Expression of Monoclonal Antibody HECA-452–Defined E-Selectin Ligands on Langerhans Cells in Normal and Diseased Skin

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The cutaneous lymphocyte-associated antigen recognized by the monoclonal antibody HECA-452 has been thought to play a major role in the homing of memory T-cell subsets to the skin by virtue of its ability to bind to E-selectin of dermal microvascular endothelial cells. Considering that the homing of different leukocyte populations to the skin may involve similar mechanisms, we studied the expression of HECA-452–reactive molecules on CD1a+ epidermal Langerhans cells. Immunofluorescence double-labeling of cryostat sections and epidermal sheets of normal skin revealed HECA-452 immunoreactivity on a subpopulation of dermal and epidermal CD1a+ cells, whereas upon flow-cytometric analysis of epidermal single cell suspensions virtually all CD1a+ cells bound HECA-452 antibodies. We observed a marked upregulation of HECA-452-antigen expression on CD1a+ epidermal cells and a pronounced increase in the number of HECA-452+/CD1a+ dermal cells in lesional skin.

Epidermal Langerhans cells (LC) are major histocompatibility complex class I/class II–bearing dendritic leukocytes with potent antigen-specific T-cell–stimulating properties [1,2]. Whereas LC were previously regarded as a rather sessile cell population, it is now clear that the fairly constant numbers of anti-CD1a–reactive human LC result from a delicately balanced interplay of three factors: a) immigration, b) mitosis, and c) emigration. Although the occurrence of these three events has been amply documented [3], the mechanisms underlying these phenomena are only partly understood. In the very recent past, we have gained insight into the factors stimulating mitotic activity in putative LC precursors [4] as well as those triggering LC emigration§ [5]; in contrast, the factors responsible for the immigration of LC/LC precursors into the skin have remained enigmatic.

The reason why one assumes that the homing of LC to the skin/epidemis occurs in a directed rather than random fashion is based on the observations i) that intravenously injected LC specifically home to the skin [6] and ii) that certain T-lymphocyte subpopulations home preferentially to the skin [7,8]. In this respect, particular attention has focused on molecules detected by the monoclonal antibody (MoAb) HECA-452. This MoAb reacts with more than 80% of T cells infiltrating inflamed areas of the skin, but only with a low percentage (less than 10%) of T cells at other sites of inflammation [8]. The T-cell structure detected by this MoAb has therefore been termed “cutaneous lymphocyte-associated antigen” (CLA) [9]. Biochemical studies have revealed that MoAb HECA-452 recognizes the carbohydrate structures i) sialyl-LewisX (sLeX), the sialylated form of LewisX (LeX; CD15), ii) sLeX, an isomeric form of sLeX, and iii) closely related carbohydrate moieties [10,11]. Various studies indicate that these carbohydrates serve as ligands for E-selectin (previously called endothelial leukocyte adhesion molecule-1 [ELAM-1]) [10–13]; this vascular adhesion molecule is constitutively expressed at low levels on endothelial cells of the dermal microvasculature and its expression is highly upregulated during inflammation [14]. These findings, together with other observations showing i) that E-selectin–transfected L cells bind to isolated CLA-antigen [11] and ii) that memory T cells expressing CLA-antigen bind to E-selectin–transfected COS cells [15], led to the hy-

**Abbreviations:** CLA, cutaneous lymphocyte-associated antigen; LeX, LewisX (CD15); RT, Room temperature; sLeX, Sialyl-LewisX; sLeX, Sialyl-LewisX; SA-PE, Streptavidin phycoerythrin; SA-T, Streptavidin Texas red. § Enk AH, Angeloni VL, Udey MC, Katz SI. An essential role for interleukin-1β in the induction of primary immune responses in skin (abstr.). J Invest Dermatol 100:447, 1993.
pothesis that the interaction of E-selectin and CLA is crucial for T-cell homing to the skin.

