Langerhans Cell Histiocytosis

Immunohistochemical Expression of Fascin, a Dendritic Cell Marker

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Key Words: Fascin; Langerhans cell histiocytosis; Langerhans cells; Dendritic cells

Abstract

Langerhans cell histiocytosis (LCH) is a clonal disorder believed to be derived from cells of the dendritic system. Fascin, a 55-kd actin-bundling protein, represents a highly selective marker for dendritic cells of lymphoid tissues and peripheral blood and is involved in the formation of dendritic processes in maturing epidermal Langerhans cells. Since lesional cells of LCH may represent Langerhans cells arrested at an early stage of activation, immunohistochemical expression of fascin in epidermal Langerhans cells and in the lesional cells of 34 cases of LCH was evaluated in paraffin sections using an immunoalkaline phosphatase technique. Though epidermal Langerhans cells were nonreactive for fascin, lesional cells in all LCH cases exhibited immunoreactivity for fascin, CD1a, and S-100 protein. Variation in staining intensity was observed in some cases, possibly reflecting differences in cell maturation or activation. Involved tissues included bone, soft tissue, lymph node, thyroid, orbit, and extradural cranial tissue. Immunoreactivity of lesional cells of LCH for fascin supports their derivation from cells of the dendritic system and represents another alteration in the phenotype of Langerhans cells that is associated with maturation, migration, culture, or clonal expansion.

Langerhans cell histiocytosis (LCH) is a unifocal or multifocal disorder characterized by a proliferation of distinctive cells with ovoid, reniform, grooved, or highly convoluted nuclei and pale eosinophilic cytoplasm. Bone is the most frequent site of disease, although skin, lymph node, lung, and other sites may be involved. The lesions contain varying proportions of Langerhans cells, macrophages, eosinophils, lymphocytes, giant cells, and, to a lesser extent, plasma cells and neutrophils. Currently, the term Langerhans cell histiocytosis is used to include a spectrum of disorders previously designated as eosinophilic granuloma, histiocytosis X, Hand-Schüller-Christian disease, and Letterer-Siwe disease.1,2 The most specific markers for the lesional cells of LCH are Birbeck granules, identified ultrastructurally, and CD1a glycoprotein, detected immunohistochemically. Although this process has been regarded as a reactive disorder of immune dysregulation, molecular genetic studies have demonstrated that all forms of LCH are clonal.3-5 However, based on long-term studies of large numbers of cases, some investigators still consider this lesion to be a disorder of altered immunity, analogous to sarcoidosis, and prefer the term Langerhans cell granulomatosis.6

Langerhans cells (LCs), the hallmark of this disease, were first described in 1868 by the investigator whose name they now bear, using a gold chloride staining technique that identified a unique nonpigmented dendritic appearing cell in the epidermis.7 These cells also are found in lymph nodes and the thymus and in limited numbers in the oral mucosa, esophagus, main bronchi, and distal colon.8 LCs are believed to be bone marrow–derived from CD34+ precursor cells and to represent part of the dendritic cell system.9,10 These cells are potent antigen-presenting cells and, after antigenic stimulation, have
the capacity to migrate from the epidermis via afferent lymphatics to the paracortical areas of lymph nodes, where they apparently complete the process of maturation to dendritic cells, including interdigitating reticulum cells. Functional as well as phenotypic and morphologic changes are coincident with this maturation process. LCs express Fc receptors and the nonpolymorphic class I molecule CD1a and ultrastructurally have cytoplasmic Birbeck granules. Following migration from skin, or in culture, these features are lost in association with up-regulation of major histocompatibility complex (MHC) class II and adhesion molecules. The lesional cells of LCH are reactive for a variety of antigens in paraffin sections, with CD1a and S-100 protein representing the most helpful markers. Immunochemical studies have demonstrated that dendritic cells in lymphoid tissues may be detected selectively with a monoclonal antibody to fascin, a 55-kd actin-bundling protein. Interdigitating reticulum cells in interfollicular T-cell zones exhibited the strongest reactivity, with weaker staining of follicular dendritic cells and nodal sinus lining cells. Histocytes (eg, epithelioid, phagocytic) typically are nonreactive. Since LCs are regarded as part of the dendritic system, rather than of the mononuclear phagocytic system, we evaluated the immunohistochemical staining profile for fascin in epidermal LCs and in tissues involved by LCH. The results of these studies potentially may provide information related to the pathogenesis of this disorder and possibly provide another phenotypic marker for these unique lesional cells.

