T6-Antigen–Bearing Cells in Eosinophilic Granuloma of Bone

Joshua L. Fox, MD, Brian Berman, MD, PhD

• **T6-antigen–bearing Langerhans' cells were detected in a touch imprint of eosinophilic granuloma involving a thoracic vertebra of an 11-year-old boy. It is interesting to note that in this localized form of histiocytosis X, no T6-antigen–bearing Langerhans' cells were found in the peripheral blood. Immunofluorescence detection of T6 antigenicity has not been described in this disease and may serve as a rapid diagnostic test.**

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HISTIOCYTOSIS X, characterized by an abnormal proliferation of histiocytes, includes the clinical spectrum of Letterer-Siwe disease, Hand-Schüller-Christian disease, and eosinophilic granuloma.[1] Eosinophilic granuloma, first described by Taratynov in 1913,[2] is most commonly seen in children, often in bone, but involvement of other organ systems, including the lungs and gastrointestinal (GI) tract, also occurs.[3] The course is usually benign, but 10% of patients manifest multifocal disease in less than six months.[4]

Nezelof et al[5] suggested that the infiltrating cell in histiocytosis X is a Langerhans' cell with its characteristic granules. Langerhans' cells, which are bone marrow derived, share many properties and activities of macrophages (reviewed in reference 6). Monoclonal antibody directed against human T6 antigen, which was first defined during delineation of human thymocyte differentiation, has recently been shown by immunoelectron microscopy to be specific for Langerhans' cells.[7] We, therefore, attempted to detect T6-antigen-bearing Langerhans' cells in a touch imprint of osseous eosinophilic granuloma and in the peripheral blood of a boy with this disease.

REPORT OF A CASE

An 11-year-old boy had development of sharp intermittent, aching midback pain during a three-month period. The pain was not related to exercise but was occasionally relieved by aspirin administration and lying prone. Roentgenograms disclosed a left scoliosis of the lower thoracic vertebrae and a bony abnormality at T-9. A bone scan disclosed technetium Tc 99m uptake at T-9. There was no history of fever, diaphoresis, weight change, extremity weakness, paresthesias, GI or urinary tract symptoms, or any other change from the patient's previously excellent health.

The spine and ribs were not tender to palpation. The differential diagnosis included osteoid osteoma, neurofibroma, osteogenic or Ewing's sarcoma, and eosinophilic granuloma of bone.

Computed tomography and myelography disclosed almost complete destruction and collapse of the T-9 vertebra without cord compression. Complete blood cell count with differential cell count, ESR, levels of C-reactive protein and serum electrolytes, liver function test results, chest roentgenogram, intravenous pyelogram, CSF analysis, urinalysis, and PPD skin test results were negative or within normal limits.

No definitive histological diagnosis could be made from frozen sections of T-9 vertebral biopsy material (Fig 1). Routine histological sections disclosed a cellular infiltrate composed of large numbers of histiocytes with one or more nuclei and numerous eosinophils, as well as neutrophils, lymphocytes, and occasional plasma cells. Small blood vessels were present throughout, and there were foci of hemorrhage and minute foci of necrosis. Numerous osteoclastic giant cells were present in some sections. These findings were consistent with a diagnosis of eosinophilic granuloma.

Three days after the biopsy, a cast was set, and, on the following day, the patient was discharged for subsequent observation.

MATERIALS AND METHODS

Antibody and Solutions

Fluorescein - isothiocyanate - conjugated mouse monoclonal antihuman T6 antigen IgG (Ortho Pharmaceutical Corp, Raritan, NJ) was used. All washings were carried out in phosphate-buffered saline.

Detection of T6-Antigen–Bearing Cells Within Vertebral Eosinophilic Granuloma

Air-dried touch preparation slides of vertebral biopsy material were incubated in 50 μL of fluoresceinated antibody in a wet chamber at 20 °C for 30 minutes, washed three times, and mounted with glycerol gelatin. Specimens were examined under epifluorescence microscopy. Routine staining of other touch-preparation slides, simultaneously prepared, showed the presence of histiocytes, polymorphonuclear leukocytes, lymphocytes, plasma cells, and an occasional eosinophil.

Detection of T6-Antigen–Bearing Cells in Peripheral Blood

Five-milliliter samples of heparinized venous blood were obtained from the patient and a sex- and age-matched control subject. Mononuclear cells, collected following Ficoll-Hypaque separation of buffy-coated cells, were washed in phosphate-buffered saline containing 5% bovine serum albumin. A quantity (1×10^6) of mononuclear cells were incubated with 20 μL of the fluoresceinated antibody for 30 minutes at 4 °C. One thousand five-hundred cells were counted and all surface fluorescence was assessed under epifluorescence microscopy.

RESULTS

Fluorescing dendritic cells were present among the cells of the touch preparation. The dendritic processes were longer and thinner than those of epidermal Langerhans' cells[8] (Fig 2).

No fluorescing cells were detected in the peripheral blood mononuclear cells in either the patient or in the control subject.

COMMENT

Ultrastructural examination of the proliferating histiocytic cells of eosinophilic granuloma, a localized, relatively benign form of histiocytosis X,[4] have been shown to contain Langerhans' cells.[3]

To our knowledge, there are no previous reports of immunofluorescent detection of T6 antigenicity in eosinophilic granuloma. Immunofluorescent identification of Langerhans' cells on touch preparations may potentially serve as a means of confirming or rapidly diagnosing histiocytosis X, especially in cases like our own in which no definitive diagnosis could be made from a frozen section. In addition to its rapidity, use of the touch preparation may enhance the visualization of Langerhans' cells, as greater cytological detail is often seen

From the Department of Dermatology, The Mount Sinai School of Medicine, New York.

