Differentiation of Langerhans cells in Langerhans cell histiocytosis

Frederic Geissmann, Yves Lepelletier, Sylvie Fraitag, Jenny Valladeau, Christine Bodemer, Marianne Debre, Michelle Leborgne, Sem Saeland, and Nicole Brousse

Langerhans cell histiocytosis (LCH) consists of lesions composed of cells with a dendritic Langerhans cell (LC) phenotype. The clinical course of LCH ranges from spontaneous resolution to a chronic and sometimes lethal disease. We studied 25 patients with various clinical forms of the disease. In bone and chronic lesions, LCH cells had immature phenotype and function. They coexpressed LC antigens CD1a and Langerin together with monocyte antigens CD68 and CD14. Class II antigens were intracellular and LCH cells almost never expressed CD83 or CD86 or dendritic cell (DC)–Lamp, despite their CD40 expression. Consistently, LCH cells sorted from bone lesions (eosinophilic granuloma) poorly stimulated allogeneic T-cell proliferation in vitro. Strikingly, however, in vitro treatment with CD40L induced the expression of membrane class II and CD86 and strongly increased LCH cell allostimulatory activity to a level similar to that of mature DCs. Numerous interleukin-10–positive (IL-10+) Langerin+, and CD68+ macrophages were found within bone and lymph node lesions. In patients with self-healing and/or isolated cutaneous disease, LCH cells had a more mature phenotype. LCH cells were frequently CD14+ and CD86+, and macrophages were rare or absent, as were IL-10–expressing cells. We conclude that LCH cells in the bone and/or chronic forms of the disease accumulate within the tissues in an immature state and that most probably result from extrinsic signals and may be induced to differentiate toward mature DCs after CD40 triggering. Drugs that enhance the in vivo maturation of these immature DCs, or that induce their death, may be of therapeutic benefit.

© 2001 by The American Society of Hematology

Introduction

Langerhans cell histiocytosis (LCH) affects mainly young children and features accumulation of CD1a+ Birbeck granule+ cells within the epidermis and dermis, the bones, and occasionally lymphoid organs, lungs, and digestive tract. A frequent clinical feature is a skin eruption in the first months or days after birth. It may spontaneously resolve (Hashimoto-Pritzker syndrome) or be part of a widespread disease (Letterer-Siwe syndrome). In the older child, chronic/granulomatous forms are more frequent (eosinophilic granuloma, Hand-Schuller-Christian disease). Eosinophilic granuloma, found in 50% to 80% of all patients with LCH, consists of chronic lytic bone lesions that may be unifocal or multifocal and may associate with the involvement of smooth tissue. It may be difficult, however, to distinguish between an active and an inactive bone lesion without serial biopsies, which are not performed in most cases for obvious ethical reasons. Treatment of severe or chronic disease, relying on cytotoxic chemotherapy, continues to be controversial and, in many cases, ineffectual. Although LCH has been proposed to be a clonal disorder, its cause remains unknown, despite an extensive search for evidence of consistent cytogenetic abnormalities or gene rearrangements. Whether LCH is reactive or neoplastic is even debated, and several features provide seemingly contradictory evidence on this point (spontaneous resolution of disease on the one hand and clonality of lesional LCH on the other). Similarly, the pathogenesis of the disease is enigmatic, although the altered expression of cytokines and cellular adhesion molecules, important for migration and homing of the normal Langerhans cell (LC), may play an important role. It has been suggested that LCH cells may be in an arrested state of activation and/or differentiation of LCs. Apparently, contrasting studies have reported that LCH cells may be activated, based on phenotypic data, whereas others have failed to detect alloantigen-presenting activity. Although no immune defect has been identified in affected children, some T lymphocyte phenotype abnormalities that suggest alterations in antigen-driven activation processes have been reported.

