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Expression of Monoclonal Antibody HML-1-Defined $\alpha^E\beta7$ Integrin in Cutaneous T Cell Lymphoma

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Considering that integrins may play a major role in the localization in distinct biological features as well as in the dissemination of several types of lymphomas, we studied the expression of the monoclonal antibody HML-1-defined $\alpha^E\beta7$ integrin (CD103) in the clinically and histologically determined stages of 53 mycosis fungoides (MF) skin biopsies and in 16 affected lymph nodes. Immunoperoxidase staining revealed HML-1 immunoreactivity with T cells in the early stages of disease (patch and plaque stage MF). HML-1 expression was more pronounced on infiltrating epidermal than on dermal T cells. In contrast to early stages, tumor stage MF and lymph nodes affected in the course of cutaneous T cell lymphoma were HML-1 negative. We found a strong association between HML-1 expression, epidermotropism of infiltrating T cells, and the stage of disease. We provide evidence that: 1) the loss of the HML-1 antigen on T cells in MF is a marker of poor prognosis and 2) because the HML-1 antigen is selectively expressed on T lymphocytes of epithelial sites such as gut and skin, our results are compatible with the view that $\alpha^E\beta7$ integrins perform homing receptor functions for epitheliotropic T cells. (Am J Pathol 1994, 145:1148–1158)

The skin is the most common site of primary extranodal non-Hodgkin's lymphoma of T cell type. The large spectrum of diseases under the general category of cutaneous T cell lymphoma (CTCL) includes the clinical syndromes mycosis fungoides (MF) and Sézary's syndrome (SS).[1,2] MF initially presents with a scaly eruption (patch stage MF) that progresses through a plaque stage leading to skin tumors. After variable time, progression may lead to involvement of lymph nodes, bone marrow, and/or visceral organs.

The histological appearance of CTCL varies with the stage of disease: in the patch stage, lymphocytes invade the epidermis and form Pautrier's microabscesses. Accumulations of lymphocytes in the upper dermis forming a band-like infiltrate are observed in the plaque stage. Progression of the disease into tumor stage MF results in the loss of epidermotropism and occasionally systemic disease. With few exceptions, CTCLs are composed of cells displaying the immunological phenotype of mature T helper cells,[1,2] ie, cells are commonly $CD2^+/CD3^+/CD4^+/CD5^+/CD8^-$. Occasionally, loss of T cell-associated antigens (eg, CD3, CD7, or antigens associated with the T cell receptor) may be observed.[3,4] On the other hand, the emergence of activation antigens of neoplastic T cells, eg, Ki-1 (CD30), is noted sporadically.[5]

The distinction of MF from reactive cutaneous inflammation, especially in early stages, can be difficult. Benign inflammatory processes (eg, large and small plaque parapsoriasis or several types of eczemas) exhibit predominantly mature T helper cell phenotypes similar to that displayed by cells of most cases of MF. Unfortunately, standard immunophenotyping with different T cell markers has not been helpful in the distinction of benign, reactive processes and the early malignant lymphoma lesions. For this reason, many investigations focused on studies on the expression of adhesion molecules by MF cells. Abel et al[6] as well as other authors consecutively[7] reported that the expression of the homing receptor molecule for peripheral lymph node T cells, L-selectin (CD62L), is uncommon in MF. In 1990, Picker et al[8] showed that

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the majority of skin-associated T cells selectively express the HECA-452 antigen; they speculated on a new homing receptor molecule for skin homing T cells, termed cutaneous lymphocyte-associated antigen (CLA), because they found only a low percentage of HECA-452$^+$ T cells in other lymphoid organs. Moreover, lymphoma cells in early stages of MF but not tumor cells in advanced stages expressed the CLA antigen. Sterry et al[9] pointed to the important role of adhesion molecules, eg, β_1-integrins, expressed on lymphocytes in the occurrence of the phenomenon of epidermotropism via binding to their ligands expressed by keratinocytes.

Our study focuses on the expression of the $\alpha^E\beta7$ integrin in MF using the monoclonal antibody (MAb) HML-1[10] (CD103). Initially, HML-1 was reported to react with intestinal intraepithelial lymphocytes (IELs)[11] and approximately 50% of lamina propria T cells, whereas only few scattered positive T cells were found within other lymphatic tissue, such as peripheral lymph nodes, tonsils, and thymus. The HML-1 antigen has recently been identified as a new member of the integrin family[12–15] with a $\beta7$-integrin chain[16,17] and an as yet uncharacterized α^E-chain.[16,18] Until now several MAbs have been purified that recognize the same antigen: HML-1,[10] B-ly7,[19] BP6,[17] LF61,[20] and Ber-ACT 8.[21] All MAbs precipitate three glycoproteins with the same molecular weights: 160 kd, 130 kd, and 105 kd unreduced, 145 kd and 120 kd reduced, respectively. Beside antigen expression on IELs and few T cells in lymph nodes, reactivity with these MAbs was also found on few B cells, activated macrophages, and on hairy cell leukemia cells.[19,21–23]

In contrast to other peripheral T cell lymphomas,[24,25] most enteropathy-associated T cell lymphomas (EATCL)[24,26,27] display HML-1 reactivity. The latter findings were not unequivocal, because Pallesen et al[28] reported that of 88 peripheral T cell lymphomas examined, only 16 (18%) were unreactive with the MAb HML-1. Sperling et al[29] reported in 1989 on eight cases of CTCL with pronounced epidermotropism, where only a minority (3 of 8) displayed HML-1 positivity.

