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

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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 sLe^x/sLe^a 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-Le^x/sialyl-Le^a · Homing mechanisms · Immunohistochemistry

This work is dedicated to Professor Dr. Thaddäus Radaszkiewicz, who died in September 1995

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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 neoplastical lymphocytic skin diseases [13]. Several investigations revealed that the moAb HECA-452 recognizes the carbohydrate structures of sialyl-$Lewis^x$(sLe^x), the sialylated form of CD15, sialyl-$Lewis^a$ (sLe^a) , an isomeric form of sLe^x, 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 sLe^x/sLe^a antigens; whether expression of HECA-452 is restricted to cutaneous 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 formalin-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 tissues were reviewed and subsequent sections were stained with the antibodies listed in Table 1, using a sensitive three-step immunoperoxidase 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.

Table 1 List of antibodies used

Antibody	Source	Specification
LCA	Dakopatts[a]	CD45
CD1a	Immunotech[b]	CD1a
Leu-22	Becton-Dickinson[c]	CD43
S-100	HCS[d]	S100
PGM-1	Dakopatts	CD68R [7]
Ki-M1p	–[e]	CD68 [19]
HLA-DR	Becton-Dickinson	MHC class II
L26	Dakopatts	CD20/cy
CD3	Dakopatts	CD3
MIB-1	Immunotech	Ki-67; proliferating cell nuclear antigen
HECA-452	–[f]	sLe^x/sLe^a structures

[a] Dakopatts, Copenhagen, Denmark
[b] Immunotech, Marseille, France
[c] Becton-Dickinson, Mountain View, Calif.
[d] HCS, Toronto, Canada
[e] Kindly provided by Professor M.R. Parwaresch, Kiel, Germany
[f] Kindly provided by Professor S.T. Pals, Amsterdam, The Netherlands

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

Localization	No. of biopsies	Reactivity			
		<5%	5–20%	20–50%	50–>90%
Skin/oral mucosa	12	0	0	3	9
Lymph node	6	0	3	3	0
Bone	26	0	10	5	11

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 greyish-blue cytoplasm on Giemsa staining. Particularly in bone lesions, multinucleated giant cells were intermingled, 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. Multinucleated 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 formalin-fixed, paraffin-embedded sections. Nuclear staining was observed on variable portions of neoplastic LC (range <5–25%). We were not able to demonstrate differences in the proliferation fraction relative to different localizations 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 LCH cells. There was no correlation between the expression of CD68-related antigens and HECA-452 staining. Similarly, the proliferation fraction did not correlate with HECA-452-antigen expression.

The most pronounced HECA-452 positivity was detected in skin biopsies (range 20–>90% reactive LCH cells). Skin lesions commonly presented with strong epidermotropism, and sometimes with formations of subcorneal abscesses of LCH cells. These cells were easily distinguished from normal resting LC, since they were round and not dendritic. HECA-452+ LCH cells tended to be localized within and/or fairly well below the epidermal/dermal boundary (Fig. 2), whereas deep dermal LCH-cell infiltrates were rather HECA-452 negative.

In the lymph node specimens, an intrasinusoidal and interfollicular infiltration pattern with LCH was generally 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