Recent progress in the field of dendritic cell (DC) biology has led us to revisit the phenotype and function of LCH cells in an attempt to better understand the pathophysiology of this disease and to explain why an accumulation of antigen-presenting cells may develop in the apparent absence of an efficient immune response. We first investigated the differentiation stages of LCH cells in the distinct clinical forms of the disease by combining phenotypic and functional studies. We concluded that LCH cells are functionally immature DCs in the chronic form of the disease. We then investigated whether immature LCH cells may be induced to become mature DCs, and we found that CD40 triggering induced their differentiation as efficiently as for normal DCs. To investigate why LCH cells remain immature in vivo, we examined their...
microenvironment. We found that non-Langerhans cells (macrophages) within bone and chronic lesions produced interleukin-10 (IL-10) in vivo. On the contrary, we have observed that LCH cells in isolated or healing skin lesions have a more mature phenotype and that IL-10–producing cells were absent from these lesions. The presence of IL-10 may contribute to the immaturity of LCH cells in bone/chronic lesions. The results of our study therefore indicate that LCH cells, in the bone/chronic form of the disease, are maintained immature in vivo, most probably by extrinsic signals. These data shed some light on the pathogenesis of LCH and may be useful for designing therapeutic strategies in this disease.

Patients, materials, and methods

Patients

Biopsy samples from 25 patients, referred to Necker Enfants Malades Hospital and diagnosed as having Langerhans cell histiocytosis, on the basis of their clinical history and the expression of CD1a by histiocytic cells, were examined by immunohistochemistry and confocal microscopy in this study. For 6 patients (numbers 7459, 10331, 10337, 9980, 9387, 564), fresh tissues were available at the time of frozen examination and were studied by flow cytometry and/or functional assays. Patients were evaluated as having various clinical forms of the disease (self-healing cutaneous histiocytosis [CCH], eosinophilic granuloma [EG], Hashimoto-Pritzker disease [HP], and multisystem disease [MS]). Twenty-nine biopsy samples from various anatomic sites were examined (Table 1).

Table 1. In situ immunophenotype of Langerhans cell histiocytosis cells in 25 patients

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age/sex</th>
<th>Clinical stage</th>
<th>Biopsy site</th>
<th>Langerin</th>
<th>CD14</th>
<th>CD68</th>
<th>CD40</th>
<th>CD80</th>
<th>CD86</th>
</tr>
</thead>
<tbody>
<tr>
<td>7459</td>
<td>7 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>3774</td>
<td>2 y/F</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>10391</td>
<td>3 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>9980</td>
<td>11 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>10337</td>
<td>6 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>2558</td>
<td>8 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>7553</td>
<td>6 y/F</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>3389</td>
<td>10 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5259</td>
<td>3 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>4188</td>
<td>8 y/M</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5149</td>
<td>6 y/F</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>9387</td>
<td>8 y/F</td>
<td>EG</td>
<td>Bone</td>
<td>++</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>6423</td>
<td>1 mo/M</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>3807</td>
<td>15 d/F</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5828</td>
<td>7 d/F</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5464</td>
<td>3 d/M</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>3771</td>
<td>4 mo/F</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>7333</td>
<td>7 mo/F</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>7089</td>
<td>1 mo/M</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>±</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>4968</td>
<td>8 mo/M</td>
<td>Skin (HP)</td>
<td>Skin</td>
<td>++</td>
<td>±</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>6193</td>
<td>1 y/M</td>
<td>MS</td>
<td>Skin</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>6146</td>
<td>1 y/M</td>
<td>MS</td>
<td>Skin</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>6277</td>
<td>3 mo/M</td>
<td>MS</td>
<td>Skin</td>
<td>++</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>8718</td>
<td>2 mo/F</td>
<td>MS</td>
<td>Skin</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>564</td>
<td>2 mo/F</td>
<td>MS</td>
<td>Skin</td>
<td>++</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

LCH cells in biopsy samples were positive for CD1a in all 25 patients. Staining with Langerin, CD14, CD68, CD40, CD80, CD86, and CD83 was evaluated on serial sections. LCH indicates Langerhans cell histiocytosis; EG, eosinophilic granuloma; HP, Hashimoto-Pritzker disease; MS, multisystem disease; LN, lymph node. Most (+ +) denotes positivity of most (more than 75%) of cells; numerous (+ +), 25% to 75%; rare (±), less than 25%; negative (−−), less than 5%; nd, not available.