In this study, we evaluate 68 CTCL specimens and 16 affected lymph nodes of patients with CTCL as well as 23 control biopsies of benign, inflammatory skin diseases of T cell type for HML-1 reactivity. We correlate the $\alpha^E\beta7$-integrin expression with the stages of MF and speculate on the possible diagnostic significance associated with the presence of HML-1$^+$ cells in the early stages of MF and the possible prognostic significance associated with the loss of the HML-1 antigen on neoplastic T cells in tumor stage MF.

Materials and Methods

Skin and lymph node biopsies were consecutively listed in the files of the Department of Dermatology and the Institute of Clinical Pathology between the years 1989 and 1993. Sixty-eight cases of primary T cell lymphoma of the skin and 16 cases of lymph nodes involved in the course of CTCL were registered and classified according to the updated Kiel classification of malignant non-Hodgkin's lymphomas.[30] In addition, 23 specimens of different benign lymphoproliferative disorders and inflammatory skin biopsies were evaluated, ie, 6 cases of chronic unspecified eczema, 3 cases of parapsoriasis small plaque, 10 cases of parapsoriasis large plaque, and 4 cases of pseudolymphoma. These specimens served as comparable biopsies, because they exhibited prominent T cell infiltration.

Skin biopsies from patients with MF (n = 53), SS (n = 3), pleomorphic T cell lymphoma (n = 4), and high grade T cell lymphoma (n = 8) (six anaplastic large cell lymphomas [ALCL], Ki-1$^+$, and two immunoblastic T cell lymphomas) were investigated. In addition, 16 infiltrated lymph nodes were tested. Biopsies were excised under local anesthesia after informed consent has been obtained from the patients.

Biopsies were divided into two pieces. They were fixed in 8% phosphate-buffered formalin and processed for routine light microscopy and immunohistochemistry snap-frozen in precooled isopentane (Merck, Darmstadt, Germany), respectively. Frozen biopsies were stored at −70 C until further use. Three-micron thick cryostat sections were cut from each block, air-dried, and fixed in acetone for 10 minutes.

Immunostaining of frozen sections was performed by applying a sensitive three-step immunoperoxidase technique.[31] Briefly, sections were overlayered with the respective primary MAbs, diluted appropriately in 5% bovine serum albumin (BSA) phosphate-buffered saline (PBS). The panel of MAbs used is listed in Table 1. As a second step, sections were incubated for 1 hour with a biotinylated sheep F(ab')$_2$ anti-mouse Ig (Amersham Intern. plc, Little Chalfont, UK) diluted 1:200 in 5% BSA-PBS. Finally, sections were incubated in a 1:200 dilution of avidin-biotinylated peroxidase complex (Dakopatts, Glastrup, Denmark) in Tris-buffered saline for 1 hour. Peroxidase staining was developed in a solution of 3-amino-9-ethyl-carbazole (Sigma, St. Louis, MO) in the presence of H_2O_2. Each incubation was followed by three washings in PBS. Sections were counterstained with hematoxylin (Merck, Vienna, Austria) and

Table 1. *MAbs*

CD	MAbs	Dilution	Ig Subclass	Source
CD1a	OKT6	1:800	IgG1	OP
CD3	Leu4	1:800	IgG1	BD
CD4	Leu3a	1:100	IgG1	BD
CD5	Leu1	1:100	IgG2a	BD
CD8	Leu2a	1:100	IgG1	BD
CD14	LeuM3	1:100	IgG2b	BD
CD20	B1	1:200	IgG2a	CI
CD30	Ki-1	1:40	IgG3k	DP
CD45	HLe-1	1:200	IgG1	BD
CD45RO	UCHL-1	1:200	IgG1	DP
CD54	ICAM-1	1:300	IgG1	IT
CD57	Leu7	1:50	IgG1	BD
	βF1 (TCR-αβ)	1:30	IgG1	TCS
	δTCR-1 (TCR-γδ)	1:100	IgG1	TCS
CD103	HML-1	1:200	IgG2a	IT
CD62L	Leu8	1:50	IgG2a	BD
	HECA-452	1:750	IgM (rat)	*
	Laminin	1:200	IgG1 (rat)	IT

BD, Becton Dickinson, Mountain View, CA; IT, Immunotech, Marseille, France; TCS, T Cell Sciences, Cambridge, MA; DP, Dakopatts, Copenhagen, Denmark; OP, Ortho Pharmaceutical Corporation, Raritan, NJ; CI, Coulter Immunology, Hialeah, FL; B, Behring, Marburg, Germany.

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mounted in Aquamount (BDH, Poole, Dorset, UK). All reaction steps were performed at room temperature.

The percentages of intraepidermal, corium lymphocytes, and cells within affected lymph nodes positive for HML-1 were compared with cells that were $CD3^+$ and were estimated by two investigators independently. In selected cases of predominant intraepithelial lymphoma spread in skin biopsies, a double immunostaining technique, according to Mason and Sammons,[32] was conducted on frozen sections. This technique was used to exclude reactivity of nonlymphoid cells, eg, Langerhans cells, with the MAb HML-1.

Results

This study focuses on 53 cases of MF that were further subdivided into three stages: 27 biopsies represented patch stage MF, 20 biopsies were plaque stage, and 6 biopsies were categorized as tumor stage MF. The patch stage MF specimens exhibited marked epidermotropism of infiltrating cells, whereas epidermotropism was absent in 50% of plaque stage biopsies. All tumor stage cases lacked epidermotropism.