Materials and Methods

A total of 34 cases of LCH were evaluated. All tissues were fixed in formalin. Electron microscopic studies were available for 2 cases and demonstrated LCs with characteristic Birbeck granules. Normal skin (8 specimens; frozen or paraffin sections) and skin lesions from inflammatory disorders (4 cases) or lymphoproliferative disorders (6 cases) also were evaluated.

Immunohistochemical studies for fascin were performed on deparaffinized sections following heat-induced epitope retrieval using citrate buffer (0.01-mol/L concentration, pH 6.0) in a steamer (model HS-80, Black & Decker, Shelton, CT) or in a microwave oven (model JE1390GV001 with 850 W microwave power output, General Electric, Louisville, KY) set at maximum probe temperature (93°C) for 25 minutes, then placed at room temperature for 25 minutes.

Slides were washed with water, then placed in a 0.05-mol/L concentration of tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.6, supplemented with 3% porcine serum. Preliminary studies of these formalin-fixed tissues (many represented bone lesions that had been decalcified) demonstrated that heat-induced epitope retrieval was required to optimize staining for fascin using the detection system described. Studies were performed using mouse antihuman fascin monoclonal antibody, clone 55K-2, an IgG1 immunoglobulin (DAKO, Carpinteria, CA), with preparation and immunohistochemical techniques as previously described. Sections were incubated for 1 hour at room temperature with antifascin monoclonal antibody (1:5,000 dilution; 1:1,000 for some cases) and subsequently incubated for 40 minutes with rabbit antimouse immunoglobulin antibodies (DAKO; 1:40 dilution using a 0.10-mol/L concentration of Tris buffer, pH 7.6, supplemented with 4% human AB serum as diluent), followed by a 40-minute incubation with alkaline phosphatase anti–alkaline phosphatase immune complexes (APAAP, DAKO; 1:60 dilution with a 0.10-mol/L concentration of Tris buffer, pH 7.6, as diluent). The latter 2 steps were repeated using 20-minute incubation times. Antibody localization was preceded with a 5-minute soak in a 0.10-mol/L concentration of Tris buffer, pH 8.2, containing a 3.00-mmol/L concentration of levamisole hydrochloride (catalog No. L9756; Sigma Chemical, St Louis, MO) as an inhibitor for endogenous alkaline phosphatase. Color development was effected using an alkaline phosphatase reaction with naphthol AS-MX phosphate (catalog No. N4875; Sigma Chemical) as substrate and New Fuchsin (color index number 42520, Cell Point Scientific, Rockville, MD) as the chromogen in a 0.10-mol/L concentration of Tris buffer, pH 8.2, containing a 3.00-mmol/L concentration of levamisole. Slides were counterstained with Mayer or Harris hematoxylin; dehydrated with organic solvents, substituting Pro-Par (Anatech, Battle Creek, MI) for xylene; and mounted with Permount (Fisher Scientific, Pittsburgh, PA). Control studies also were performed using isotype-specific mouse immunoglobulin (IgG1, Coulter, Hialeah, FL) on sequential sections. Positive control slides (either reactive lymph node or cases of Hodgkin disease) also were processed.

Studies for CD1a (prediluted; clone 010; Immunotech, Westbrook, ME) were performed after heat-induced epitope retrieval using citrate buffer or target unmasking fluid (Signet Lab, Dedham, MA) as noted for fascin studies. Slides were incubated with primary antibody overnight at room temperature, then processed with the DAKO LSAB+ peroxidase detection system (40-minute incubation with each reagent; DAKO). Antibody localization was effected using a peroxidase reaction with 3,3’-diaminobenzidine tetrahydrochloride (Sigma Chemical) as the chromogen. For S-100 studies, slides were not pretreated and were processed on a Ventana ES automated immunostainer (Ventana Medical Systems, Tucson, AZ) using rabbit polyclonal S-100 antibody.
antibody (1:500 dilution; 32-minute incubation; DAKO) and a biotin and peroxidase-conjugated avidin detection system (Ventana detection kit, Ventana), with diaminobenzidine as the chromogen.