Cells

Only samples from eosinophilic granuloma met the conditions for separation of LCH cells (sufficient numbers of lesional cells and availability of fresh tissue at the time of diagnosis). After frozen section examination, sterile tissue from eosinophilic granuloma was harvested in RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% heat-inactivated fetal calf serum (FCS) myocyte (all from GIBCO BRL, Gaithersburg, MD), referred to below as complete medium. Tissues were immediately gently dissociated through a nylon mesh. The cell suspension was washed 3 times and incubated with human IgG to block Fc receptor, and anti-CD1a antibody (BL6; Immunotech, Marseille, France). The cells were washed twice, incubated with antismme microbeads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) for 15 minutes at 4°C. Cells were washed again, and then CD1a+ and CD1a- LCH cells were separated by positive immunomagnetic selection by using a magnetic cell separator (MACS) according to the manufacturer’s instructions. Between two 106 and six 105 CD1a+ cells were recovered from each sample. Purity of CD1a+ and CD1a- sorted fraction was 80% or greater and mortality 10% or less.

LC-type dendritic cells were prepared as previously described. Briefly, fresh CD14+ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) of healthy volunteers obtained by the standard Ficoll-Hypaque method and immediately separated by negative magnetic depletion by using hapten-conjugated CD3, CD7, CD19, CD45RA, CD56, and anti-IgE antibodies (MACS; Miltenyi Biotec) and a MACS according to the manufacturer’s instructions, routinely resulting in more than 95% purity of the CD14+ cells. Cells were cultured in flasks or in 6- or 24-well tissue culture plates (Costar Corp, Cambridge, MA) for 5 to 7 days in complete medium supplemented with 100 ng/mL.

From www.bloodjournal.org by guest on October 31, 2017. For personal use only.
granulocyte-macrophage colony-stimulating factor (GM-CSF), 10 ng/mL IL-4, and 10 ng/mL transforming growth factor beta 1 (TGF-β1).

Murine fibroblast cell lines transfected with human CD40L (LeCD40L) or CD32 (LeCD32) were kindly provided by Dr J. Banchereau and Dr F. Brière (Schering-Plough, Dardilly, France). T cells were isolated from the PBMCs of healthy volunteers by the standard Ficoll-Hypaque method, followed by magnetic depletion of non-T cells (MACS; Miltenyi Biotec).

**Antibodies**

Uncoupled antibody to CD1a (clone BL6) used for cell separation and immunocytochemistry on tissue sections was purchased from Immunotech (Marseille, France). Fluorescein isothiocyanate (FITC)–conjugated anti-CD1a (clone VVM-35) used for flow cytometry was purchased from TEBU (Marseille, France). Fluorescein isothiocyanate (FITC)–conjugated anti-CD14, CD86, and FITC-conjugated HLA-DR were obtained from Immunotech. Antibodies to CD40, CD80, CD83, or DC-Lamp, or CD86 mouse primary antibodies at the appropriate concentration as determined by titration, and then labeled with a goat antirat alkaline phosphatase-conjugated antibody. Fast red (Sigma, St Louis, MO) was used as a substrate for staining of numerous (25%-75%) LCH cells; scored as follows: ++ staining of most (more than 75%) LCH cells; + staining of numerous (25%-75%) LCH cells; ± staining of few LCH cells (less than 25%); and − absence of staining of LCH cells (less than 5%).

**Flow cytometry**

For 2-color flow cytometry, 5 × 10⁴ to 1 × 10⁵ cells were incubated in 96-well plates (Becton Dickinson) for 15 minutes at 4°C in phosphate-buffered saline (PBS), 2% human AB serum, and 0.01 M NaN₃, with FITC-conjugated monoclonal antibodies (mAbs) at the appropriate concentration, or with control isotype-matched irrelevant mAbs at the same concentration (Becton Dickinson). After washing, 10⁴ events were analyzed with a FACScanLibur (Becton Dickinson) using CellQuest software (Becton Dickinson).