Based on nearly identical staining patterns in serial sections of 53 MF biopsies, the following phenotype of infiltrating cells predominated: $CD45^+/CD45RO^+/CD3^+/CD4^+/CD8^-$. T cells did not express natural killer cell markers, ie, they were $CD57^-$. In 50 of 53 biopsies equipment of $CD3^+$ cells with the T cell receptor (TCR)-αβ was confirmed by reactivity with the MAb βF1. $CD8^+$ cells were interspersed among the infiltrating cells to a varying extent; however, we could not identify a single case predominated by $CD3^+/CD8^+$ cells. Two cases differed from the other MF specimens in that the malignant cells were $CD45^+/CD45RO^+/CD3^+/CD4^-/CD8^-$ and were equipped with the TCR-γδ instead of the TCR-αβ (Figure 1). These two lymphoma specimens represented so-called double negative T cell lymphomas, and thus shared phenotypic similarities (concerning the double negativity) with the EATCLs.[26] Both showed very pronounced epidermotropism of infiltrating lymphoma cells and were classified as patch stage MF.

Skin

HML-1 Reactivity (Table 2, Figure 2)

Patch Stage (Figure 3) In 22 of 27 specimens, infiltrating cells exhibited marked epidermotropism with formation of Pautrier's microabscesses and/or a linear distribution of infiltrating cells along the epidermal basement membrane with additional infiltration of the suprabasal layers. Within the dermis, patchy T cell infiltrates were seen.

In 18 of 27 (66%) of patch stage biopsies, >90% of intraepidermal T cells were $HML\text{-}1^+$. The $HML\text{-}1^+$ cells were distributed along the dermoepidermal junction and spread throughout the epidermis. However, staining of lymphocytes within Pautrier's microabscesses was inhomogeneous. In the two double negative $TCR\text{-}\gamma\delta^+$ lymphoma specimens, almost all infiltrating cells were $HML\text{-}1^+$, again being located in a pearl chain appearance along the basement membrane and infiltrating concurrently all epidermal layers (Figure 1B).

More than 90% of dermal T cells were $HML\text{-}1^+$ in only seven (≈25%) cases of patch stage biopsies tested (among them the two double negative $TCR\text{-}\gamma\delta^+$ T cell lymphomas). In 2 of 27 of patch stage biopsies only single scattered or no $HML\text{-}1^+$ epidermal and dermal T cells were observed.

Plaque Stage (Figure 4) Twenty biopsies of plaque stage MF were examined immunophenotypically; epidermotropism was noted in 50% of cases tested. Samples showed dense subepidermal band-like infiltrates and accumulations of T cells within the epidermis, occasionally forming Pautrier's microabscesses. In 20% (n = 4) of the biopsies investigated, $HML\text{-}1^+$ T cells comprised >90% of epidermal infiltrating T cells. Again, $HML\text{-}1^+$ cells tended to spare abscess formations and were distributed diffusely

