In Vivo Neutrophil and Lymphocyte Function Studies in a Patient With Leukocyte Adhesion Deficiency Type II

By Thomas H. Price, Hans D. Ochs, R. Gershoni-Baruch, John M. Harlan, and Amos Etzioni

We investigated in vivo neutrophil and lymphocyte function in a patient who lacks Sialyl-Lewis-X, a ligand for the selectin family of leukocyte adhesion molecules (leukocyte adhesion deficiency II, LAD II). As assessed by skin chamber and skin window techniques, in vivo chemotaxis of neutrophils was markedly impaired (less than 6% of normal values). A marginal pool was present as determined by an increase in circulating neutrophils after epinephrine injection and calculated recovery of infused radiolabeled autologous neutrophils. Kinetic studies showed a reduced half-life of 3.2 hours (normal 7 hours) and markedly increased turnover rate (cells/kg/dl) of approximately eight times the normal value. A normal antibody response to the T-cell-dependent antigen bacteriophage φX174 showed that T/B-cell interaction is not affected in LAD II. These findings provide direct evidence that the selectin family and its ligands play an important role in neutrophil function.

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MATERIALS AND METHODS

Patient. YW is a 5-year-old boy who was noted within the first year of life to have severe mental retardation, short stature, a distinctive facial appearance, neutrophilia, recurrent bacterial infections, and the Bombay (hh) blood phenotype. A detailed clinical description of this patient was previously reported. The patient’s neutrophil count is typically 20 to 35 × 10^9/L when he is free of infection and usually exceeds 70 × 10^9/L during episodes of infection. Severe periodontitis has persisted from early life. In the last 3 years, the frequency of infection decreased and prophylactic antibiotics were discontinued. Informed consent for all studies was given by the patient’s parents. All studies were approved by either the University of Washington or the Rambam Medical Center Human Subjects Review Committee.

Hematologic values. Hematocrit, platelet count, white blood cell count, and differential counts were determined using standard hematologic techniques. Lymphocyte subsets were determined by flow cytometry using appropriate monoclonal antibodies (MoAbs).

In vivo chemotaxis. The neutrophil response to cutaneous inflammation was assessed by both skin-chamber and skin-window techniques. Skin-chamber studies were performed as previously described. A 1-cm^2 area on the left-forearm was abraded with a scalpel blade and the abrasion covered with a 10-ml sterile glass chamber that was fastened in place by adhesive compound. The chamber was filled with fluid consisting of 10% autologous serum

From the Departments of Medicine and Pediatrics, University of Washington; the Puget Sound Blood Center, Seattle, WA; Departments of Pediatrics and Clinical Immunology, Rambam Medical Center; and the B. Rappaport Faculty of Medicine, Technion, Haifa, Israel.

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Address reprint requests to Thomas H. Price, MD, Puget Sound Blood Center, 921 Terry Ave, Seattle, WA 98104.

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in normal saline to which 100 U/mL streptokinase/streptodornase had been added. The fluid was removed after 24 hours and the cell count and differential count determined. Skin-window leukocyte accumulation was assessed by the technique of Dale et al. A 3-× 3-mm abrasion was created on the right-volar forearm by scalpel blade as above. Sterile 18-mm round cover slips were applied to the abrasion, covered with a sterile cardboard square, and changed at 1, 3, 5, 7, 9, 12, and 24 hours. Differential counts were performed after staining with Wright’s stain. The number of cells in the most cellular 1 mm² of the coverslip was quantitated by counting the cells in a photograph of the field. Results were expressed as the geometric mean response, as described by Dale et al.

**Blood neutrophil kinetics.** Neutrophil kinetics were determined as previously described. Seventy-five milliliters of blood was withdrawn from the patient and labeled in vitro with 3H-diisopropylfluorophosphate (3H-DFP; New England Nuclear, Boston, MA; 10 Ci/mmol). After removal of an aliquot for determination of neutrophil count and specific activity, the blood was reinfused over a 10-minute period. Three-milliliter blood samples were then obtained at 0, 1, 4, 8, and 24 hours after infusion. Neutrophils were isolated from each sample by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) sedimentation and contaminating red blood cells were removed by dextran sedimentation and lysis with ammonium chloride. Neutrophil specific activity (cpm/10⁶ cells) was plotted on semi-log paper. The calculated neutrophil turnover rate (cells/kg/d) was approximately 8 times the normal value.

**Epinephrine test.** Epinephrine (0.2 mL; 1 mg/mL; 1:1000) was injected subcutaneously and blood samples were obtained for neutrophil count at 0, 5, 10, and 30 minutes.

**Immunoassay with bacteriophage φX174.** Bacteriophage φX174 (phage) was produced as previously described. The final concentration was adjusted to 1 × 10¹⁰ plaque-forming units (PFU)/mL. After obtaining informed consent, phage was administered intravenously at a dose of 2 × 10⁹ PFU/kg body weight. A second immunization was given 6 weeks later. Normal controls and the patient with LAD I received a tertiary immunization 6 or more weeks after the secondary immunization. Blood samples for antibody titers were collected immediately before immunization and at 1, 2 and 4 weeks after each immunization. Antibody activity was determined by a neutralizing antibody assay and expressed as the first order rate constant (Kv), or phage inactivation, as described.