**Confocal microscopy**

Cells were washed in Ca²⁺/Mg²⁺-free PBS and centrifuged onto glass slides by using Cytospins (Shandon, Pittsburgh, PA), dried for 1 hour at room temperature, fixed in acetone for 10 minutes, and stored at −20°C. Frozen tissue section (5 μM thick) were also fixed in acetone for 10 minutes and stored at −20°C. For staining, slides were rehydrated for 5 minutes in PBS with 2% pooled normal human serum AB (staining medium), and then incubated for 1 hour at room temperature with mouse antihuman Langerin, followed by goat antirat Cy3 or FITC-conjugated mAb to Langerin, FITC-conjugated mAb to HLA-DR, PE-conjugated mAb to CD1a, CD14, CD86, and CD68, biotinylated rat antianimal IL-10 and streptavidin Cy3 and Cy5 (Jackson Laboratories, Bar Harbor, ME). Slides were mounted with Fluoprep (Biomerieux SA, Marcy l’Etoile, France) and analyzed with a confocal laser microscope system attached to a microscope.

**Allogeneic lymphocyte proliferation**

Patient CD1a⁺ cells and LC-type DCs were reexpanded in 24-well tissue culture plates at a concentration of 5.10⁵ cell/mL in complete medium supplemented with 100 ng/mL GM-CSF and 10 ng/mL IL-4 for stimulation. Fibroblastic L-cells transfected with either CD40L, or CD32 as control, were irradiated at 80 Gy and added to the culture wells at a proportion of 25%. Cells were collected after 40 hours of stimulation, washed 3 times in PBS, resuspended in RPMI with 10% human AB serum, and added in triplicate at various concentrations to 10⁵ autologous T cells per well in 96-well tissue culture plates (Falcon, Amersham, Freiburg, Germany). [³H]Thymidine (Amersham Life Science, Buckinghamshire, United Kingdom) incorporation was measured in newly synthesized DNA over 18 hours, by using plates initiated at day 5 of the culture with 0.037 MBq (1 μCi) per well of [³H]thymidine. Cells were then harvested with a 96-well Harvester (Pharmacia, St. Quentin, France), collected on glass-fiber filter (Pharmacia), and the incorporation of thymidine was measured with a beta-plate microscintillation counter (LKB, Pharmacia).

**Results**

**Patients**

As shown in Table 1, 12 patients presented with bone disease (eosinophilic granuloma), either unifocal or multifocal, 8 patients presented with LCH restricted to the skin, and among them 4 had self-healing cutaneous histiocytosis (Hashimoto-Pritzker syndrome) diagnosed. Five patients presented with multifocal LCH involving more than 2 organs.

In patients with skin LCH, lesional cells constituted an homogeneous dermoepidermal infiltrate of CD1a⁺ cells (Figure 1) also characterized by their round shape, admixed with various amounts of small lymphocytes. A few polymorphonuclear eosinophils were present in some patients’ biopsy specimens, and CD1a⁻ macrophages were absent or very rare in all cases. In the bone biopsy specimens (eosinophilic granuloma), round-shaped CD1a⁺ LCH cells were admixed with relatively numerous CD68⁺ CD1a⁻ macrophages (Figures 1, 4), occasional multinucleated giant cells, eosinophilic polymorphonuclear cells, and scattered lymphocytes. Lymph node biopsy specimens revealed the presence of CD1a⁺ cells within the sinuses and the T-cell areas, and the presence of CD1a⁻, CD68⁻ macrophages. These features fitted with previously described characteristics of cutaneous, bone, and lymph node lesions of LCH.²⁻⁴

**Results**

**Langerhans cell histiocytosis cells express Langerin, CD14, and CD68**

In all 25 patients, more than 75% CD1a⁺ positive cells stained for Langerin in serial sections (Figure 1; Table 1). Confocal microscopy study on eosinophilic granuloma samples (n = 3) further demonstrated that antibodies against CD1a and Langerin labeled the same cells (Figure 2A). Langerin expression was a constant feature of CD1a⁺ LCH cells, whatever the site of the biopsy (skin, bone, or lymph node), or the stage or clinical form of the disease.