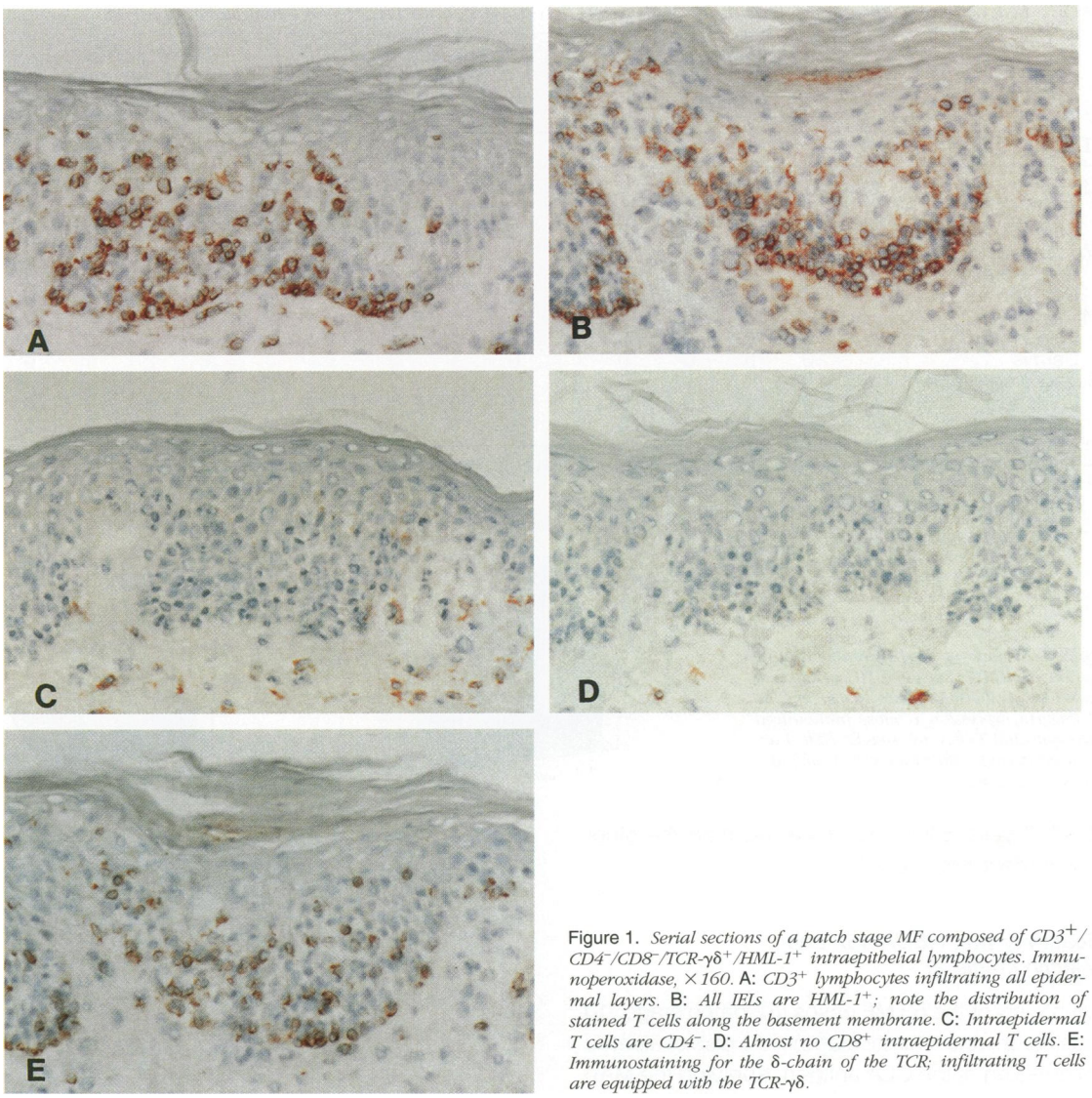

Figure 1. *Serial sections of a patch stage MF composed of CD3$^+$/CD4$^-$/CD8$^-$/TCR-γδ$^+$/HML-1$^+$ intraepithelial lymphocytes. Immunoperoxidase, ×160.* A: *CD3$^+$ lymphocytes infiltrating all epidermal layers.* B: *All IELs are HML-1$^+$; note the distribution of stained T cells along the basement membrane.* C: *Intraepidermal T cells are CD4$^-$.* D: *Almost no CD8$^+$ intraepidermal T cells.* E: *Immunostaining for the δ-chain of the TCR; infiltrating T cells are equipped with the TCR-γδ.*

over all epidermal layers. Dermal infiltrating cells were >90% HML-1$^+$ in only one case, >50% HML-1$^+$ in five (25%) cases. Thirty-five percent (n = 7) of biopsies tested (all of them showing no epidermotropism of T cells) were virtually HML-1$^-$.

Tumor Stage (Figure 5) Six biopsies of tumor stage MF were examined: none showed epidermotropism of T cells, whereas a dense and deep dermal T cell infiltrate was observed. In all six specimens tested, lymphoma cells did not express the HML-1 antigen.

SS (Figure 6) Two of three SS biopsies showed pronounced epidermotropism of infiltrating cells, whereas epidermotropism was no longer observed in one specimen due to PUVA therapy of this patient. All specimens presented with a dense band-like dermal T cell infiltrate. The two SS samples with epidermotropic T cells showed reactivity of the MAb HML-1 with >50% infiltrating T cells in both epidermis and dermis, whereas the remaining specimen exhibited less HML-1$^+$ cells, may be due to the patient's preceding therapy or progression of the disease.

Biopsies of 13 CTCLs other than MF (ie, pleomorphic T cell lymphomas or high grade T cell lymphomas) did not exhibit epidermotropism and were virtually HML-1$^-$. For comparison, 23 specimens of benign inflammatory and lymphoproliferative skin disorders were investigated for HML-1 antigen expression. Infiltrates were composed primarily of T helper cells. In all eczema (Figure 7), small plaque parapsoriasis, and pseudolymphoma specimens, only <5% of infiltrating cells (within epidermis and/or dermis) were HML-1$^+$. Five of 10 large plaque parapsoriasis specimens presented considerably more

Table 2. *Reactivity of Infiltrating Cells with the MAbs HML-1, HECA-452, and Leu8 in Different Stages of MF/SS*

	Expression of HML-1					Expression of HECA-452					Expression of Leu8				
	No. of Cases*					No. of Cases*					No. of Cases*				
Diagnosis Skin	n	–	+/–	+	++	n	–	+/–	+	++	n	–	+/–	+	++
MF patch	27					12					18				
Epidermis		2	4	3	18		0	0	4	8		16	2	0	0
Dermis		6	5	9	7		0	0	5	7		9	6	3	0
MF plaque	20					8					10				
Epidermis		10	1	5	4		0	0	4	4		6	4	0	0
Dermis		7	7	5	1		0	1	3	4		3	4	3	0
MF tumor	6					4					4				
Epidermis		6	0	0	0		1	1	1	1		4	0	0	0
Dermis		6	0	0	0		0	1	2	1		1	2	1	0
Sezary's syndrome	3					3					3				
Epidermis		0	1	2	0		1	0	1	1		0	3	0	0
Dermis		0	1	2	0		1	0	1	1		0	1	2	0

* – Equals only single cells; +/– equals 20 to 50% + cells; + equals 50 to 90% + cells; ++ equals >90% + cells.

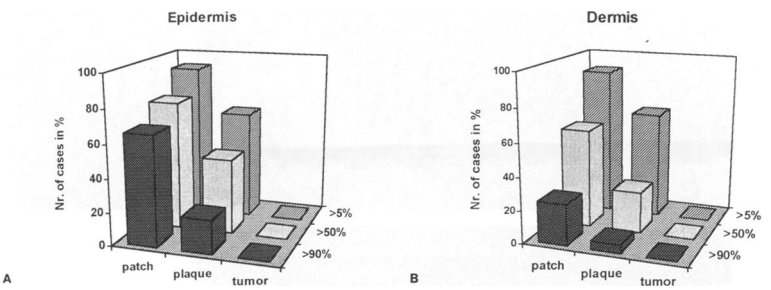

Figure 2. *Schematic of the differential HML-1 expression in distinct stages of MF; results are expressed as positivity of cases in percent.* **A:** *$\alpha^E\beta 7$ integrin expression is more pronounced on intraepithelial T cells, whereas* **B:** *HML-1 expression on dermal infiltrating cells is only apparent in early stages.*

HML-1$^+$ T cells within the epidermis than the other biopsies (data not shown).

Reactivity with HECA-452 and L-Selectin (Table 2)

Twenty-six of the 53 MF cases (12 patch stage, 10 plaque stage, and 4 tumor stage) were also evaluated for expression of the CLA antigen, applying the MAb HECA-452.[33] The range of infiltrating T cells that were HECA-452$^+$ varied from >90% of cells to only a minority of cells expressing CLA. We could not demonstrate a clear association between the stage of disease and HECA-452 expression, because the antigen was not lost in the tumor stage biopsies tested.