Results

A total of 34 cases of LCH were evaluated for fascin, CD1a, and S-100 protein. The available clinical data and results of phenotypic studies for the lesional cells are summarized in Table 1. Patient age ranged from 1 to 54 years (mean, 11.0 years; median, 5 years). Males were affected more frequently than females (26:8). Bone was the most common site of involvement (total, 22; skull, 9; femur, 5; rib, 3; tibia, pelvis, ischium, acetabulum, clavicle, 1 each). Other sites included soft tissue (6), lymph node (3), orbit (1), thyroid (1), and extradural cranial tissue (1).

In all cases, the lesions were composed of LCs with generally complex, clefted, grooved, irregular, or convoluted nuclei with fine chromatin and 1 or more small nucleoli and moderate to abundant quantities of eosinophilic cytoplasm, admixed in varying proportions with eosinophils Image 1. Multinucleolated forms were apparent in some cases. Other cells noted in varying numbers were neutrophils, lymphocytes, histiocytes, and giant cells, frequently of the osteoclastic type.

Most lesional cells were reactive for CD1a Image 2 and S-100 protein Image 3, with some variation in staining intensity. More variation in the percentage of reactive cells and the intensity of staining was observed for fascin Image 4 and Image 5. In all cases, however, at least 50% of cells were positive for fascin. The variation in staining intensity and numbers of immunoreactive LCs in the bone lesions possibly may be attributed to some loss of antigenicity related to the decalcification procedures required for processing. However, variation in staining intensity also was noted for nondecalcified specimens, suggesting that this finding may reflect true differences in antigen expression by the lesional cells. Cells reactive for CD1a demonstrated

Table 1
Langerhans Cell Histiocytosis: Clinical Features and Phenotypic Studies of Lesional Cells

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Site of Involvement</th>
<th>CD1a</th>
<th>S-100</th>
<th>Fascin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/1</td>
<td>Tibia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2/M/1</td>
<td>Skull</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3/M/2</td>
<td>Bone, pelvis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4/M/2</td>
<td>Lymph node, inguinal (disseminated disease)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5/M/2</td>
<td>Skull</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6/F/2</td>
<td>Femur</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7/M/2</td>
<td>Skull</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8/M/2</td>
<td>Femur</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9/M/2</td>
<td>Soft tissue, mandible</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11/F/3</td>
<td>Clavicle</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>12/M/3</td>
<td>Soft tissue, mastoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13/F/4</td>
<td>Lymph node, cervical</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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</tr>
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<td>15/F/4</td>
<td>Soft tissue, right temporal</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16/F/5</td>
<td>Soft tissue, occipital</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17/M/5</td>
<td>Skull</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18/M/5</td>
<td>Acetabulum</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19/M/6</td>
<td>Femur</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20/M/7</td>
<td>Femur</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21/M/8</td>
<td>Skull</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22/M/12</td>
<td>Ischium</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23/M/12</td>
<td>Orbital mass</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24/F/13</td>
<td>Thyroid (also skin, lymph node)</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>25/M/15</td>
<td>Skull</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>26/M/17</td>
<td>Bone and soft tissue, skull</td>
<td>+</td>
<td>+</td>
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<td>Soft tissue, skull</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>28/M/22</td>
<td>Extradural cranial tumor (disseminated disease)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>29/M/22</td>
<td>Soft tissue, scalp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>30/M/23</td>
<td>Femur</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>31/M/25</td>
<td>Lymph node, cervical</td>
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<td>+</td>
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<td>32/F/26</td>
<td>Rib</td>
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<td>Rib</td>
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</tr>
<tr>
<td>34/M/54</td>
<td>Rib and soft tissue</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

+, positive.

* Majority of cells immunoreactive, with variable staining intensity.

† Weak immunoreactivity.
membrane accentuation with weak cytoplasmic reactivity, frequently with focal localized Golgi-type cytoplasmic staining (Image 2). Studies for S-100 protein demonstrated diffuse nuclear and cytoplasmic staining (Image 3). Lesional cells reactive for fascin exhibited uniform cytoplasmic staining, generally of varying intensity within the cell population (Image 4). In some cases, particularly those associated with disseminated disease, the staining pattern was strong and appeared more uniform within the lesional cell population (Image 5). Eosinophils, neutrophils, lymphoid cells, and histiocytes were nonreactive. Endothelial cells revealed variable reactivity. Osteoclasts were nonreactive or occasionally weakly reactive. Multinucleated cells, which appeared cytologically related to the mononuclear lesional cells based on nuclear features, were identified in 5 cases and were positive for fascin (Image 4). In some cases, interstitial spindle/dendritic cells were reactive for fascin. In an involved lymph node, dendritic cells in residual follicular centers were positive for fascin. In occasional lesions, strongly staining cells with dendritic features, morphologically consistent with interdigitating reticulum cells, were noted. Thyroid epithelial cells, pulmonary macrophages, and skeletal muscle were nonreactive. In some cases, nuclei of proliferating bone revealed weak staining. Control sections, substituting mouse IgG1 immunoglobulin for the antibody to fascin, were negative.

