A New Leukocyte Hyperadhesion Syndrome of Delayed Cord Separation, Skin Infection, and Nephrosis

abstract

Leukocyte adhesion deficiency (LAD) I is a well-described genetic disorder in which leukocytes are unable to migrate to sites of inflammation due to mutations in the ITGB2 gene coding for the β subunit of β2 (CD18) leukocyte integrins. The classic symptoms of the disease present in the newborn period as failure of separation of the umbilical cord and recurrent bacterial infections, which continue throughout life. We report on a patient with these clinical manifestations but with normal ITGB2 gene sequencing excluding LAD-I, normal carbohydrate-deficient transferrin testing excluding LAD-II, and normal platelet function excluding LAD-III. With testing for CD18 integrin function by flow cytometry, adhesion assay analysis, and time-lapse microscopy, we found the patient’s T lymphocytes to express normal levels of β1 and β2 integrins but to be highly adhesive to integrin ligands and to display decreased migration compared with control T lymphocytes. The hyperadhesiveness of the cells suggests that they might be prevented from reaching infected tissues. Interestingly, administration of glucocorticoids, for the patient’s nephrotic syndrome, alleviated the patient’s chronic diarrhea and decreased the incidence of skin infections. The hyperadhesiveness rather than adhesion deficiency of the patient’s leukocytes suggests that a novel lesion in a pathway regulating integrin adhesion is responsible for the patient’s unique LAD-I–like symptoms. Pediatrics 2014;133:e257–e262
Three leukocyte adhesion deficiency (LAD) disorders have been recognized thus far. LAD-I is caused by mutations in the ITGB2 gene coding for the β2 (CD18) subunit responsible for membrane expression of the leukocyte integrins: lymphocyte function–associated antigen-1 (LFA-1), macrophage-1 antigen (Mac-1), p150,95, and αXB2, which are all exclusively expressed by leukocytes. The LAD-I leukocytes display deficient adhesion and migration and have difficulty reaching inflamed tissues due particularly to lack of LFA-1 and Mac-1 expression. Without the interaction of leukocyte integrins with the endothelial intracellular adhesion molecules (ICAMs), the leukocytes are unable to adhere to the blood vessel wall and therefore unable to extravasate into tissues. The defective adhesion caused by this mutation presents clinically as delayed separation of the umbilical cord in the newborn period and recurrent bacterial infections throughout life. LAD-II is a rare disorder resulting from a deficiency in a guanosine diphosphate–fucose transporter in the Golgi apparatus, which is essential for fucosylating the ligands of the selectin adhesion receptors. In LAD-II, the leukocytes fail to roll along the vasculature and integrin-mediated adhesion stabilization cannot take place. In the most recently described LAD, termed LAD-III, the integrins on bone marrow–derived leukocytes and platelets are correctly expressed but fail to function due to mutation in the FERMT3 gene coding for the kindlin-3 protein. The patients exhibit a severe bleeding disorder as well as widespread infections as in LAD-I. We report a patient with features of LAD-I, including delayed separation of the cord and recurrent infections, in whom a newly identified defect of leukocyte hyperadhesion was detected, representing a potentially novel condition.

**PATIENT PRESENTATION**

Our patient is a 2-year-old white girl whom we first encountered during an admission for nephrotic syndrome; a renal biopsy performed during admission revealed diffuse mesangial hypercellularity. At this point in her life, the patient had already experienced multiple skin infections with methicillin-resistant *Staphylococcus aureus* including some episodes of secondary cellulitis, recurrent episodes of otitis media, and other upper respiratory infections. She had undergone testing for immunodeficiency as well as for common causes of failure to thrive, including esophageal-gastro-duodenoscopy and colonoscopy to rule out Shwachman-Diamond syndrome and sweat testing to rule out cystic fibrosis.

At our initial encounter, a thorough history was obtained from the patient’s mother, which revealed the following information. The patient was born to a 34-year-old gravida 2 para 1 mother and a 31-year-old father at 39 weeks’ gestation. No consanguinity was noted in the pedigree. The pregnancy was complicated by borderline diabetes controlled by diet and by maternal asthma treated with albuterol. There were no other complications or medications reported. During the pregnancy, ultrasound studies were normal and the patient was delivered via cesarean section secondary to previous cesarean delivery. The patient had some issues with elevated bilirubin in the neonatal period but did not require phototherapy. She was discharged from the hospital at 2 days of age.