Staining for the human peripheral lymph node homing receptor L-selectin (CD62L) with the MAb Leu8[34] was performed in 32 of 53 specimens. Intraepidermal T cells failed to express Leu8 in the majority of biopsies, whereas dermal infiltrating T cells were reactive in up to 50%, independent of the stage of disease (Table 2).

Lymph Nodes

Sixteen involved lymph nodes of patients with CTCL (four with MF, four with SS, four with pleomorphic T cell

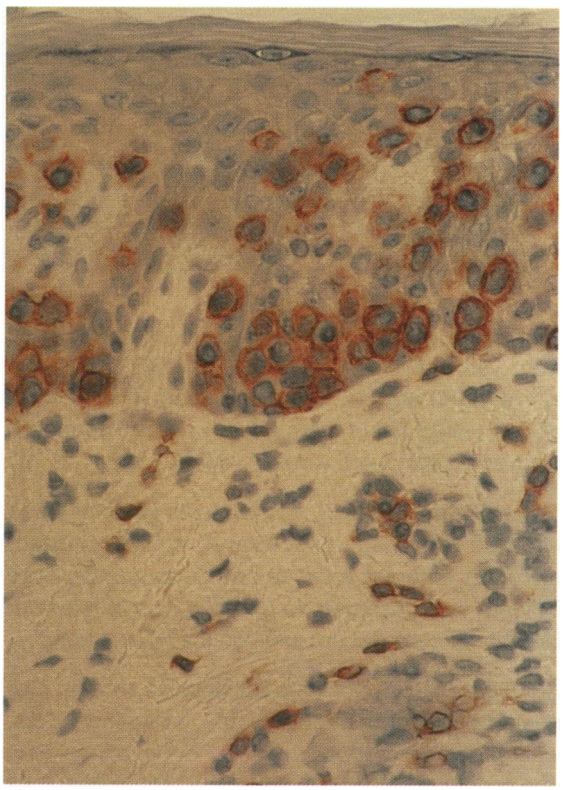

Figure 3. *Patch stage MF: intraepidermal T cells show marked HML-1 positivity. Immunoperoxidase, ×320.*

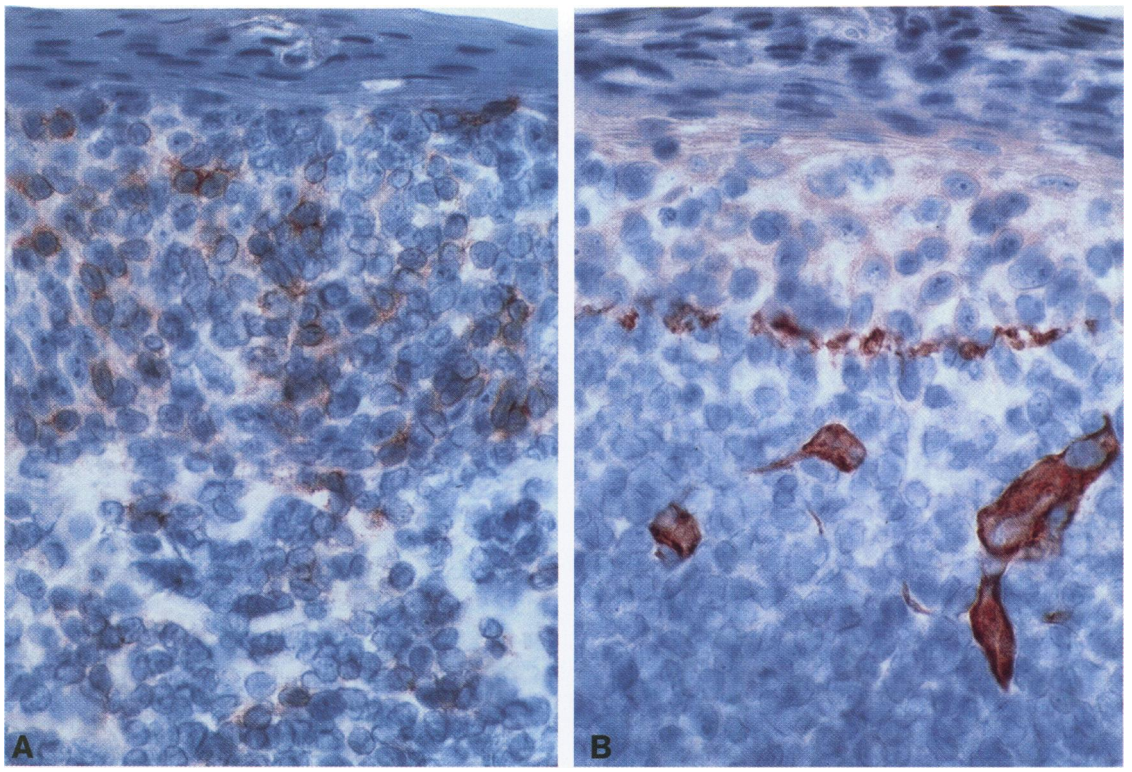

Figure 4. *Plaque stage MF: immunoperoxidase, ×320.* **A:** *The epidermis presents with numerous epidermotropic HML-1$^+$ lymphocytes, whereas dermal T cells show inhomogenous antigen expression.* **B:** *Laminin staining provides focal disruption of the basement membrane caused by epidermotropic infiltrating T cells.*