Immunohistochemical studies also were performed on cryostat sections (3 cases) and paraffin sections (5 cases) of nonneoplastic skin and revealed reactivity of epidermal LCs for CD1a and S-100 protein. However, these cells appeared nonreactive for fascin, with only a rare weakly positive dendritic cell noted. Weak ill-defined epidermal staining was observed, occasionally with accentuation of basal epidermis, and some skin adnexal structures were positive for fascin. Additional studies of paraffin sections of skin from patients...
with nonspecific inflammatory skin disorders (4 cases) or lymphoproliferative disorders of the T-cell (4 cases) or B-cell (2 cases) type revealed only rare fascin-positive epidermal cells with dendritic morphologic features and occasional dermal dendritic cells. In 1 case of mycosis fungoides, frequent fascin-positive dermal dendritic cells were noted.

Discussion

**Langerhans cell histiocytosis** is the term adopted by the Histiocyte Society in 1987 to define the disorder characterized by the proliferation of cells similar to LCs of the epidermis. A definitive diagnosis requires the presence of Birbeck granules or detection of CD1a glycoprotein for the lesional cells. Although initially thought to be related to the neural system, LCs are now known to express MHC class II molecules and represent one of the most potent antigen-presenting cells in the body. These cells are part of the dendritic cell system, with the important function of immune surveillance, ie, for the detection of foreign antigens entering the body through the skin.

LCs are freely mobile with the ability to migrate from the epidermis to regional nodes via efferent lymphatics. Migration is an integral part of the function of LCs. Epidermal LCs and the lesional cells of LCH exhibit similarities as well as differences. Both express high levels of MHC class II molecules and the CD1 complex, and both are positive for S-100. The proto-oncogene *bcl-2* is reportedly not observed in normal epidermal LCs but is up-regulated in LCH. However, *bcl-2* gene rearrangement is not observed. Freshly isolated epidermal LCs express Fc receptors and CD1a and contain Birbeck granules, features that are lost after culture, or after migration of these cells. Interestingly, freshly isolated epidermal LCs are not active antigen-presenting cells for the mixed lymphocyte reaction. However, activity develops after 1 to 3 days in culture during which time other morphologic and phenotypic changes also occur, including those noted. Ultimately, cultured LCs fully resemble lymphoid dendritic cells, suggesting that epidermal LCs represent immature precursors of lymphoid dendritic cells in lymph nodes.

In LCH, lesional cells reveal little or no function in alloantigen presentation. The possibility has been raised that lesional cells of LCH represent LCs arrested at an early stage of activation. The heterogeneity of nodal dendritic cells, which are believed to derive from epidermal LCs, as defined by a variety of markers (CD1a, CD83, CD86, Birbeck granules) and their phenotypic changes related to culture also have been described. In the latter study, 3 subsets of cells believed to be derived from LCs were defined. Individual round to ovoid cells in lymph node sinuses and contiguous parenchyma (CD1a+, CD83– or dim, CD86–).
CD86– or dim, Birbeck granule–) apparently represent these cells initially following migration. The other 2 subsets include interdigitating reticulum cells (CD1a–, CD83+, CD86+, Birbeck granule–) in normal T-cell zones and activated interdigitating reticulum cells (CD1a+ bright, CD83+, CD86+) in hyperplastic T zones. It could be shown that with culture, the first subset of cells became larger and evolved to typical interdigitating reticulum cells (CD1a dim, CD83+, CD86+), which formed large complexes with many T cells and exhibited characteristic ultrastructural features. However, culture of the first subset in the presence of granulocyte-macrophage colony-stimulating factor produced very large dendritic cells with features consistent with stimulated interdigitating reticulum cells. These findings suggest that the latter 2 subsets of nodal dendritic cells are derived from the first subset. The phenotypic changes observed for LCs, nodal dendritic cells, and lesional cells of LCH are summarized in Table 2.

