-affected dog. Neutrophils isolated from human or canine peripheral blood were stained with an isotype control antibody (indicated in red) or an anti-human CD18 MAb (which cross-reacts with the canine CD18 molecule). Staining of CD18 on normal human or dog neutrophils is indicated in green and staining for CD18 on neutrophils from an LAD patient and a CLAD dog is shown in blue.

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LAD AND CLAD

Allogeneic hematopoietic stem cell transplantation represents the only definitive therapy for LAD. In the largest study involving allogeneic hematopoietic stem cell transplantation for LAD, 14 children with LAD received a myeloablative conditioning regimen followed by bone marrow grafts from either HLA-identical siblings (five patients) or from partially matched family members (nine patients) (15). Of the five children who received HLA-identical grafts, two died of transplant-related complications (infection and GVHD). Although the transplant ultimately resulted in engraftment in 10 patients, 5 required a second transplant due to graft failure, and the overall mortality was 28% (15). Of note, three of the patients who underwent stem cell transplantation became long-term mixed hematopoietic chimeras, and appeared to be free of disease despite having less than full donor chimerism.

There are subsequent reports in which children with LAD have been cured with allogeneic marrow transplantation using marrow from matched unrelated donors (16), and cord blood from an HLA-identical sibling donor (17). All of these studies used a myeloablative conditioning regimen, and were accompanied by significant regimen-related toxicity and GVHD (18, 19). Complications from GVHD tend to increase in proportion to the degree of mismatch, and because only about 25% of patients with hematologic diseases will have HLA-identical siblings, a large proportion of patients are at risk of GVHD after hematopoietic stem cell transplantation.

The evidence from the patients with LAD who were mixed donor-host chimeras after myeloablative conditioning before allogeneic transplant suggests several possibilities. First, patients with LAD appear to require a more intensive conditioning regimen than individuals with other hematologic diseases to achieve full donor chimerism, most likely due to the expanded myeloid bone marrow in LAD patients (12). Second, less than full donor chimerism appears to be sufficient to reverse the disease phenotype in LAD, suggesting that a nonmyeloablative conditioning regimen before allogeneic transplant in LAD may be efficacious (15). Nonmyeloablative conditioning regimens result in reduced transplant-related mortality and secondary side effects due to decreased amounts of total body irradiation and chemotherapeutic drugs (20). These reduced conditioning regimens were designed to allow a wider age range of patients to be treated by marrow transplantation, as well as to allow marrow transplantation to be extended to individuals with major organ dysfunction that would preclude the administration of an ablative conditioning regimen.

Nonmyeloablative conditioning regimens have been used successfully to treat other immunodeficiency diseases, including chronic granulomatous disease (21), Wiskott-Aldrich syndrome (22), and Fanconi anemia (23). In those transplants, a minimally toxic regimen of either total body irradiation or cyclophosphamide plus fludarabine resulted in sufficient myelosuppression and immunosuppression to permit engraftment in the majority of patients (21). Of note, a number of these patients became stable, mixed chimeras.

Recently, nonmyeloablative bone marrow transplantation, which resulted in mixed donor-host chimerism, has been performed in CLAD pups (24). These animal studies were approved by the Institutional Animal Care and Use Committee of the National Cancer Institute, National Institutes of Health. Results in two CLAD dogs are shown (Fig. 4). The two dogs with CLAD were transplanted using matched littermate donors after a nonmyeloablative dose of 200 cGy total body irradiation. Both dogs received posttransplant immunosuppression