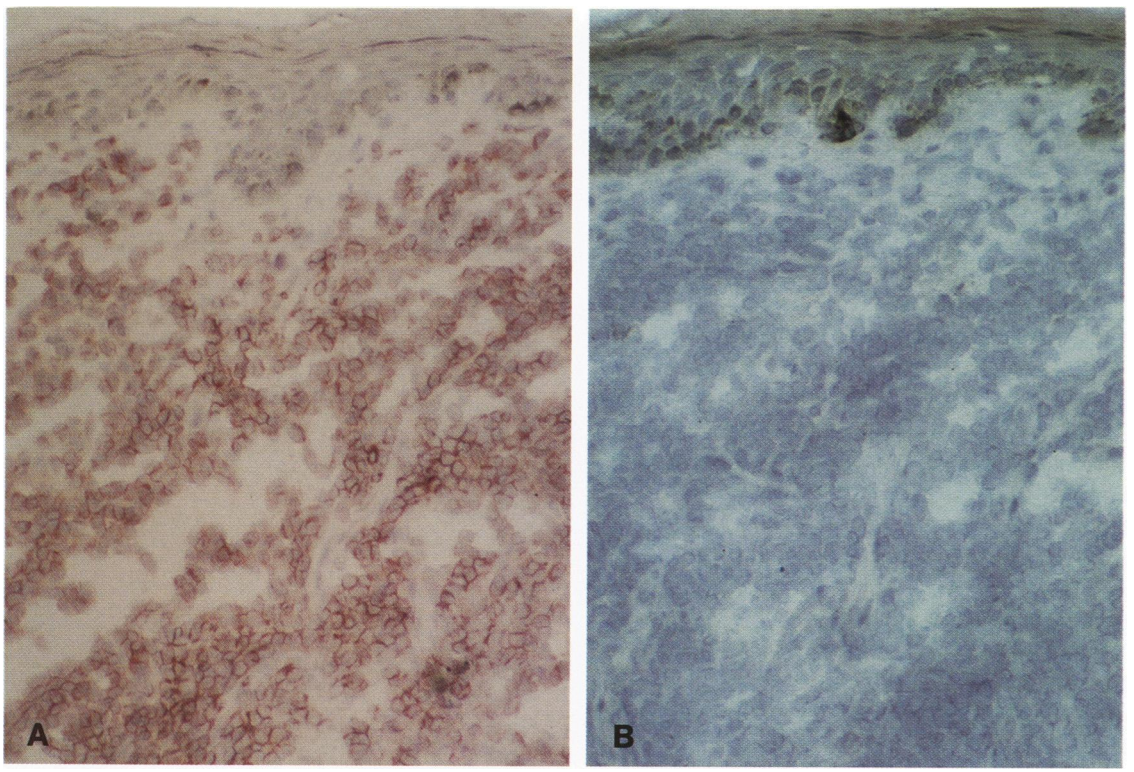

Figure 5. *Tumor stage MF: serial sections. Immunoperoxidase, ×160.* **A:** *Dense CD3$^+$ dermal infiltrate exhibiting no epidermotropism.* **B:** *Tumor cells are completely HML-1$^-$.*

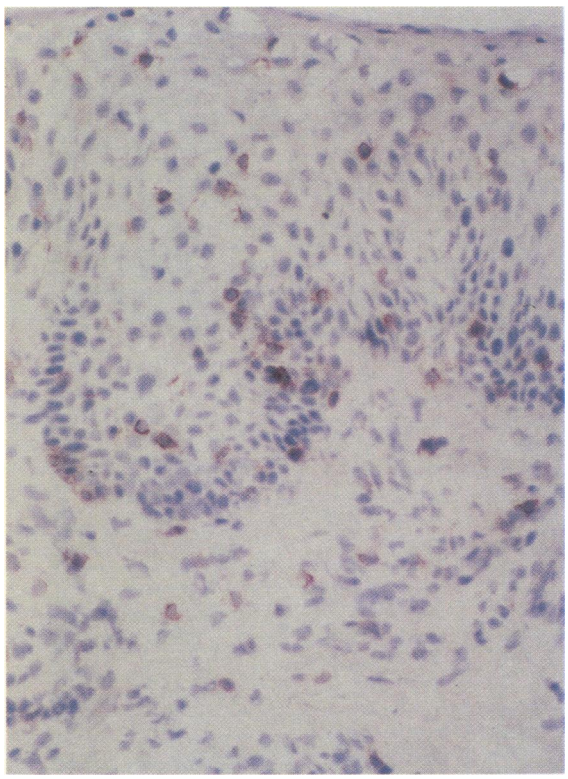

Figure 6. *SS: Many intraepidermal HML-1$^+$ lymphocytes, several HML-1$^+$ dermal T cells. Immunoperoxidase, ×160.*

lymphoma, one with ALCL, Ki-1$^+$, and three unclassified T cell lymphomas) were investigated for reactivity with several MAbs (Table 1). Skin and lymph node biopsies of the same patient were available in five cases. These skin biopsies were subcharacterized as follows: two cases with tumor stage MF (with lymph node involvement by MF cells), two cases with SS in skin and lymph nodes, and one case with patch stage MF skin lesions and a lymph node infiltrated with a secondary ALCL, Ki-1$^+$. With the exception of this case, lymph node biopsies showed cells with the phenotype of T helper cells (CD45$^+$/CD45RO$^+$/CD3$^+$/CD4$^+$/TCR-$\alpha\beta^+$). In the secondary ALCL, Ki-1$^+$, infiltrating cells lost CD3 antigens, TCR antigens, and partially CD4 antigens.