Fascin is a 55-kd monomeric globular actin-bundling protein, initially isolated from HeLa cells, which makes F-actin aggregate side by side into bundles.17 Globular actin-bundling proteins are responsible for the formation of granulocyte-macrophage colony-stimulating factor produced very large dendritic cells with features consistent with stimulated interdigitating reticulum cells. These findings suggest that the latter 2 subsets of nodal dendritic cells are derived from the first subset. The phenotypic changes observed for LCs, nodal dendritic cells, and lesional cells of LCH are summarized in Table 2.

**Image 5** Langerhans cell histiocytosis (LCH), lymph node in a 4-year-old girl. A, In this case of disseminated LCH, nearly all cells exhibit strong immunoreactivity for fascin characterized by diffuse cytoplasmic staining (immunoalkaline phosphatase [alkaline phosphatase anti–alkaline phosphatase, APAAP] technique, hematoxylin counterstain, ×400). B, Higher magnification highlights the characteristic nuclear grooves and reniform appearance of the lesional cells and the strong cytoplasmic staining pattern (immunoalkaline phosphatase [APAAP] technique, hematoxylin counterstain, ×1,000).

**Table 2**
Comparison of Phenotypic Markers of Epidermal, Cultured, and Activated Langerhans Cells, Langerhans Cell Histiocytosis, and Nodal Dendritic Cells

<table>
<thead>
<tr>
<th>Marker</th>
<th>Epidermal Langerhans Cells</th>
<th>Cultured or Activated Langerhans Cells</th>
<th>Nodal Interdigitating Cells</th>
<th>Langerhans Cell Histiocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>+</td>
<td>+</td>
<td>Variable†</td>
<td>+</td>
</tr>
<tr>
<td>S-100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fascin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Birbeck granules</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major histocompatibility class II</td>
<td>–</td>
<td>+</td>
<td>No data</td>
<td>+</td>
</tr>
<tr>
<td>Fc receptor (IgG)</td>
<td>+</td>
<td>+</td>
<td>No data</td>
<td>+</td>
</tr>
<tr>
<td>Alloantigen presentation</td>
<td>+</td>
<td>+</td>
<td>No data</td>
<td>Weak/–</td>
</tr>
<tr>
<td>bcl-2</td>
<td>–</td>
<td>No data</td>
<td>No data</td>
<td>+</td>
</tr>
</tbody>
</table>

+, positive; –, negative; ±, lost during activation or culture.
† Excludes follicular dendritic cells.
‡ Several subsets of dendritic cells (see text).
§ In culture, increased expression with time (see text).
++ Marked increase in antigen-presenting function.
microfilament bundles in microspikes. Certain microspike structures are extremely mobile, a feature that permits the lamellipodia of cells to extend and retract. Dynamic cell changes of this type are integral to the function of cells of the dendritic system, which are involved in homing, migration, and antigen presentation.

A previous immunohistochemical study of various lymphoid tissues including lymph node, thymus, and spleen, as well as peripheral blood mononuclear cells, demonstrated that reactivity for fascin was highly restricted to cells of the dendritic system in these tissues. Although the intensity of staining varied, reactivity was observed for peripheral blood dendritic cells, thymic medullary dendritic cells, nodal interdigitating reticulum cells, follicular dendritic cells, nodal sinus lining cells, mononuclear cells (probably veiled cells) in afferent lymphatics, and dendritic cells of splenic white pulp (particularly periarteriolar and marginal zones). Endothelial cells, including lining cells of splenic sinuses, also were positive for fascin. Fascin-positive cells with dendritic morphologic features also were identified in the bone marrow (G.S. Pinkus, J.L. Pinkus, unpublished observations). Our studies of normal skin failed to demonstrate reactivity for LCs in paraffin or cryostat sections, although occasional fascin-positive dendritic cells were identified in the epidermis or dermis of biopsy specimens of patients with mycosis fungoides.