Reasoning that analogous mechanisms could also facilitate the homing of other leukocytes to the skin, we searched for the presence of HECA-452-reactive epitopes on epidermal LC in normal and diseased skin.

**MATERIALS AND METHODS**

**Specimen Collection, Tissue, and Cell Preparation**

Four-millimeter punch biopsies of normal skin from the upper arm were taken from three healthy volunteers with normal-appearing skin and no history of any skin disease. Samples of lesional skin were obtained from patients suffering from various inflammatory and neoplastic lymphocytic skin diseases (see Table II). A small portion of each biopsy was processed for routine histopathology and the rest of the specimens were embedded in Tissue-Tec II (Laboratory Inc., Elkhart, IN), frozen in 2-methylbutane (Merck, Darmstadt, Germany) for flow cytometry, split-thickness skin appearing skin obtained from patients undergoing cosmetic surgery or from patients with lepromatous leprosy (TWEEN-20, Bio Rad, Richmond, CA) and 2% (v/v) each of fetal calf serum and milk proteins, 0.1% (w/v) polyethylene oxide/urea, and 5% (w/v) monovalent sugars (Amersham) or from autologous MoAb HECA-452, Leu-M1, or IOT6, followed by either biotinylated mouse anti-IgM antibody (Amersham) or biotinylated mouse anti-IgM (F(ab')2 fragments (Amersham) for 1 h at RT. Detection of antibody binding was achieved with peroxidase-conjugated StreptABComplex (Dako A/S, Glostrup, Denmark) and 3-amino-9-ethyl carbazole (Sigma) in acetate-buffer (pH 5.0). Sections were counterstained with HARRIS' hematoxylin (Merck) and finally cover-slipped with a mixture of PBS/glycerol and Mowiol (Hoechst, Hoechst, Germany).

Double stainings were performed by incubating the cryostat sections overnight at 4°C in staining-buffer containing MoAb HECA-452. Antibody binding was detected by consecutive incubations with biotinylated sheep-anti-Ig antibody (Amersham) and Streptavidin Texas Red (SA-TR, Dako), each for 1 h at RT. For counterstaining, sections were then overlaid with MoAb IOT6, the binding of which was visualized by consecutive incubations with fluorescein-isothiocyanate (FITC)–labeled rabbit-anti-mouse Ig F(ab')2 fragments (Dako) diluted in staining buffer (see above) containing 2% (v/v) rat serum and FITC-labeled swine-anti-rabbit Ig antibody (Dako) diluted in staining-buffer containing 2% (v/v) rat serum and 2% (v/v) pig serum. Epithelial sections were labeled with MoAb HECA-452/SA-TR as described above and counterstained with OKT6-FITC for 1 h at RT. After final washes in PBS, sheets were mounted on glass slides and cover-slipped with a mixture of PBS/glycerol and Mowiol (Hoechst). In additional experiments, sections were incubated overnight at 4°C with anti-CD3 MoAb UCHT1 (Dako) and antibody binding was detected by consecutive incubations with biotinylated sheep-anti-mouse Ig F(ab')2 fragments (Amersham) and SA-TR, each for 1 h at RT. Sections were then counterstained with OKT6-FITC for another 1 h at RT.

Cross reactivity between antibodies or unspecific binding via Fc-receptors were ruled out by appropriate positive and negative controls (see Table I). Stained sections and sheets were evaluated with a Nikon Epi-F3 microscope overlaid with MoAb HECA-452 or anti-CL1 single stainings as described above.

**Neuraminidase Digestion of Skin Sections**

Neuraminidase digestion of cryostat sections was performed for 30 min at 37°C using 40 mM of neuraminidase of *Vibrio cholerae* (Behring, Marburg, Germany) in PBS containing 4 mM CaCl2 and 1% (w/v) bovine serum albumin (Sigma). Mock digestion was performed in digestion buffer without neuraminidase. After washing in PBS, sections were subjected to HECA-452 or anti-CD15 single stainings as described above.