The interaction between neutrophils and endothelial cells, critical for neutrophil localization to and migration across endothelium, is mediated by surface molecules on both neutrophils and endothelial cells. Three families of adhesion molecules involved in several phases of emigration have been described, acting at different steps of the adhesion process.
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Fig 1. Disappearance of neutrophil radioactivity after intravenous injection of 3H-DFP-labeled autologous neutrophils.

cess; selectins, integrins, and the Ig superfamily. The selectins (E, L, and P-selectin) mediate neutrophil "rolling" wherein, under conditions of flow, the neutrophils become loosely adherent to the endothelium. One neutrophil surface ligand for E and P-selectins has recently been determined to be the tetrasaccharide, SLex.28-34 Once the neutrophil, through rolling, is brought into contact with the endothelial cell, the β2 integrin (CD11/CD18) on the surface of the neutrophil and the Ig superfamily molecule (intracellular adhesion molecule 1) on endothelial cells interact to produce firm attachment and to facilitate neutrophil migration between the endothelial cells to tissue.12,35 The importance of the integrin receptors for neutrophil emigration has been shown by the identification of patients whose neutrophils lack these molecules. The leukocyte adhesion deficiency (LAD I) syndrome is characterized by a defective CD18 gene, absence or deficiency of the CD11/CD18 complex, inability to deliver neutrophils to sites of infection, and recurrent bacterial infection.96

A second leukocyte adhesion molecule defect, LAD II, has recently been described in two unrelated children.17,18 Neutrophils from these patients have been shown in vitro to have both motility and adherence defects. The LAD II patients apparently have a general defect in fucose metabolism. As a consequence, they exhibit the Bombay blood phenotype and lack the carbohydrate SLex on the surface of their neutrophils. SLex is a ligand for both E-selectin and P-selectin,32 and in vitro studies have shown that neutrophils from these patients are incapable of binding to the endothelial selectins.18,30a

The present studies were undertaken to determine the effect of this abnormality in the selectin system on in vivo leukocyte function. The skin-window and skin-chamber studies clearly demonstrate a marked abnormality in both neutrophil and monocyte migration to inflammatory sites. The magnitude of the abnormality in emigration seen in this patient is similar to that previously reported in patients with CD18 deficiency15 in spite of the fact that the latter appear to have more serious problems with bacterial infection. This discrepancy may reflect the limited ability of the skin-chamber and skin-window assays to predict neutrophil traffic to sites of infection, but may also be attributed to the fact that the defect in LAD I also affects lymphocyte function by interference with T/B-cell interaction.16,37 It remains to be determined to what extent T/B cell interactions are affected in LAD II.

While a similar defect in in vivo chemotaxis was observed between LAD I and LAD II patients, our findings in regards to neutrophil kinetics in the LAD II patient are different from previously described findings in LAD I patients. Several reports described a normal marginal pool in patients with LAD I.38,39 We used both the epinephrine test and the neutrophil labeling technique to show that the LAD II pa-

Fig 2. Antibody responses to bacteriophage 4x174. Phage was given at the time indicated (i). Responses presented are in normal controls (e-e, geometric mean; -----, 95% confidence interval), in a patient with LAD II (SLex deficiency) (□-□) and in a patient with LAD I (β2 subunit [integrin] deficiency) (○-○). Antibody is expressed as phage neutralizing activity (Kv). Percent IgG of this activity is indicated for various time points.
tients also had a marginal pool that appears somewhat reduced. Normally, the marginal and circulating pools are approximately equal in size and are in dynamic equilibrium. The anatomic location of the marginal pool is uncertain. It may represent neutrophils transiently sequestered in the lung or other organs, or perhaps cells rolling along the vessels in the general microcirculation. The degree to which selectin receptors are important for the determination of the marginal pool is not clear. Our results could imply that there are two marginal pools. The first, accounting for approximately 20% of the blood neutrophils, is comprised of neutrophils sequestered in the lungs or other organs, may depend upon leukocyte size and deformability, is selectin independent, and is intact in our patient. The second, accounting for approximately 30% of the blood neutrophils, represents neutrophils rolling in the microvascular, is selectin mediated, and is absent or reduced in our patient. Such a construct would be consistent with observations that the total marginal pool size is normal in LAD I patients—neither component is integrin dependent—but reduced in LAD II patients.

Both LAD I and LAD II patients exhibit persistent neutropenia. In LAD I, a prolonged intravascular neutrophil survival was observed, which in part may be explained by the inability of the cells to migrate from the blood vessels to the sites of infection. In our LAD II patient, the neutrophil intravascular half-disappearance time, which is normally 6 to 9 hours, was 3.2 hours. This shortened intravascular residence time was unexpected. The neutrophil turnover rate, calculated from the t1/2 and the blood neutrophil pool size, was markedly elevated and indicated a marrow neutrophil production of approximately 8 times normal. A continuing stimulus to marrow neutrophil production does not seem unreasonable in a patient unable to deliver neutrophils to sites of inflammation, but the fact that the blood neutrophil count did not increase over the period of study suggests that neutrophils were destroyed or used at a high rate somewhere other than in the tissues. It is also possible that the underlying abnormality in fucose metabolism, in addition to causing the apparent inability of the cells to migrate from inflammatory sites and suggests that these are being destroyed or used elsewhere at a high rate. Further studies are needed to investigate these questions.

REFERENCES

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