In the first few weeks of life she developed an umbilical cord infection and subsequently delayed separation of the umbilical cord at 6 weeks of age. Over the next 2 years the patient developed multiple skin infections. Of note, she did not display problems with wound healing or scarring. Her white blood cell count 1 day before steroid administration revealed a total leukocyte number of 6300 per mm$^3$ with a differential of 1575 per mm$^3$ (25%) neutrophils, 3906 per mm$^3$ (62%) lymphocytes, 441 per mm$^3$ (7%) monocytes, and 315 per mm$^3$ (5%) eosinophils. An immunology workup including quantitative immunoglobulins, oxidative burst testing, and T- and B-lymphocyte subsets was normal. In addition, the patient had both eczema and chronic diarrhea.

A review of the family history revealed that both of the patient’s siblings, an older sister and younger brother, have suffered from otitis media and that her sister has also experienced methicillin-resistant *Staphylococcus aureus* skin infections. The patient’s mother has occasional boils and asthma and suffered from a prolonged course of thrombotic thrombocytopenic purpura as a child. The patient’s father has gout, degenerative disc disease, and hypertension.

**RESULTS AND DISCUSSION**

Given the negative workup for failure to thrive and immunodeficiency, additional testing was initiated to find a cause for the patient’s immunodeficiency. Bidirectional genomic sequencing of the CD18 exons 2 through 16 with splice sites revealed no abnormality (GeneDx, Gaithersburg, MD; data not shown). The lack of mutation in this gene that codes for the CD18 subunit of leukocyte integrins indicated that LAD-I was an unlikely explanation. Carbohydrate-deficient transferrin testing to discern the number of sialylated N-linked oligosaccharide residues revealed no defect in fucosylated glycoproteins, thereby ruling out the diagnosis of LAD-II. In the LAD-III disorder, both platelet and leukocyte function are affected leading to severe bleeding and infection because bone marrow–derived leukocyte integrin function is minimal. Thus, more detailed studies of the patient’s white blood cells and platelets were next carried out. Platelet function tests revealed normal platelet adhesion and aggregation with normal closure times on both collagen-epinephrine (patient:...
136 seconds; versus a normal range of <183 seconds) and collagen-adenosine diphosphate–coated membranes (patient: 81 seconds; versus a normal range of <122 seconds). Normal platelet function indicated that the platelet β1 and β3 integrins were active, thus ruling out classic LAD-III as an explanation for the patient’s disorder.

A biopsy of the gastrointestinal tract for suspected reflux esophagitis, characterized by an abundance of eosinophils in the esophageal tissue, revealed only a few eosinophils in the upper portion of the esophagus, none in the middle, and a minimal number of eosinophils in the lower esophagus. The minimal numbers of tissue eosinophils suggested the possibility that the cells were unable to reach their target of inflammation in the reflux disease, potentially because of an adhesion disorder.

To investigate the possibility of aberrant leukocyte integrin function, we first investigated integrin expression focusing on the T lymphocytes (T cells). Phenotypic analysis using flow cytometry revealed normal levels of LFA-1 subunit αL (CD11a) and Integrin α4β1 (Very Late Antigen-4 [VLA-4]) subunit α4 (CD49a) on the patient’s T cells compared with age-appropriate normal control T cells (Fig 1); the β subunits of both integrin classes (CD18 and CD29, respectively) were also normally expressed (data not shown). We next performed adhesion assays to test for the ability of these integrins to adhere to their ligands. Investigation of adhesion to the LFA-1 ligand, ICAM-1, and the α4β1 ligand fibronectin revealed that the integrins on the patient’s T cells compared with control T cells were highly activated without requiring any additional stimulus to adhere (Fig 2). Treating the T cells with diverse integrin-activating stimuli such as phorbol ester (PdBu), thapsigargin, Mg2+/ethyleneglycotetraacetic acid (EGTA) and cross-linking the T-cell receptor only slightly increased the already optimal adhesive responses of the patient’s T cells. LFA-1 also acts as a promigratory receptor. When migration was viewed by using time-lapse microscopy, the patient’s T cells were observed to migrate slowly on ICAM-1 compared with control T cells, with a speed of 4.05 μm per minute compared with a normal rate of 16.97 μm per minute (Fig 3). The patient’s T cells were extensively spread on ICAM-1, consistent with an activated integrin phenotype (Fig 3). However, of note, was the normal expression of the epitopes found on activated LFA-1 (data not shown). This finding would seem to rule out a direct effect on the integrins themselves. Thus, the patient’s T cells showed normal β1 and β2 integrin expression, yet abnormally strong adhesion and slow migration.