**Enumeration of Epidermal and Dermal Cell Populations**

To determine the percentage of HECA-452* LC, cryostat sections were subjected to HECA-452/IOT6 immunofluorescence double-labeling (see above). HECA-452*/CD1a* and HECA-452*/CD1a* epithelial cells were then enumerated at 400X magnification, whereby only cells with a clearly visible cell body were counted. 160–1100 CD1a* cells were evaluated on three or more non-seral sections of the biopsy and the percentage of HECA-452* cells was calculated from the total number of CD1a* cells. To determine absolute numbers of CD1a* epithelial and dermal cells, perox-
Table II. Quantification of CD1a\(^+\) Epidermal Cells Expressing HECA-452–Reactive Molecules

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Specimens</th>
<th>% HECA-452(^+) of CD1a(^+) Cells</th>
<th>Estimated Percentage of Suprabasally Located, Dendritic Cells Reactive with MoAb HECA-452(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal skin</td>
<td>3</td>
<td>20 ± 18(^\circ)</td>
<td>1</td>
</tr>
<tr>
<td>Atopic eczema</td>
<td>3</td>
<td>72 ± 12</td>
<td>0</td>
</tr>
<tr>
<td>Contact eczema</td>
<td>1</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>Pseudolymphoma</td>
<td>1</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>1</td>
<td>400</td>
<td>0</td>
</tr>
<tr>
<td>Parapsoriasis</td>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Viral exanthem</td>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CD30(^+) secondary LCAL(^+)</td>
<td>1</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>CTCL (Mf-type)</td>
<td>1</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>Seborrheic keratosis(^+)</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^+\) Serial sections were stained with either HECA-452 or OKT6 MoAbs. The percentage of HECA-452\(^+\) LC was estimated by the comparison of the number of OKT6\(^+\) and HECA-452\(^+\) suprabasally located dendritic cells. Only data from specimens not evaluated by immunofluorescence double-labeling are included in this part of the table.

\(^\circ\) CD30\(^+\) secondary large cell anaplastic lymphoma. HECA-452\(^+\) and CD1a\(^+\) cells were counted within the epidermis above the barely epidermotropic neoplastic infiltrate.

\(^\circ\) HECA-452\(^+\) and CD1a\(^+\) cells were counted in the lesional epidermis partly infiltrated by neoplastic T cells.

\(^\circ\) HECA-452\(^+\) and CD1a\(^+\) cells were counted within the epidermal cords of the lesion, as well as, in the perilesional epidermis. In both instances 4% of the CD1a\(^+\) cells coexpressed HECA-452-antigens.

\(^\circ\) Results are means ± SD.

Flow-Cytometric Analysis of Epidermal Cells

Epidermal cells were prepared for flow-cytometric analysis by washing in azide containing buffer (PBS/1% fetal bovine serum/0.1% sodium azide/0.5 mM ethylene diamine tetraacetic acid) followed by incubation with normal sheep serum (5%, v/v) and further washes in azide containing buffer. 1 x 10\(^6\) cells/100 \(\mu\)l were reacted with first-step antibodies (HECA-452, CSLEX1, or control IgM) for 30 min on ice and washed once in azide containing buffer. Antibody-binding was visualized by incubation with either biotinylated sheep–anti-rat Ig antibody (Amersham) or biotinylated sheep–anti-mouse Ig (Amersham) for 20 min on ice followed by Streptavidin Phycoerythrin (SA-PE; Becton Dickinson, San Jose, CA). Cells were then counterstained for 20 min with either MoAb OKT6-FITC, HLe-l-FITC, or species/iso­
type-matched FITC-labeled control MoAb. After two washes fluorescence was measured in a FACSscan (Becton Dickinson). Dead cells were identified by 7-amino-actinomycin D (7-AAD; Sigma) uptake and electronic gates were first set to acquire 10,000 living cells and then set to acquire only CD1a\(^+\) living cells.