We found that CD1a⁺ cells frequently coexpressed CD14 in situ on serial sections in all patients with extracutaneous disease (Figure 1A,B; Table 1). To exclude that the CD14 positive staining may be solely due to expression of CD14 by macrophages that are present in LCH lesions, flow cytometry analysis was performed in 3 patients and confirmed the expression of various levels of CD14 by CD1a⁺ LCH cells (Figure 3A). In addition, confocal microscopy (Figure 2B) showed that besides Langerin⁻ CD14⁺ macrophages, both Langerin⁺ CD14⁻ and Langerin⁺ CD14⁺ Langerhans cells were observed. CD14 expression appeared to depend on the clinical form of the disease. Numerous CD1a⁻ cells were stained in bone lesions (eosinophilic granuloma, a chronic lesion) (11 patients) and numerous LCH cells were also CD14⁺ on serial sections from involved lymph nodes (n = 3, Figure 1B).

However, many fewer cells were stained in skin samples (Figure 1C), and CD14⁺ cells were numerous in only 3 of 7 tested patients
with pure cutaneous disease (Table 1). In addition, in all patients, most CD1a
1
Langerin
1
cells coexpressed CD68, although at a lower level than do macrophages (Table 1; Figure 1; also Figure 5B). This is in accordance with previous studies. CD68 is a lysosomal antigen expressed (at high levels) in monocyte/macrophages, and (at low levels) in immature skin LCs, and down-regulated on maturation. In skin lesions, CD68, CD1a, and Langerin staining patterns were very similar on serial sections. In bone and lymph node lesions, similarly to what was observed for CD14 staining, some CD1a
2
Langerin
2
cells were CD68
1
, indicating the presence of macrophages admixed with Langerhans cells (Figure 5B). Altogether, these data suggested that LCH cells have features of immature LCs, and we therefore investigated the expression of costimulatory molecules and the cellular localization of major histocompatibility complex (MHC) class II molecules in these cells.

Major histocompatibility complex class II and costimulatory molecules

Although CD80 was frequently detected (Table 1), CD86 (B7-2) expression was undetectable on most LCH cells in the majority of bone lesions (10 of 11), in 2 of 3 cases of lymphadenopathy, and in skin lesions from patients with multisystem disease (Table 1, Figure 1, Figure 3A). CD83, a marker of mature DCs, was expressed only by scattered cells in all these samples, except for one case of lymphadenopathy (Table 1, Figure 1). In accordance, DC-Lamp, another molecule selectively expressed by mature DCs, was only expressed by scattered cells (Figure 2C). Moreover, although it has been reported that LCH cells express MHC class II molecules,27,28 we showed by confocal microscopy on sorted LCH cells from bone lesions that most class II resides within intracellular vesicular compartments (Figure 3B), as observed in immature LCs. In contrast, among patients with pure cutaneous disease, including patients with Hashimoto-Pritzker syndrome, CD86 was expressed by the majority of LCH cells in skin lesions of all tested patients (6 of 6) (Table 1, Figure 1C) and CD83 was expressed by a majority of cells in 2 of 7. Moreover, DC-Lamp was also expressed by the majority of cells in 3 of 3 patients tested (Figure 2D). This confirms that the phenotype of LCH cells differs between cases of isolated skin involvement and bone/disseminated diseases, being more immature in the latter. This suggests that LCH cells, although most frequently immature, may become mature in some circumstances. This may possibly occur through CD40/CD40L interaction, because in all patients, LCH cells expressed CD40 at an even higher level than did normal epidermal Langerhans cells (Table 1, Figure 4A). We therefore investigated the functional properties of LCH cells.

LCH cells from bone lesions are functionally immature, but can mature after CD40 triggering