Staining with the HML-1 MAb revealed that tumor cells did not express the antigen in all 16 cases tested (Figure 8). Only single scattered cells were found around high endothelial venules. Most interestingly, the patient with patch stage MF skin lesions (with >90% HML-1$^+$ infiltrating cells in the skin biopsy) showed no HML-1$^+$ infiltrating T cells within the lymph node biopsy of an ALCL, Ki-1$^+$. The other specimens, where skin and lymph node material was available, showed the same pattern: in the case of the two SS specimens, skin biopsies were HML-1$^+$ to varying degrees, whereas lymph nodes were virtually HML-1$^-$.

In addition, staining for the homing receptor antigens L-selectin and CLA was performed; at least >50% of T cells expressed CD62L. The expression of HECA-452 varied in the lymph node lymphoma specimens; we noted only one case (MF involvement of the lymph node) where infiltrating T cells failed to express CLA. All other cases, regardless of lymphoma type, displayed considerable portions of HECA-452 reactive T cells. Three lymph nodes were almost entirely HECA-452$^+$.

Discussion

The antigen recognized by the MAb HML-1 is expressed on virtually all intestinal IELs and on roughly 40% of lamina propria T cells,[10] whereas in other lymphoid tissues, such as thymus, spleen, and lymph nodes, it is rarely or not expressed.[10] Based on the

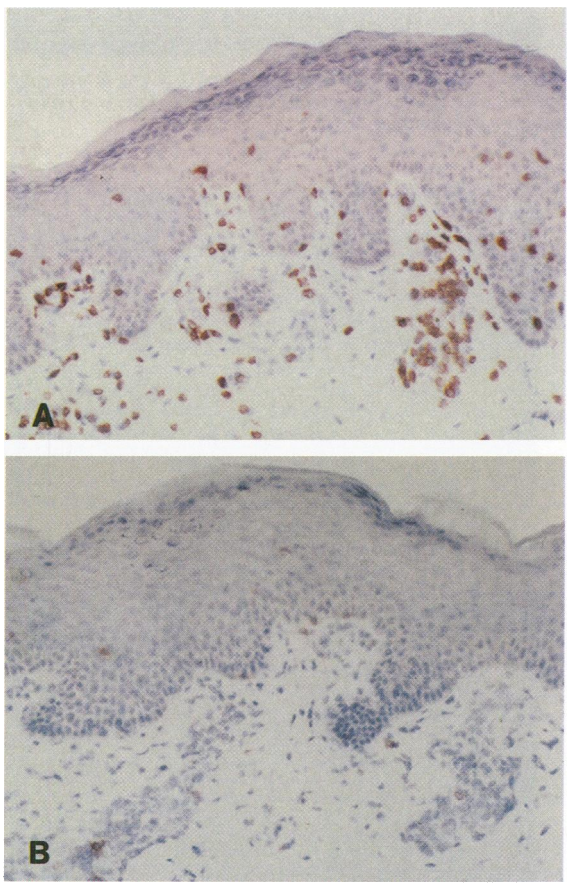

Figure 7. *Chronic unspecified eczema: serial sections. Immunoperoxidase, ×80.* **A:** *Staining with CD3 reveals numerous CD3$^+$ T cells within the epidermis and a patchy dermal T cell infiltration, whereas* **B:** *the vast majority of the T cell infiltrate is unreactive with MAb HML-1.*

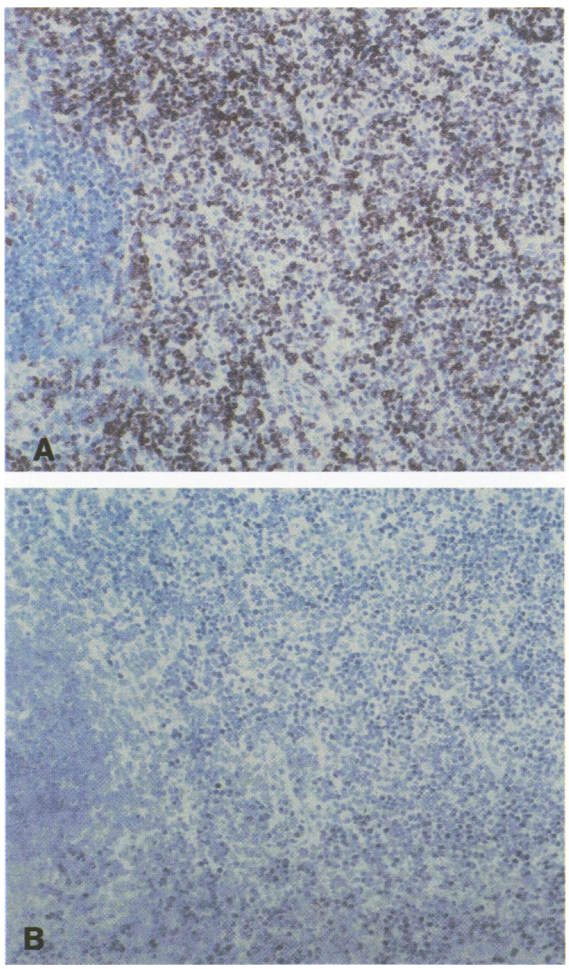

Figure 8. *Immunohistochemical staining of a lymph node affected in the course of MF: serial sections, one residual B follicle on the left. Immunoperoxidase, ×80.* **A:** *Diffuse CD3⁺ T cell infiltration.* **B:** *HML-1: infiltrating T cells are negative.*

first reports, HML-1 expression appeared to be restricted to cells of the T cell lineage,[10] either normal, nonneoplastic IELs in the gut or malignant T cells in EATCL.[24,26,27] The expression of the HML-1 antigen is not only independent of the type of accessory molecules, such as CD4 or CD8, on gut T lymphocytes,[10] but also independent of the TCR used. Similarly, in this study on cutaneous lymphomas, HML-1 was not restricted to any lymphocyte subset, ie, we observed antigen expression on $CD3^+/CD4^+$ T cells and double negative, TCR-$\gamma\delta^+$ T cells.