RESULTS

HECA-452–reactive Cells in Normal Skin

Exposure of cryostat sections of normal human skin to MoAb HECA-452 revealed the presence of stained cells scattered throughout the epidermis and dermis. Within the dermis, the majority of labeled cells were located in the vicinity of vessels, displayed a round shape, and, thus, presumably represent T lymphocytes [7, 8, 16]. As previously reported [16–18], dermal endothelial cells were essentially non-reactive with MoAb HECA-452. Epidermal HECA-452\(^+\) cells consisted of i) a few round cells scattered throughout the basal layer (again probably representing T cells [8, 16]) and ii) suprabasally located dendritic cells (data not shown). To see whether the latter cells are LC, we subjected cryostat sections and sheets of normal skin to an immunofluorescence double-labeling protocol using MoAb HECA-452 and anti-CD1a MoAb IOT6. Results obtained revealed that 5 to 40% (20 ± 18%, mean ± SD; Table II) of CD1a\(^+\) epidermal cells specifically bound MoAb HECA-452. Although the majority of these cells exhibited only a weak HECA-452 reactivity, a few brightly stained cells were also detected (Fig 1). The intensity of the HECA-452 staining did not correlate with the level of CD1a expression, because LC that stained weakly with anti-CD1a MoAb sometimes expressed high levels of HECA-452-antigens and vice versa. As opposed to the results obtained on tissue preparations, we found that most, if not all, CD1a\(^+\) cells reacted with the MoAb HECA-452 when single-cell suspensions of normal epidermis were stained and analyzed by flow cytometry (Fig 2).

HECA-452–reactive Cells in Diseased Skin

In view of reports of increased numbers of HECA-452\(^+\) cells in inflamed skin [18], we asked whether this increase could be, at least partially, due to an increase in the number of HECA-452\(^+\) LC. When we analyzed skin sections from various inflammatory and neoplastic lymphocytic skin disorders for HECA-452–antigen expression, we noted considerably larger numbers of HECA-452\(^+\) cells in both dermis and epidermis of diseased skin as compared to normal skin. Double-staining with anti-CD1a MoAb revealed that i) approximately 50% of the HECA-452\(^+\) cells in the dermis belong to the LC-lineage and ii) that 50% to 80% of all CD1a\(^+\) dermal cells exhibited a strong labeling with the MoAb HECA-452 (Fig 3).

The increase in absolute numbers of dermal HECA-452\(^+\) cells was therefore not only due to an elevation in HECA-452\(^+\)/CD1a\(^+\) cells (Fig 3), which according to the literature are likely T cells [8, 16] and monocytic cells [16, 18], but was also due to an increase in the absolute numbers of CD1a\(^+\) dendritic cells (Table III). In contrast, the marked increase in HECA-452–reactive epidermal cells was not accompanied by elevated numbers of CD1a\(^+\) cells (Table III), was not due to a substantial increase in HECA-452\(^+\) non-LC (except in MF, data not shown), but rather a consequence of enhanced HECA-452–antigen expression by CD1a\(^+\) dendritic cells (Fig 3). Enumeration of HECA-452\(^+\)/CD1a\(^+\) and HECA-452\(^+\)/CD1a\(^+\) cells in the epidermis of the various inflammatory and neoplastic lymphocytic dermatoses studied revealed that 53–95% (71 ± 17%, mean ± SD) of the CD1a\(^+\) cells expressed HECA-452–reactive epitopes (Table II, left columns).