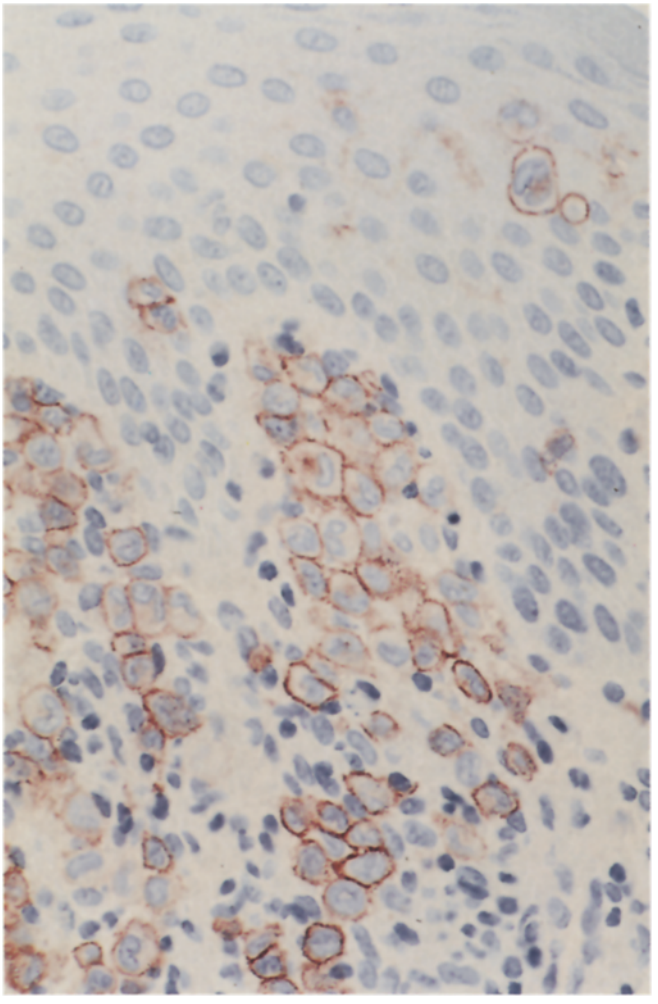

Fig. 1 Paraffin section of a LCH infiltrate in the skin, showing numerous LCH cells in the papillary dermis and single epidermal infiltrating cells, all stained positive for CD1a. (Immunoperoxidase stain; ×1200)

some sections, whereas areas without eosinophilic granulocytes rarely showed HECA-452+ LCH cells. Interestingly, multinucleated giant cells failed to express HECA-452 in all cases.

Discussion

The diagnosis of LCH is based on the reactivity of morphologically characteristic LCH cells with antibodies against CD1a, HLA-DR and S100 proteins as well as the demonstration of ultrastructurally detectable Birbeck granula [28]. Up to now, the detection of CD1a+ cells was limited to frozen sections; on application of the recently available moAb CD1a on formalin-fixed, paraffin-embedded sections, all 44 biopsies investigated in this series were found to fulfil the immunohistochemical criteria required for a diagnosis of LCH.

Normal epidermal LC are phagocytic, process antigen and may express macrophage-associated markers. In re-

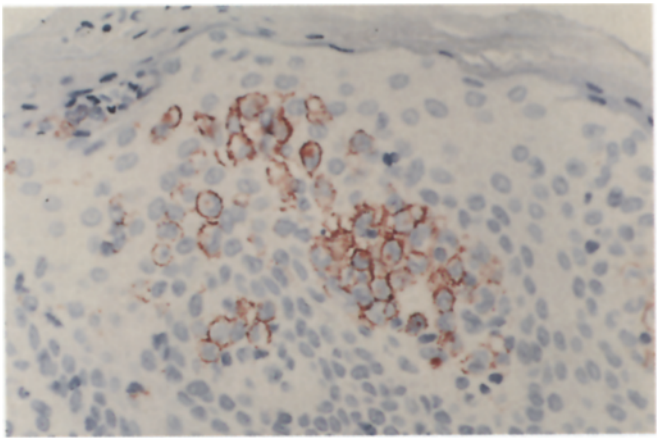

Fig. 2 Paraffin section of a cutaneous LCH infiltrate stained for HECA-452; almost all intraepidermal LCH-cells are HECA-452+, one subcorneal abscess in the *left corner* with single stained LCH -cells. (Immunoperoxidase stain; ×460)

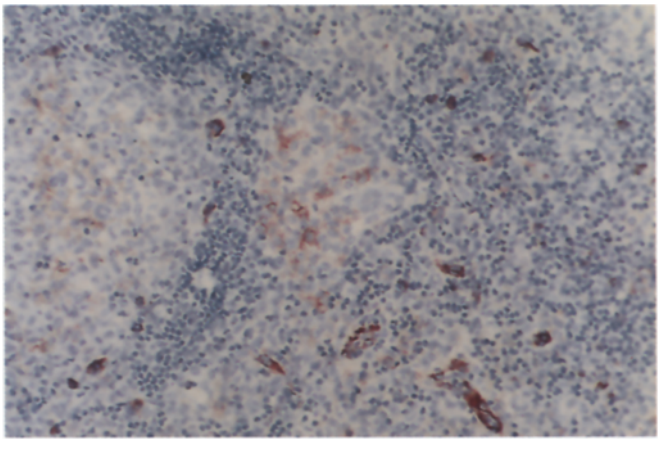

Fig. 3 Paraffin section of a lymph node involved by LCH and stained for HECA-452; HEV are strongly positive, whereas cells of the LCH-infiltrate are more weakly stained for HECA-452. (Immunoperoxidase stain; ×230)

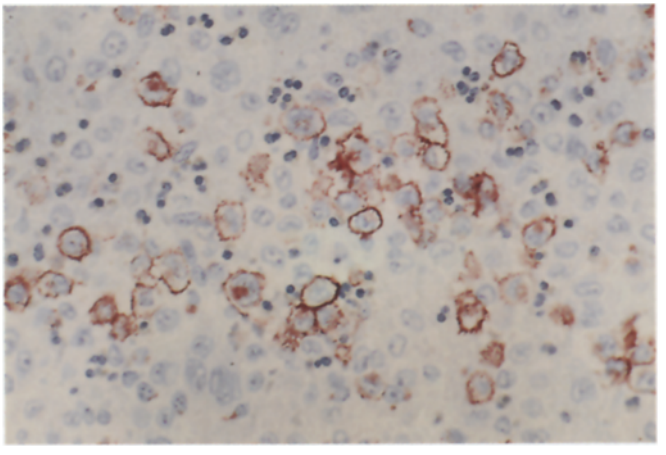

Fig. 4 Paraffin section of a bone lesion of LCH. Numerous eosinophilic granulocytes are intermingled, and many LCH cells display strong, occasionally cytoplasmic reactivity with HECA-452. (Immunoperoxidase stain; ×920)

