Expression of the monoclonal antibody HECA-452 defined E-selectin ligands in Langerhans cell histiocytosis

Abstract The cutaneous lymphocyte associated antigen (CLA) recognized by the monoclonal antibody (moAb) HECA-452 plays a major role in the homing of lymphocyte subpopulations to the skin by binding to E-selectin on dermal microvessels. The factors responsible for the immigration of Langerhans cells (LC) and their precursors into the skin are still unknown, but because normal resting LC are also capable of expressing CLA, the antigen was proposed as a candidate LC-homing structure. To gain insight into these mechanisms, the expression of HECA-452 on neoplastic LC within and outside the skin was investigated in paraffin-embedded sections from 44 patients with localized and disseminated forms of Langerhans cell histiocytosis (LCH) presenting with proliferating cells positive for CD45, CD1a, S100 and HLA-DR. Irrespective of the clinical presentation or the type of organ involved, HECA-452-positive LC were detected in all biopsies tested (range 5–90%). The most prominent HECA-452 reactivity was observed in skin lesions and in areas with accumulations of eosinophilic granulocytes. Our data provide evidence for a heterogeneous expression of sLex/sLeα structures in various stages of activated and/or differentiated LCH cells. Remarkably, CLA-antigen expression on LCH-cells was not restricted to cutaneous sites. In view of recent findings on the expression of HECA-452 on resting epidermal LC, our data are compatible with the view that local cytokine production by keratinocytes or cells from the surrounding infiltrate induce and/or modulate CLA expression on LC in both cutaneous and extra-cutaneous sites.

Key words Langerhans cell histiocytosis • HECA-452 • Sialyl-Leα/Sialyl-Leβ • Homing mechanisms • Immunohistochemistry

Introduction

Langerhans cell histiocytosis (LCH), formerly termed histiocytosis X, is a disorder in which Langerhans cells (LC) and/or their immigrating precursors are the predominant proliferating cells [28]. The clinical spectrum of LCH includes rather benign (solitary lesions in one organ) or fatal (multisystemic disease) clinical pictures; three clinical syndromes are distinguished: eosinophilic granuloma [14], Hand-Schüller-Christian disease [12], and Abt-Letterer-Siwe disease [1]. The LC infiltrates are accompanied by variable numbers of inflammatory cells, such as eosinophilic granulocytes, plasma cells and lymphocytes. In some cases (mainly in bone lesions), multinucleated giant cells are intermingled. The proliferating cells (LC) in each category display the same ultrastructural and immunophenotypic features of “normal” Langerhans cells, including expression of CD1a and class II antigens and, ultrastructurally, an abundance of Birbeck granules [4]. However, the phenotype of LC can vary [8, 9, 11, 20], depending upon the differentiation stage and/or the activation status. Recently, the clonal origin of LC in LCH was demonstrated and the neoplastic nature of the disease in all three particular clinical entities was confirmed [27, 29].

In a recent study, the expression of the cutaneous lymphocyte-associated antigen (CLA) [17] recognized by the monoclonal antibody (moAb) HECA-452 [6] was demonstrated on normal resting epidermal LC in lesional skin from patients with inflammatory and neoplastic lymphocytic skin diseases [13]. Several investigations revealed that the moAb HECA-452 recognizes the carbohydrate structures of sialyl-Leα(sLeα), the sialylated form of CD15, sialyl-Leα (sLeα), an isomeric form of sLeα, and closely related carbohydrate moieties [2, 3]. These carbohydrate structures serve as ligands for E-se-
lectin (CD 62E, previously called endothelial leucocyte
adhesion molecule-1, ELAM-1); this adhesion molecule
is expressed at low levels on dermal endothelial cells
and is highly upregulated during inflammation [10].

We have carried out an immunophenotypic study on
an LCH series with different organic involvement; the
purpose was to establish whether the clonal proliferating
cells have the capacity to express sLeα/sLeα antigens;
whether expression of HECA-452 is restricted to cutane-
ous LCH lesions; and, finally, whether expression of this
moAb is different in different clinical presentations.

Materials and methods

Forty-four specimens diagnosed as LCH were investigated on for-
malin-fixed, paraffin-embedded sections. The cases were obtained
from the files of the Department of Dermatology and the Institute
of Clinical Pathology. The sites of the lesions were skin and tissue
of the oral mucosa in 12 cases, lymph nodes in 6 cases, and bone
in 26 cases. Routine HE- and Giemsa-stained sections of all tis-
ues were reviewed and subsequent sections were stained with the
antibodies listed in Table 1, using a sensitive three-step immuno-
peroxidase technique as previously described [24]. The proportion
of LC positive for HECA-452 and other moAbs was estimated by
two of the authors independently, comparing the cell population
stained with CD1a, HLA-DR and S100 moAbs, respectively.

Results

Light microscopy showed that lesions were composed of
cells with typical LCH morphology – cells were large
and had folded, partly grooved nuclei and abundant grey-
ish-blue cytoplasm on Giemsa staining. Particularly in
bone lesions, multinucleated giant cells were intermin-
gled, whereas they were absent in skin lesions. Infiltrates
were accompanied by variable numbers of eosinophilic
granulocytes, lymphocytes and plasma cells.