To confirm that the enhanced expression of HECA-452–reactive moieties on CD1a\(^+\) cells is representative of the examined disease entities, we subjected a larger panel of sections obtained from additional patients to HECA-452 single-labeling. Results obtained showed again that, compared to the situation in the skin of normal healthy volunteers, the lesional epidermis from patients with pseudolymphoma, atopic and contact eczema, psoriasis,
parapsoriasis, viral exanthem, and cutaneous T-cell lymphoma (CTCL) contained substantially elevated numbers of HECA-452+ dendritic cells (Table II, right columns). In contrast, in seborrheic keratosis, a proliferative disorder of keratinocytes not accompanied by T-cell infiltration, the percentage of HECA-452+ epidermal CD1a+ cells was comparable to that seen in normal skin (Table II, left column). To exclude the possibility that changes in the cytokine profile of lesional skin compared to normal skin could induce CD1a expression on skin T cells, we double labeled sections of the same specimens as used for the evaluation of HECA-452 expression with anti-CD1a and anti-CD3 MoAbs. Because these MoAbs reacted with mutually exclusive cell populations (data not shown), we conclude that the HECA-452+/CD1a+ cells in the lesional epidermis represent LC.

Figure 1. Demonstration of HECA-452 reactivity of epidermal Langerhans cell of normal skin. Cryostat sections were reacted with MoAb HECA-452/SA-TR and counterstained with anti-CD1a (IOT6)/FITC. Single photographic exposures with the appropriate filters show HECA-452+ cells in red (A,B) and CD1a+ cells in green (a,b). In some epidermal areas most of the CD1a+ cells react with the HECA-452 MoAb (A,a), whereas in other areas only few CD1a+ cells coexpressed the HECA-452-antigen (B,b). White dots, dermal epidermal junction. Bar, 30 μm.

Figure 2. Flow-cytometric analysis of HECA-452-antigen expression on epidermal cells. Cells of normal epidermis were labeled with MoAb HECA-452 (A,B) or control IgM (C) followed by appropriate second step MoAb/SA-PE and counterstained with HLe-1-FITC (anti-CD45; A) or OKT6-FITC (anti-CD1a; B,C). Ten thousand cells were recorded in A, demonstrating that nearly all CD45+ cells express HECA-452 antigens (upper right), whereas keratinocytes are essentially negative (lower left). Six thousand CD1a+ cells were recorded in B and C, demonstrating that more than 90% of CD1a+ cells react with the MoAb HECA-452 (B) and not with the control rat IgM (C). Data of one of four similar experiments are shown.
Serologic Characterization of the HECA-452-Reactive Molecule on LC The reactivity of LC with MoAb HECA-452 could be indicative of the presence of sLe\(^\alpha\), sLe\(^\beta\) or related molecules on these cells. To address this question we performed a series of experiments the results of which strongly imply that the HECA-452-reactive epitope on LC is sLe\(^\alpha\). First, epidermal single-cell suspensions were double-labeled with anti-sLe\(^\alpha\) MoAb CSLEX1 and anti-CD1a MoAb OKT6. All CD1a\(^+\) cells reacted with the CSLEX1 MoAb and the staining pattern observed was similar to that obtained with MoAb HECA-452 (Fig 4). Second, skin sections were treated with neuraminidase or control buffer and then either stained with MoAb HECA-452 or anti-CD15. Whereas epidermal cells in the mock-digested samples reacted with MoAb HECA-452, but not with anti-CD15 MoAb Leu-M1, neuraminidase digestion of the sections led to the loss of HECA-452 reactivity and to the gain of anti-CD15 reactivity (Fig 5). These data show that the epitope recognized by the MoAb HECA-452 on epidermal LC and T cells is a sialylated form of Le\(^\alpha\). In concordance with the absence of HECA-452-reactivity on keratinocytes, we could not detect binding of MoAb CSLEX1 on this epidermal cell population (Fig 4), and keratinocytes in the neuraminidase digested sample did not label with MoAb Leu-M1 (Fig 5).