sponse to antigens, dendritic LC leave the epidermis and migrate via lymphatics to regional lymph nodes. This migration is accompanied by enhanced T cell stimulatory capabilities as well as by phenotypic changes [8, 9, 20]. In agreement with other authors [11, 16, 19, 22], we observed the expression of CD68 antigens (PGM-1, Ki-M1p) on a variable portion of LCH cells, and especially on CD1a/S100+ multinucleated giant cells. Our findings are consistent with the view that proliferating Langerhans cells pass through different stages of differentiation or activation [8, 9, 11, 20] and show similarities with closely related cells from the monocyte/macrophage lineage.

It has recently been reported that most lymphocytes in inflammatory skin diseases express CLA that is recognized by the moAb HECA-452. It has been suggested that this molecule is the homing receptor for lymphocytes migrating into the skin [17]. Normal resting epidermal LC are capable of the expression of both sLe^x [5, 21, 25] and sLe^x/sLe^a structures [13], the latter recognized by the moAb HECA-452. The expression of these structures on LC is highly upregulated during inflammation and in cutaneous lymphoid neoplastic processes, indicating a role in LC-endothelial cell adhesion during LC migration into and out of the skin through interactions with selectins expressed on endothelial cells. Because it was demonstrated that skin-homing lymphocytes require HECA-452 antigens on their surface to bind to their ligand E-selectin on dermal microvessels [2, 3], this was also thought to be true for HECA-452 expressing LC. The ligand(s) or mechanisms involved in this are still unknown. In this report, we demonstrate that neoplastic LCH cells are able to express the HECA-452 antigen in a similar way to their normal counterparts within the skin. Antigen expression was observed in 44 LCH lesions from patients with different clinical manifestations of LCH. Irrespective of the type of organ involved, at least parts of the cellular infiltrate (range 5–>90% of LCH cells) showed immunoreactivity with moAb HECA-452. The most pronounced positivity was observed within skin lesions, especially in lesions with marked epidermotropism of the LCH cells. Our data on skin lesion LCH provide further evidence for the expression of HECA-452 on skin-associated dendritic cells. It is possible that both LC and their neoplastic counterparts in LCH migrate into the skin via the interaction between sLe^x/sLe^a and E-selectin expressed on dermal microvessels. In accordance with other authors [25], we have observed heterogeneous antigen expression, pointing to a different stage of activation or maturation of the clonally proliferating cells.

The expression of the HECA-452 antigen on proliferating LC in extracutaneous manifestations of LCH can be interpreted in different ways. The expression of the HECA-452 antigen on T cells can be upregulated in vitro by mitogen stimulation in combination with interleukin-6 or transforming growth factor-ß [18]. By analogy, one might conclude that the HECA-452-reactive structure(s) on LC and their counterparts on LCH cells might also be induced by the presence of antigen and/or cytokines, which is accompanied by an increased activation status. This observation may be correlated with the findings of van Dinther-Janssen et al. [26], who reported that activated dendritic cells in the inflamed synovium are HECA-452+. One might speculate about local cytokine secretion by inflammatory cells such as lymphocytes or eosinophilic granulocytes, because LCH-lesions showing an abundance of eosinophilic granulocytes exhibited pronounced HECA-452 positivity. The expression of the HECA-452 molecules on LCH cells may therefore be regulated by various cytokines released in the vicinity of proliferating LCH cells, either by epithelial cells such as keratinocytes or by cells of the concomitant infiltrate.

As already mentioned, LC pass through a different differentiation status when leaving the epidermis to migrate to lymph nodes and vice versa. This change in activation is usually accompanied by a varying immunophenotype of migrating LC, depending on a given environmental condition. Neoplastic LCH cells retain the capacity for mimicking various stages of activated and/or differentiated normal LC [9]; it is therefore conceivable that variable numbers of LCH cells do not lose their capacity to express sLe^x/sLe^a structures, even in extracutaneous sites. In keeping with this assumption, it has been reported that the neoplastic cells of primary cutaneous T cell lymphomas may retain their HECA-452 positivity in lymph node deposits [15, 23].

At the moment, a functional role for the HECA-452 antigen in adhesive processes of LC cannot be excluded on the basis of our data. Further studies are needed to identify potential ligands and mechanisms of sLe^x/sLe^a expressing LC and their precursors to gain further insight into the homing mechanisms of LC.

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