Sorted CD1a
1
cells from the bone lesion (eosinophilic granuloma) of 3 patients (7459, 10391, 3774) were studied for their ability to
In situ, cells from these patients were CD1a+, Langerin+, CD40+, CD14+, CD68-. CD80 cells were rare (10391) or numerous (3774, 7459), and CD86 and CD83 cells were rare in all patients. Sorted cells exhibited the same phenotype (Figure 4E; data not shown). In patient 7459 (Figure 4B), the CD1a+ and CD1a− fractions were purified as described in the “Materials and methods” section, and cocultured with sorted allogeneic T lymphocytes. As a control, immature DCs (Langerhans cell type) and CD40L-treated mature DCs, generated as described, were also cultured in the same experiment with allogeneic T lymphocytes from the same donor. Patients’ CD1a+ cells and control cultured immature DCs had comparable effects on T lymphocytes and failed to induce significant thymidine incorporation at a 1% stimulator/effector ratio. This is clearly different from the vigorous T-cell proliferation induced by mature DCs (Figure 4B). In further experiments (Figure 4C,D, patients 10391 and 3774, respectively), sorted CD1a+ LCH cells and control immature DCs were cultured either with CD40L or with CD32 transfected fibroblasts for 2 days before being added to allogeneic lymphocytes. Strikingly, although both LCH cells and control immature DCs cultured with CD32 transfected cells equally (poorly) stimulated lymphocyte proliferation, both LCH cells and control immature DCs stimulated via CD40 showed a strong increase in their capacity to stimulate lymphocytes at low stimulator/effector ratio. Confocal microscopy examination of patients’ CD1a+ cells, either fresh or cultured with LcCD32 stimulate T-cell proliferation in a similar manner to that of control immature monocyte-derived DCs, whereas LCH CD1a+ cells cultured with LcCD40L stimulate T-cell proliferation in a similar manner to that of control mature monocyte-derived DCs cultured with LcCD40L. (D) The same experiment was performed with fresh tissue from eosinophilic granuloma from patient 3774. (E) Sorted CD1a+ cells from eosinophilic granuloma from patient 10391 cultured with LcCD32 or with LcCD40L for 2 days were also analyzed by confocal microscopy for expression of HLA-DR (green) and CD86 (red). Note that green DR staining increased and is present at the cell surface, in cells cultured with LcCD40L, and that red CD86 staining, absent on cells cultured with LcCD32, is induced on cells cultured with LcCD40L.
Discussion

This study aimed to define phenotypic and functional characteristics of LCH cells that may account for the pathogenesis of the disease, and in particular, their ability to induce an immune response. We have studied the in situ phenotype of LCH cells in a large series of patients, relative to the low incidence, that represents the various clinical courses of the disease. However, flow cytometry analysis and functional studies could be performed only in a smaller number of patients, due to the difficulty to obtain fresh lesional tissue at the time of diagnosis. Despite the widely accepted use of CD1a antibodies to confirm the diagnosis of LCH, CD1a expression is not restricted to Langerhans cells, and accurate diagnosis of Langerhans cell histiocytosis, required in such a study, may be questionable in the absence of electron microscopy. The results presented here are reinforced by the 100% concordance observed between positivity of Langerin and of CD1a stainings in the 25 patients who had various clinical forms and stages of LCH diagnosed, at various biopsy sites. Assuming that Langerin is a specific LC marker associated with Birbeck granules at the ultrastructural level, this indicates that all studied patients indeed presented with “Langerhans cell” histiocytosis. Our findings also establish Langerin as a useful marker for diagnosis of LCH.

It appears clearly from our results that LCH cells express CD14 at least in bone and lymph node involvement. CD14 expression may depend on the clinical form of the disease because fewer cells are stained in skin lesions. Earlier studies have reported that LCH cells may, or may not, express CD14. Such a phenotype, CD1a+, Langerin+, CD14+, is unusual because normal Langerhans cells do not, or very poorly, express CD14. It is, however, reminiscent of the phenotype of recent immigrant Langerhans cells within the epidermis after a bone marrow graft as described by Murphy et al. It is noteworthy that (i) during monocyte to DC differentiation in vitro, CD14 and CD68 are down-regulated, whereas CD1a is induced, due to the effect of IL-4 (or IL-13) and TGFβ1, and that (ii) Langerhans cells, markedly diminished in patient skin after allogeneic bone marrow transplantation, are replenished with epidermal dendritic cells exhibiting coexpression of monocyte/macrophage and Langerhans cell surface antigens during the first 4 weeks after this intervention. It is thus conceivable that the expression of CD14 by LCH cells reflects their immature stage of differentiation, and therefore their CD14+ origin, although it can be also argued that CD14 expression might have been acquired by lesional cells, or delineate distinct cellular origin or differentiation pathways. However, the heterogeneous level of CD14 expression by CD1a+ cells present in one single lesion (Figures 2B, 3A) argues against the latter hypothesis.