Irrespective of the clinical presentation or the type of
organ involved, virtually all LCH cells were CD45+ and
CD1a+ (Fig. 1), reacted with antibodies against the S100
protein and expressed MHC class II antigens (HLA-
DR+). Cells were negative when tested with T- and B-
cell markers (CD3-/L26-). In the majority of specimens,
a variable portion (5–90%) of LCH cells reacted with
moAbs directed against monocyte/macrophage (CD68)
antigens, i.e. PGM-1 [7] and KiM1p, respectively. Multi-
nucleated giant cells, in particular, were strongly CD68
positive, whereas CD1a and S100 staining of the giant
cells was rather faint in serial sections. The proliferation
fraction of LCH cells was determined using the moAb
MIB-1, which represents the Ki-67 equivalent for forma-
lin-fixed, paraffin-embedded sections. Nuclear staining
was observed on variable portions of neoplastic LC
(range <5–25%). We were not able to demonstrate differ-
ences in the proliferation fraction relative to different lo-
calizations or clinical presentation.

Irrespective of the organ involved, HECA-452+ cells
were observed in all cases tested (Table 2). The range of
HECA-452-reactive cells varied from 5% to over 90% of

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCA*</td>
<td>Dakopatts</td>
<td>CD45</td>
</tr>
<tr>
<td>CD1a</td>
<td>Immunotech</td>
<td>CD1a</td>
</tr>
<tr>
<td>Leu-22</td>
<td>Becton-Dickinson</td>
<td>CD43</td>
</tr>
<tr>
<td>S-100</td>
<td>HCS*</td>
<td>S100</td>
</tr>
<tr>
<td>PGM-1</td>
<td>Dakopatts</td>
<td>CD68R [7]</td>
</tr>
<tr>
<td>Ki-M1p</td>
<td>-e</td>
<td>CD68 [19]</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Becton-Dickinson</td>
<td>MHC class II</td>
</tr>
<tr>
<td>L26</td>
<td>Dakopatts</td>
<td>CD20cy</td>
</tr>
<tr>
<td>L23</td>
<td>Dakopatts</td>
<td>CD3</td>
</tr>
<tr>
<td>MIB-1</td>
<td>Immunotech</td>
<td>Ki-67: proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>HECA-452</td>
<td>-f</td>
<td>sLeα/sLeα structures</td>
</tr>
</tbody>
</table>

Table 1 List of antibodies used

<table>
<thead>
<tr>
<th>Localization</th>
<th>No. of biopsies</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Skin/oral mucosa</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Bone</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

In the lymph node specimens, an intrasinusoidal and interfollicular infiltration pattern with LCH was gen-
erally observed. HECA-452+ LCH cells were detected chiefly
in the vicinity of high endothelial venules (HEV) and
within dilated sinuses. The staining intensity of HECA-
452+ LCH-cells was lower than that of strongly positive
endothelial cells (Fig. 3). In general, lower proportions
of LCH cells stained positively for HECA-452 than in
skin specimens (range 5–50% HECA-452+ LCH-cells).

In the bone lesion specimens, we detected varying
numbers of HECA-452+ cells. HECA-452 reactivity was
predominantly observed in areas with accumulations of
eosinophilic granulocytes (Fig. 4). Antigen expression
was observed in these areas in over 90% of LCH cells in

Table 2 HECA-452 expression in different lesion sites of LCH

Materials and methods

Irrespective of the clinical presentation or the type of
organ involved, virtually all LCH cells were CD45+ and
CD1a+ (Fig. 1), reacted with antibodies against the S100
protein and expressed MHC class II antigens (HLA-
DR+). Cells were negative when tested with T-and B-
cell markers (CD3-/L26-). In the majority of specimens,
a variable portion (5–90%) of LCH cells reacted with
moAbs directed against monocyte/macrophage (CD68)
antigens, i.e. PGM-1 [7] and KiM1p, respectively. Multi-
nucleated giant cells, in particular, were strongly CD68
positive, whereas CD1a and S100 staining of the giant
cells was rather faint in serial sections. The proliferation
fraction of LCH cells was determined using the moAb
MIB-1, which represents the Ki-67 equivalent for forma-
lin-fixed, paraffin-embedded sections. Nuclear staining
was observed on variable portions of neoplastic LC
(range <5–25%). We were not able to demonstrate differ-
ences in the proliferation fraction relative to different lo-
calizations or clinical presentation.

Irrespective of the organ involved, HECA-452+ cells
were observed in all cases tested (Table 2). The range of
HECA-452-reactive cells varied from 5% to over 90% of

<table>
<thead>
<tr>
<th>Localization</th>
<th>No. of biopsies</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Skin/oral mucosa</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Bone</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

In the lymph node specimens, an intrasinusoidal and interfollicular infiltration pattern with LCH was gen-
erally observed. HECA-452+ LCH cells were detected chiefly
in the vicinity of high endothelial venules (HEV) and
within dilated sinuses. The staining intensity of HECA-
452+ LCH-cells was lower than that of strongly positive
endothelial cells (Fig. 3). In general, lower proportions
of LCH cells stained positively for HECA-452 than in
skin specimens (range 5–50% HECA-452+ LCH-cells).

In the bone lesion specimens, we detected varying
numbers of HECA-452+ cells. HECA-452 reactivity was
predominantly observed in areas with accumulations of
eosinophilic granulocytes (Fig. 4). Antigen expression
was observed in these areas in over 90% of LCH cells in