Recently, the HML-1 antigen has been identified as a new member of the integrin family,[12–15] where it contains the β7-chain[16,17] and thus performs adhesive functions.[14,35–37] Sterry et al[9] have demonstrated that there is an overexpression of distinct β_1-integrins on T cells in MF, pointing to a prominent role of adhesion molecules in the phenomenon of epidermotropism. Sperling et al[29] reported that of eight epidermotropic CTCLs, three were HML-1^+, whereas the other cases showed only single HML-1^+ T cells.

In this study, we correlate the expression of the $\alpha^E\beta$7-integrin in CTCL with the stage of disease and the prognosis. We categorized MF into cases with and without epidermotropism and subdivided them into the histologically and clinically determined patch, plaque, and tumor stages. Patch stage MF exhibited pronounced epidermotropism of infiltrating lymphoma cells; 18 of 27 (66%) biopsies, showed more than 90% infiltrating HML-1^+ T cells within the epidermis, whereas within the dermis, in only 7 of 27 ($\simeq$25%) biopsies up to >90% of infiltrating T cell specimens were strongly HML-1^+. In plaque stage, epidermotropism and HML-1 positivity was noted in only 50% of biopsies tested. Finally, in the tumor stage, we did not detect HML-1^+ cells, either within the epidermis or in the dermis, or in the lymph nodes. However, data for circulating lymphocytes in SS and MF are lacking, because peripheral blood was not available for testing HML-1 reactivity. Sperling et al[29] reported that HML-1 expression was found predominantly, if not exclusively, on intraepidermal infiltrating T cells, whereas corium lymphocytes remained unlabeled. This is in contrast to our findings, because we found a strong HML-1 expression on dermal lymphocytes in early (patch) stages in seven ($\simeq$25%) biopsies. Expression, however, was more pronounced within the epithelium in patch and plaque stage MF.

We found a clear correlation between HML-1 expression, epidermotropism of infiltrating T cells, and the stage of disease. HML-1 expression is no longer observed in the tumor stage of MF. Thus, the loss of epidermotropism in conjunction with HML-1 negativity may represent a marker of poor prognosis.

The fact that we were not able to demonstrate any association between the stage of disease and the expression of the HECA-452 antigen on neoplastic cells in CTCL does not sustain the observations made by Picker et al;[8] moreover, in our lymph node specimens investigated, only one specimen was completely HECA-452^- the other specimens displayed HECA-452 positivity in varying quantities. Our findings are in good correlation with the findings of Noorduyn et al,[38] where peripheral T cell lymphomas outside the skin displayed variable HECA-452 positivity.

In accordance with the results of Abel et al[6] and Wood et al,[7] $Leu8^+$ (CD62L) intraepidermal T cells were not (or only neglible) seen in 53 MF biopsies, whereas dermal T cells expressed the antigen in some cases tested. L-selectin is known to be the homing receptor for peripheral lymph node-homing leukocytes[39] but not for lymphocytes homing to any epithelial sites and thus Leu8 fails to be expressed on

skin-associated epidermal T cells. Tumor cells in affected lymph nodes in the course of CTCL, however, were commonly Leu8$^+$ in our study. Thus, our data provide further evidence for the concept of lymphoma dissemination in the course of CTCL, when lymphoma cells express (*de novo*?) the Leu8 antigen spreading to lymph nodes.

Several adhesion molecules are known to be inducible by the presence of antigen and/or cytokines on peripheral blood cells and distinct cell lines *in vitro*.[35] One might presume a similar situation is true for the HML-1 antigen, because the induction of the HML-1 antigen on peripheral blood lymphocytes with mitogens, phorbolester, or interleukin-2 *in vitro* has been reported previously.[40] Hence authors concluded that HML-1 belongs to the family of activation antigens. In the murine system, transforming growth factor-β has been identified to be capable of inducing a mouse IEL antigen, recognized by the MAb M290, on stimulated T cells.[41,42] Our results are not entirely compatible with the assumption that cytokines released by T cells in CTCL are the solitary responsible agents for the expression of the HML-1 antigen, because control biopsies, such as eczemas or parapsoriasis, where infiltrates are mainly composed by mature T helper cells, did not show pronounced HML-1 positivity.

The HML-1 antigen was identified as a new member of the integrin family. With regard to its restricted tissue distribution in the epithelium (gut and skin), it may well be that besides performing adhesive functions, it performs homing receptor[35,43] functions for epitheliotropic T cells and thus serves as an adhesion molecule that mediates localization and/or anchoring of IEL in the epithelium.[18,36,37,44] Based on the results obtained in this study it is conceivable that the HML-1 antigen is associated with a selective homing of neoplastic T lymphocytes not only to the gut, but also into the skin.

The former reports on the almost exclusive expression of the HML-1 antigen on gut IEL suggested a role of this antigen as a homing receptor for specialized gut homing T cells. However, blocking studies[11] performed with the rat analogue MAb to the human HML-1 antibody, RGL-1,[11] were unsuccessful: anti-rat IEL MAb was unable to block the migration of lymphocytes into the intestinal rat epithelium. Therefore, authors concluded that HML-1 is not a homing receptor for gut-homing T cells. In the mouse system, however, the homing receptor properties of β_7-integrins have been demonstrated.[45] It remains to be clarified for the human system whether homing receptors other than those classical homing receptors, which interact with vascular addressins, exist. Thus, the identification and distribution of HML-1 and its putative ligand probably on epithelial cells, may be a valuable tool to discern a new class of homing molecules or to identify dual functions performed by particular members of the integrin family.

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