The major finding in this study is that LCH cells are in an immature stage of differentiation in the bone/chronic forms of the disease, but are able to trigger an immune response if they receive a maturation signal such as CD40L in vitro. Although only 3 patients could be studied in functional assay, the results were clear and

Macrophages produce IL-10 in eosinophilic granuloma lesions and involved lymph nodes

The above results suggested that the immature phenotype of LCH cells in bone lesions did not result from an intrinsic maturation blockade. We investigated whether inhibitory signals may be found in the vicinity of LCH cells within lesions. With the use of confocal microscopy examination of patients’ tissue sections, no IL-10 was detected within LCH lesions from patients with localized cutaneous disease (Figure 5A, n = 3). In contrast, relatively numerous IL-10-expressing cells were detected within bone and lymph node lesions (Figure 5A, n = 4). Interestingly, the cells that expressed IL-10 were found to be very large sized, did not express CD1a or Langerin (Figure 5A,B), and were also CD3 negative (data not shown) but strongly expressed CD68 (Figure 5B), and therefore were identified as macrophages. These IL-10–expressing macrophages were found to be frequently in close contact with LCH Langerin+ cells (Figure 5A,B) and T lymphocytes (data not shown). Examination of control reactive lymph nodes did not reveal the presence of these IL-10–expressing cells (data not shown). These observations suggest that Langerin+ LCH cells and infiltrating T cells are exposed to IL-10, mainly produced by surrounding macrophages.
consistent with phenotypic studies. Both in situ and ex vivo results presented here argue against the hypothesis of an intrinsic matura-
tion blockade of LCH cells, and indicate that LCH cells can be
induced to elicit an immune response. Indeed, in the spontaneously
regressive form of the disease, LCH cells do frequently exhibit
CD86 and DC-Lamp expression in situ, suggesting that they
represent more mature DCs. Functional studies in the latter form,
however, could not be performed due to the lack of sufficient
material, and in addition would have been difficult to interpret
because of the probable contamination by normal epidermal LCs.
In contrast, CD1a+ LCH cells sorted from bone lesions do not
express membrane MHC class II or costimulatory CD86, and
poorly stimulate T cells. In vivo, and even in vitro after a 2-day
culture with fibroblasts, these cells remain in an immunologically
immature stage. Strikingly, however, CD1a+ LCH cells differenti-
ate toward mature DCs on CD40 triggering in vitro.

This is somewhat surprising, because patients with LCH do not
present with CD40L deficiency, and because, in addition to the
presence of T cells within the lesions, LCH lesions, especially
eosinophilic granuloma, abundantly express inflammatory cyto-
kines such as TNFα and IL-1β,12 that are believed to induce LC
migration and maturation. However, such cytokines may not be
sufficient stimuli to induce a functional maturation, and other
cytokines such as TGFB1 or IL-10 may prevent LC matura-
tion.13-39 In particular, IL-10 is a cytokine capable of down-
regulating the expression of B7 molecules and class II antigens by
DC/LC in vitro.38-39 The presence of active TGFB1 within LCH
lesions is likely, because Langerhans cell differentiation requires
TGFB1. However, latent TGFB1 is abundantly produced by many
cell types, and it is not possible to quantitate the presence of active
TGFB1 within tissues. We were able to detect IL-10 in the vicinity
of LCH cells in bone and lymph node lesions by confocal
microscopy. Interestingly, IL-10–expressing cells in eosinophilic
granuloma were most predominantly large-sized CD3−, Langerin−,
CD68− cells, and therefore were neither LCH cells themselves nor
T cells, but macrophages. In contrast, within skin lesions from
patients with limited or self-healing disease, macrophages are very
rare, and there are consistently no IL-10− cells. These results are
consistent with those of 2 recent studies that investigated the
presence of numerous cytokines within LCH lesions by immunohis-
tochemistry. In one study, IL-10 was detected in the vicinity of
LCH cells in 9 of 11 biopsy specimens studied (all from eosino-
philic granuloma or lymph nodes).14 In contrast, in the other study
IL-10 was not detected in 5 of 5 LCH lesions restricted to the lung
in adults patients who expressed CD86.17 It is possible that
pulmonary LCH of the adult, which is a clinically distinct disease,
may have a distinct pathophysiology. It is also possible that the
same mechanism that could be responsible for the healing of skin
lesions, ie, maturation of LCH cells, may be responsible for the
pathogenesis of lung lesions because inflammation and subsequent
fibrosis, but not tumoral involvement, are unique features of
pulmonary LCH of the adult.17