Concordant with our tissue section results for epidermal LCs, studies of LCs isolated from fresh human epidermis have demonstrated that following initial isolation, LCs are not immunoreactive for fascin (Erik Langhoff, MD, Milton S. Hershey Medical Center, Hershey, PA, unpublished data). However, after culture, reactivity for fascin is demonstrated readily, with a progressive increase in the number of fascin-positive cells as a function of time in culture (30% positive after 1 day, 55% at 2 days, and 83% at 3 days). This change in phenotype after culture is analogous to other phenotypic changes observed for epidermal LCs, suggesting that normal LCs are negative for fascin, while activated LCs and, based on the results of the present study, those of LCH are positive for fascin. In further agreement with these observations, analysis of differential gene expression in maturing LCs demonstrated that fascin is expressed differentially in these cells. In the latter study, competitive reverse transcriptase–polymerase chain reaction studies confirmed that fascin is expressed highly in cultivated epidermal LCs, but no signals were observed in freshly isolated LCs. Differential expression of fascin in LCs correlated with the formation of numerous dendritic processes. The formation of these processes could be inhibited by incubation of LCs with fascin antisense oligonucleotides during culture. Other studies also have demonstrated that fascin expression correlates with dendritic morphologic features and cell differentiation and, ultimately, with the antigen-presenting activity of dendritic cells.

Our cases of LCH exhibited typical clinical findings and characteristic histologic features, and all were immunoreactive for the definitive marker CD1a and for S-100 protein (Table 1; Images 1-3). Immunoreactivity for fascin also was observed in all cases (Images 4 and 5). However, variation in staining intensity was observed for lesional cells of LCH, a feature that may relate to true differences in fascin expression, possibly related to stage of cell maturation or activation, or, perhaps, to some extent, also to technical considerations, particularly for decalcified specimens. Optimal reactivity required heat-induced epitope retrieval. Jaffe et al. evaluated 12 cases of LCH and observed fascin reactivity only for rare lesional cells. In their study, however, antigen-retrieval techniques were not used, one factor that may contribute to these discrepant results. Also, the 5-step immunohistochemical technique (APAAP) used in our study may permit greater sensitivity for the detection of immunoreactive cells compared with the 3-step method used in the latter report. In the study by Jaffe et al., fascin reactivity was observed for lesional cells of Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy), a finding we also have observed (unpublished observations). This finding is not surprising since lymph node sinus lining cells are positive for fascin and are believed to have dendritic features. The cells of Rosai-Dorfman disease seem to have hybrid dendritic-macrophage characteristics (S-100 protein, macrophage enzymes).

Fascin expression represents another example of a phenotypic overlap between LCH and Rosai-Dorfman disease. Jaffe et al. also noted that the lesional cells of juvenile xanthogranuloma of skin and deep lesions that appear to have features of dendritic cells rather than macrophages are strongly reactive for fascin, as well as for factor XIIIa. Reactivity for fascin also has been described for lesional cells of interdigitating dendritic cell sarcomas and extra-nodal follicular dendritic cell sarcomas.

Immunoreactivity for fascin in lesional cells of LCH supports their pathogenesis from cells of the dendritic system and provides another marker that may be detected for these unique cells in paraffin sections. The lack of reactivity of epidermal LCs for this protein may reflect yet another instance of a phenotypic difference observed between freshly isolated LCs and those in situ in the epidermis, as compared with LCs following culture, migration, maturation, or differentiation or, in cases of LCH, following apparent clonal expansion. It should be noted, however, that staining intensity for fascin in cases of LCH may vary. While fascin expression has been shown to correlate with dendritic morphologic features, cell differentiation,
and antigen-presenting activity of normal dendritic cells,27-29
in LCH, although lesional cells are positive for fascin, they
are functionally defective in antigen presentation.29 Fascin
expression, however, may contribute to the ability of these
cells to migrate. The staining profile for fascin includes
dendritic cells of various types as previously described,15 as
well as endothelial cells, some epithelial cells,35,36 and Reed-
Sternberg cells of Hodgkin disease.15 Fascin reactivity repres-
ents part of the staining profile of LCH and provides insight
into the pathogenesis of this disorder; however, this actin-
bundling protein is not a feature of normal epidermal LCs
and lacks the specificity of CD1a as a marker for the lesional
cells of LCH.

NOTE: Since the completion of this study, we have had
the opportunity to evaluate skin biopsy specimens from 4
pediatric patients with cutaneous LCH. In all cases, lesional
cells were strongly immunoreactive for CD1a, S-100 protein,
and fascin, an immunohistochemical profile similar to our
noncutaneous cases.

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