**Treatment With Glucocorticoids**

Our patient experienced both improvement in her chronic diarrhea and decreased incidence of skin infections while receiving steroids for nephrotic syndrome. It is of interest that the administration of glucocorticoid leads to downregulation of adhesion molecule expression.
expression on leukocytes in vitro.\textsuperscript{12–14} Ligation of glucocorticoid receptors attenuates downregulation of \( \varepsilon \)-selectin and upregulation of CD11/CD18 adhesion molecules, contributing to inhibition of neutrophil migration by glucocorticoids.\textsuperscript{17} Thus, administration of glucocorticoids to our patient may have decreased the adhesiveness of her leukocytes, thereby allowing them increased mobility and the ability to reach sites of inflammation. Evidence for this possibility comes from the change in counts after steroids were administered. Leukocyte counts on day 3 of steroid administration increased substantially to a total count of 12,600 with a differential of 7,580 neutrophils and 3,906 lymphocytes. On day 8 of steroid administration, the total leukocyte count continued to increase.

**FIGURE 2**

T-cell adhesion to the LFA-1 ligand ICAM-1 and the \( \alpha_4 \beta_1 \) ligand fibronectin. Assays showing adhesion of T lymphoblasts to ICAM-1 (A) and fibronectin (B). The patient’s T cells show a background level of adhesion to both ligands that is similar to stimulated controls, suggesting an increased ability to adhere compared with normal T cells. Mean (\( \pm \)SD) data from 1 representative experiment of 2 experiments are shown. MgEGTA, (Mg\(^{2+}\)/ethylene glycol tetraacetic acid), RPMI (RPMI 1640 medium), PdBu (Phorbol 12,13-dibutyrate), monoclonal antibody UCHT1, EDTA.
and reached 16,400 with a differential of 10,824 neutrophils and 5084 lymphocytes. Whereas the exact mechanism causing the hyperadhesiveness of the integrins is not known, the symptoms bear resemblance to mice expressing constitutively active LFA-1 in which excessive adhesion prevents leukocytes from entering injured tissues and, when tested in vitro, causes increased adhesion and stalled migration. 

Insight into the mechanisms of glucocorticoid action might also help to explain the diminished recurrence of skin infections. 

**Leukocyte Hyperadhesiveness**

Hyperadhesiveness is predicted to prevent leukocytes from reaching sites of inflammation, thus explaining the recurrent skin infections and the minimal numbers of eosinophils in the esophageal biopsy sample in our patient. Because our patient’s leukocytes appear to be highly activated compared with control leukocytes, they may be more sensitive to inflammatory signals and become prematurely arrested. Whereas the exact mechanism causing the hyperadhesiveness of the integrins is not known, the symptoms bear resemblance to mice expressing constitutively active LFA-1 in which excessive adhesion prevents leukocytes from entering injured tissues and in vitro causes increased adhesion and stalled migration. 

Thus, increased adhesion has the same phenotypic effect as lack of adhesive activity such as that displayed by the leukocytes of LAD-I or LAD-III patients. Additionally, the lack of consanguinity, as is characteristic of LAD patients, indicates autosomal recessive inheritance is less likely, although not excluded. The hyperadhesiveness of the patient’s T cells suggests a novel mechanism by which leukocytes and their integrin activity is regulated. A better understanding of the signaling mechanisms by which leukocyte integrins are activated may provide guidance as to where to investigate the cause of the patient’s hyperadhesion. However, the strengthening of adhesion is dependent not only on the conformation of the integrins themselves but is heavily influenced by their connection to the actin and myosin cytoskeletal machinery. Cytoskeletal adaptors, of which the best understood are talin and the kindlins, serve as linker molecules and such links are essential for turnover of integrins. Although the regulation of turnover of adhesions controls leukocyte spreading and migration, the mechanisms that govern integrin release or deadhesion are not as well studied as those that promote adhesion. Further research into the disorder could potentially uncover a mechanism of integrin turnover or perhaps a novel factor in the leukocyte adhesion pathway not yet identified. 