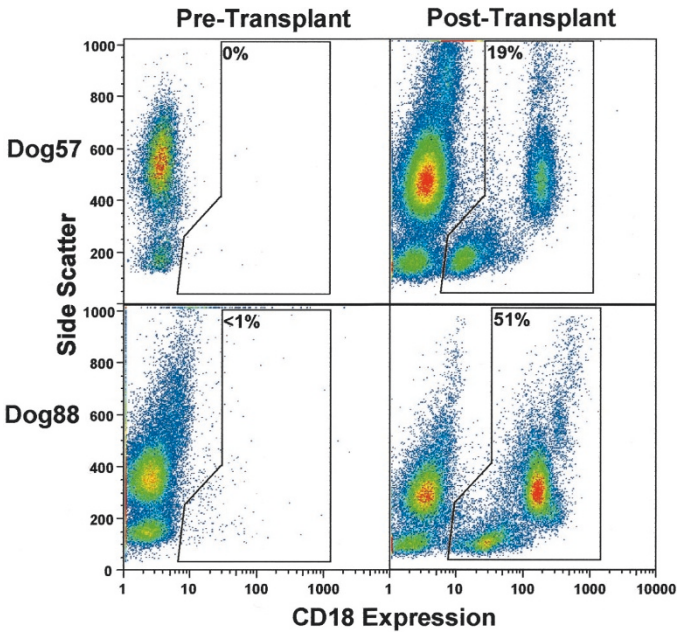

Figure 4. Mixed chimerism after nonmyeloablative conditioning in two CLAD transplanted dogs. Dot plot analysis after flow cytometry of leukocytes from two CLAD dogs before and 1 y (Dog 57) or 9 mo (Dog 88) after hematopoietic stem cell transplantation from a matched littermate donor. The leukocytes that are CD18+, and hence donor-derived, are indicated by the box. Side scatter is displayed on the *y* axis, which differentiates the populations including neutrophils and monocytes (high side scatter) from those containing lymphocytes (low side scatter). CD18 expression is displayed on the *x* axis.

with cyclosporine for 2 mo and mycophenolic acid for 1 mo. Neither dog rejected their graft nor developed GVHD. Moreover, both CLAD dogs have been free of any signs or symptoms of CLAD since the time of engraftment.

Both CLAD dogs became stable, long-term donor-host chimeras, with the percentage of donor leukocytes in the peripheral blood averaging from 19% in the first dog to 51% in the second dog (Fig. 4). Mixed chimerism in the transplanted dogs was assessed by flow cytometric analysis of CD18+ cells in the peripheral blood, which can only be donor-derived, and by comparative DNA analysis of PCR amplification of donor and host microsatellite repeat DNA markers.

HEMATOPOIETIC STEM CELL GENE THERAPY IN LAD

Gene therapy has been attempted as a therapeutic approach in two patients with LAD. In a clinical trial of retroviral-mediated gene therapy for LAD, two patients with the severe deficiency form of LAD received a single infusion of autologous, CD18 gene-corrected stem cells (25). Despite the presence of a small (<0.1%) population of CD18+ neutrophils in the peripheral blood of each patient after the infusion of CD18 gene-corrected CD34+ cells, there was no persistence of the CD18 gene marked neutrophils beyond 63 d after the infusion of transduced cells. These results are consistent with the results in a number of other human gene therapy clinical trials in which the infused hematopoietic stem cells did not possess a selective proliferative advantage.

RELEVANCE OF THE CANINE MODEL OF LAD

CLAD represents an attractive model for the investigation of novel hematopoietic stem cell transplant regimens as well as hematopoietic stem cell gene therapy approaches for several reasons. First, there is a close relationship between the clinical phenotype of CLAD and the clinical phenotype of LAD. Thus, therapeutic approaches that reverse the disease phenotype in CLAD would be expected to be efficacious in LAD. Second, because reversal of the disease phenotype remains the cornerstone of any therapy in CLAD, and because most CLAD dogs die or are euthanized by 6 mo of age due to infection, this outcome represents a clear end point against which experimental therapies can be compared. Third, the presence and number of donor-derived CD18-positive leukocytes in the peripheral blood is easily detected and quantified by flow cytometry. Therefore, the presence of CD18-positive leukocytes in a treated CLAD dog, after either an allogeneic hematopoietic stem cell transplant or an infusion of autologous, CD18 gene-corrected cells, can be correlated with reversal of the disease phenotype.

The canine model itself is well established as an experimental system in which novel hematopoietic approaches to therapy, such as hematopoietic stem cell transplantation and hematopoietic stem cell gene therapy, can be evaluated (26, 27). For hematopoietic stem cell transplantation in particular, the dog MHC, known as the dog leukocyte antigen (DLA) locus, has been defined, and has been shown to be similar to the MHC region of humans (28, 29); regimens for conditioning before stem cell transplant have been extensively tested in canines; GVHD in the setting of genotypic MHC compatibility was first described in dogs and, as a consequence, therapy to treat GVHD was developed in the dog (30); and insights into issues of hematopoiesis and transplantation derived from canine studies have been successfully translated to humans.

The CLAD model described in this report now provides a disease-specific, large-animal model for assessing new therapeutic approaches that may be translated to LAD.

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