Altogether our findings in this functional study attempting to
draw a picture of the pathogenesis of LCH may account for a
maturation blockade of LCH cells due to extrinsic signals and
reconcile contrasting studies on a few cases that either reported that
LCH cells may be activated or mature on phenotypic data17-19 or
failed to detect alloantigen-presenting activity by LCH cells.20

In our study, LCH cells from bone/chronic lesions are undoubt-
edly immature Langerhans-type dendritic cells that express higher
levels of CD68 and CD14 than normal LCs, intracellular MHC
class II, are frequently negative for CD86 and DC-Lamp and have
the same allostimulatory activity as immature normal DCs. It is,
however, clear that LCH cells are not by themselves “frozen” in an
arrested state of activation/differentiation because we show that
LCH cells may become activated in vitro in response to CD40
triggering. Moreover, in some cases in vivo, especially, and
interestingly, in self-healing cutaneous lesions, a more mature
phenotype can be observed and LCH cells appear to down-regulate
CD14 and up-regulate CD86 and DC-Lamp. Although a direct role
of IL-10 cannot be demonstrated here, IL-10 produced by CD1a−
macrophages may contribute to the maintenance of LCH CD1a+
cells in an immature stage of differentiation.

LCH has been advocated to be a malignancy or a viral disease;
however, both the search for a viral cause and for molecular
abnormalities are still unsuccessful.40,41 Several viruses have
been shown to interfere with DC functions, and it is conceivable
that an inadequate response to a viral challenge may result in the
LCH features described: local recruitment of immature LCs or their
precursors, their abnormal homing, and their persistence in the
absence of efficient maturation.

Finally, our results may contribute to explain the paradox of an
“antigen presenting-cell tumor” that does not induce its own
rejection by the immune system. In bone/chronic forms, LCH cells
are maintained in an immature stage by factors from their
environment. This may open the way for new strategies in the
treatment of LCH. Whether drugs that enhance in vivo the ability of
LCH cells to become mature may lead to their killing by activated
CTL and may be beneficial to some patients should be investigated.
Alternatively, pharmacologically induced death of immature DCs
may also be considered.

Acknowledgments

We are grateful to the French Histiocytosis Study Group and to Pr
F. Jaubert for support, to Dr Aucouturier for critical reading of
the manuscript, and to Mr Y. Goureau for help with confocal
microscopy.

References

1. Nezelof C, Basset F, Rousseau MF. Histiocytosis
X: histogenetic arguments for a Langerhans cell
1994;84:2840-2853.
3. Egeler RM, D’Angio GJ. Medical progress: Lang-
erhans cell histiocytosis. J Pediatr. 1995;127:1-
11.
tract involvement in Langerhans cell histiocyt-
5. Hashimoto K, Pritzker MS. Electron microscopy
study of reticulohistiocytoma: an unusual case of
1973;170:263-270.
6. Hashimoto K, Bale GF, Hawkins HK, Langston C,
Pritzker MS. Congenital self-healing reticulohis-
tiocytosis (Hashimoto-Pritzker type). Int J Derma-
Disseminated histiocytosis X: analysis of pronos-
tic factors based on a retrospective study of 50
8. Lichtenstein L. Histiocytosis X: integration of eo-
sinophilic granuloma of bone, “Letterer-Siwe dis-
 ease” and “Schuller-Christian disease” as related
manifestation of a single nosologic entity. Arch
Pathol. 1953;56:84-102.
ticentre retrospective survey of Langerhans’ cell
histiocytosis: 348 cases observed between 1983
and 1993. The French Langerhans’ cell histocy-
10. Willman CL, Busque L, Griffith BB, et al. Langer-
hans cell histiocytosis (histiocytosis X): a clonal
154-160.
Differentiation of Langerhans cells in Langerhans cell histiocytosis

Frederic Geissmann, Yves Lepelletier, Sylvie Fraitag, Jenny Valladeau, Christine Bodemer, Marianne Debré, Michelle Leborgne, Sem Saeland and Nicole Brousse