**ACKNOWLEDGMENTS**

We thank the patient and her family for allowing us to publish her case.
REFERENCES


ERRATA


An error occurred in the article by Eric Biondi et al, titled “Epidemiology of Bacteremia in Febrile Infants in the United States” published in the December 2013 issue of Pediatrics (2013;132(6):990–996; originally published online November 11, 2013; doi:10.1542/peds.2013-1759). On page 990, in Authors, this reads: “Vivan Lee; Children’s Hospital of Los Angeles.” This should have read: “Vivian Lee; Children’s Hospital Los Angeles.”

doi:10.1542/peds.2013-4017


An error occurred in this article by Pickering et al, titled “The Red Book Through the Ages” published in the November 2013 issue of Pediatrics (2013;132(5):898–906; originally published online October 14, 2013; doi:10.1542/peds.2013-2538). On page 899, under Early History of the C0ID, the first line reads: “After the establishment of the AAP in 1930 in the library of Harber Hospital…” This should have read: “After the establishment of the AAP in 1930 in the library of Harper Hospital…”

doi:10.1542/peds.2013-4105


doi:10.1542/peds.2014-0173


Two errors occurred in the article by Simpson et al, titled “A New Leukocyte Hyperadhesion Syndrome of Delayed Cord Separation, Skin Infection, and Nephrosis” published in the January 2014 issue of Pediatrics (2014;133(1):e257–e262; doi:10.1542/2013-0884). On page e261, under Treatment With Glucocorticoids on line 31, this reads: “Whereas the exact mechanism causing the hyperadhesiveness of the integrins is not known, the symptoms bear resemblance to mice expressing constitutively active LFA-1 in which excessive adhesion prevents leukocytes from entering injured tissues and, when tested in vitro, causes increased adhesion and stalled migration.15,16n” This should have not been inserted. Additionally, on the same page (e261) in the next section, Leukocyte Hyperadhesiveness, on line 12, this reads: “Whereas the exact mechanism causing the hyperadhesiveness of the integrins is not known, the symptoms bear resemblance to mice expressing constitutively active LFA-1 in which excessive adhesion prevents leukocytes from entering injured tissues and, when tested in vitro, causes increased adhesion and..."
stalled migration.\textsuperscript{12,13} This should have read: “Whereas the exact mechanism causing the hyperadhesiveness of the integrins is not known, the symptoms bear resemblance to mice expressing constitutively active LFA-1 in which excessive adhesion prevents leukocytes from entering injured tissues and, when tested in vitro, causes increased adhesion and stalled migration.\textsuperscript{15,16}”

doi:10.1542/peds.2014-0181


An error occurred in the article by Lanzieri et al, titled “Breast Milk-Acquired Cytomegalovirus Infection and Disease in VLBW and Premature Infants” published in the June 2013 issue of \textit{Pediatrics} (2013;131[6]:e1937–e1945; originally published online May 27, 2013; doi:10.1542/peds.2013-0076). On page e1937, in the abstract, on line 3–4, this reads: “including CMV-related sepsis-like syndrome (CMV-SLS) for which in the United States are lacking.” This should have read: “including CMV-related sepsis-like syndrome (CMV-SLS), for which estimates in the United States are lacking.”

doi:10.1542/peds.2014-0217
A New Leukocyte Hyperadhesion Syndrome of Delayed Cord Separation, Skin Infection, and Nephrosis

Brittany N. Simpson, Nancy Hogg, Lena M. Svensson, Alison McDowall, William Daley, Kilby Yarbrough and Omar A. Abdul-Rahman

Pediatrics; originally published online December 16, 2013;
DOI: 10.1542/peds.2013-0884

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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Errata
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