Reprint requests to Department of Dermatology, The Mount Sinai School of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029 (Dr Berman).

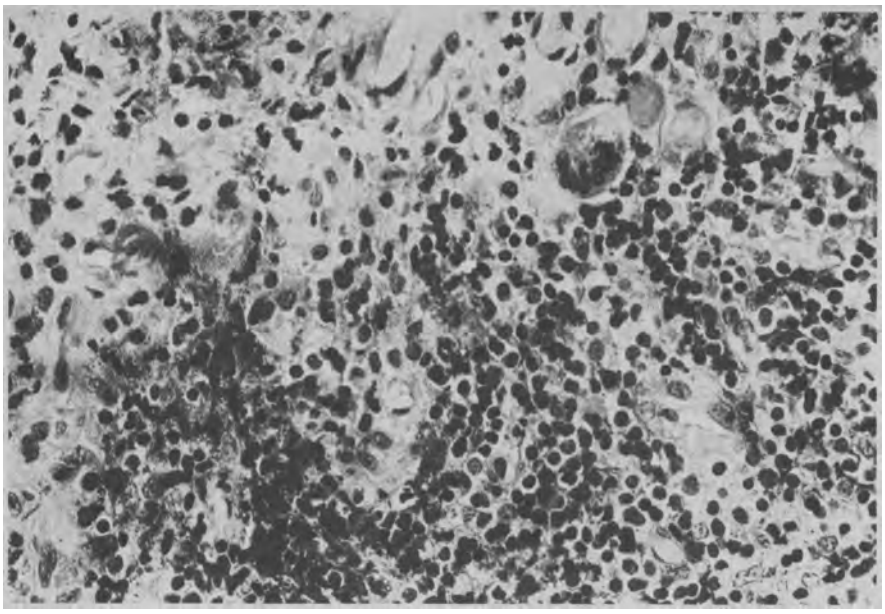

Fig 1.—Frozen section of bone biopsy. Note multinucleated giant cells, histiocytes, lymphocytes, and rare eosinophils (hematoxylin-eosin, original magnification ×100).

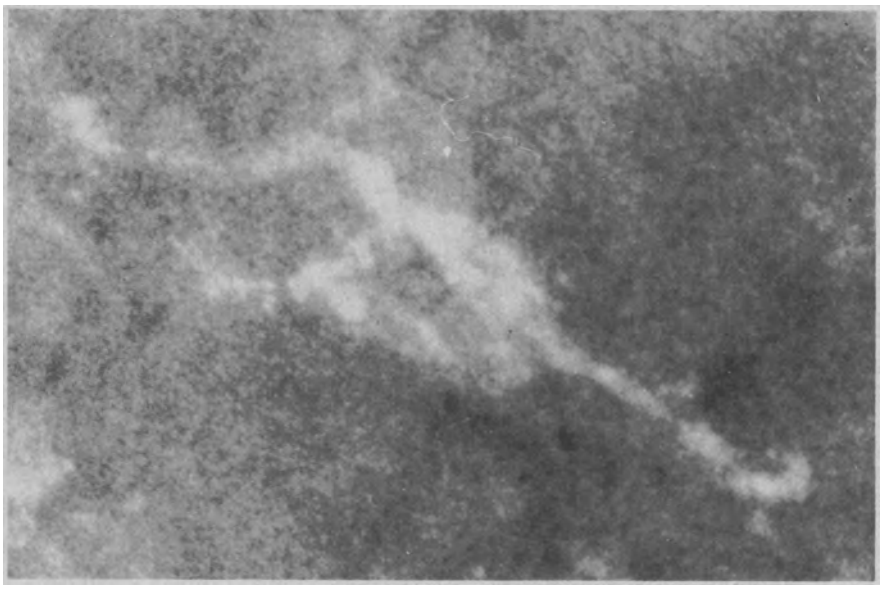

Fig 2.—Fluorescent, T6-antigen-bearing dendritic cell as seen in touch preparation of eosinophilic granuloma of bone (original magnification ×1,000).

in these preparations than in routine histological sections.

We were unable to detect T6-bearing cells in the mononuclear cell fraction of the peripheral blood of either the patient with localized eosinophilic granuloma of bone or of the age- and sex-matched control subject. Similarly, when buffy-coated cells of a patient with eosinophilic granuloma localized to skin were examined ultrastructurally, no cells containing characteristic Langerhans' granules were detected.[9] There are at least three possible explanations for the lack of detectable Langerhans' cells within the peripheral blood. The first is that T6-bearing Langerhans' cells are indeed not present in peripheral blood. This could be the result of the requirement of a microenvironment conducive to the phenotypic expression of these antigens on Langerhans' cells. Support of this hypothesis comes from the finding of bone marrow–derived T6-bearing Langerhans' cells in normal skin, yet these have not been detected within normal bone marrow and peripheral blood.[10] In fact, elaboration of T-cell differentiation factors by epidermal cells has recently been reported.[11] Another possibility is the requirement of a finite amount of time for the ultimate differentiation and complete phenotypic expression by Langerhans' cells, which would not occur while in transit.

A second possible explanation for the lack of detection of T6-bearing Langerhans' cells is that although they exist in peripheral blood, they constitute less than 0.06% of the mononuclear cells. It is conceivable that with more extensive disease, such as occurs in Letterer-Siwe disease, the percentage of these cells will reach detectable levels. We have recently examined a patient with bone and skin involvement and exophthalmos, in whom 0.4% of mononuclear cells bore T6 antigens.

A third possible explanation is that direct extension of Langerhans' cells from the bone marrow to the bony lesion may have occurred, bypassing the systemic circulation.

Fluorescent detection of T6-antigen–bearing cells may be a useful adjunct in the rapid diagnosis of eosinophilic granuloma.

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