DISCUSSION

The rat MoAb HECA-452 has been described to recognize carbohydrate moieties on skin-prone memory T cells [7]. We have now detected similar antigens on human epidermal LC. Immunolabeling of cryostat sections and epidermal sheets of normal skin revealed the presence of HECA-452 antigens on some suprabasally located dendritic CD1a\(^+\) cells and sensitive flow-cytometric studies of single cell suspensions indicated that almost all CD1a\(^+\) epidermal cells express the HECA-452-reactive epitope. In lesional skin of inflammatory as well as neoplastic lymphocytic skin diseases we found a marked upregulation of HECA-452 expression compared to normal skin, with up to 95% of the CD1a\(^+\) cells showing positive immunolabeling on cryostat sections. Concerning the nature of the HECA-452-reactive epitope on LC, we have shown that neuraminidase treatment of cryostat sections leads to the loss of HECA-452 reactivity and unmasking of CD15 molecules and that CD1a\(^+\) epidermal cells react with the anti-sLe\(^\alpha\) MoAb CSLEX1.

At first glance, the detection of HECA-452-reactive epitopes on LC may be surprising because Bos et al [16] recently reported HECA-452 MoAb binding to T-cell and macrophage subpopulations of normal skin, but absence of HECA-452 immunolabeling of epidermal CD1a\(^+\) cells. In addition, Jalkanen et al [18] reported enhanced numbers of HECA-452\(^+\) and CD1a\(^+\) dendritic cells in diseased versus normal skin, but stated that the populations recognized by MoAbs HECA-452 and anti-CD1a were not identical. We would presume that the sensitivity of the staining system used may account for the discrepancies between these observations and our finding. In this context, it should be emphasized that i) the HECA-452 labeling we detected on approximately 20% of CD1a\(^+\) cells in cryostat sections of normal skin was generally rather dull and ii) that uniform HECA-452 labeling of LC was only revealed by flow-cytometric analysis of epidermal single cell suspensions. In principle this difference could be due to i) the higher sensitivity of flow-cyto-

**Table III.** Number of CD1a\(^+\) Epidermal and Dermal Cells in Normal and Diseased Skin

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Number of Specimens</th>
<th>Number of CD1a(^+) Epidermal Cells/mm(^2) Basement Membrane</th>
<th>Number of CD1a(^+) Epidermal Cells/mm(^2) Epidermal Area</th>
<th>Number of CD1a(^+) Dermal Cells/mm(^2) Basement Membrane</th>
<th>Number of CD1a(^+) Dermal Cells/mm(^2) Dermal Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal skin</td>
<td>3</td>
<td>27 ± 2(^a)</td>
<td>583 ± 127</td>
<td>5 ± 1</td>
<td>47 ± 10</td>
</tr>
<tr>
<td>Atopic eczema</td>
<td>4</td>
<td>32 ± 9</td>
<td>307 ± 108</td>
<td>28 ± 15</td>
<td>253 ± 49</td>
</tr>
<tr>
<td>Contact eczema</td>
<td>3</td>
<td>37 ± 31</td>
<td>460 ± 441</td>
<td>63 ± 65</td>
<td>512 ± 385</td>
</tr>
<tr>
<td>Pseudolymphoma</td>
<td>3</td>
<td>33 ± 18</td>
<td>518 ± 318</td>
<td>14 ± 11</td>
<td>116 ± 85</td>
</tr>
<tr>
<td>Parapsoriasis</td>
<td>3</td>
<td>46 ± 8</td>
<td>744 ± 293</td>
<td>52 ± 17</td>
<td>583 ± 180</td>
</tr>
<tr>
<td>Viral exanthem</td>
<td>1</td>
<td>45</td>
<td>727</td>
<td>17</td>
<td>205</td>
</tr>
<tr>
<td>CD30(^+) secondary LCAL(^+)</td>
<td>1</td>
<td>27</td>
<td>376</td>
<td>9</td>
<td>84</td>
</tr>
<tr>
<td>CTCL (MF type)</td>
<td>4</td>
<td>52 ± 19</td>
<td>567 ± 200</td>
<td>43 ± 24</td>
<td>385 ± 183</td>
</tr>
</tbody>
</table>

* With the exception of contact eczema and parapsoriasis, data were obtained from sections cut from the same biopsies as used for the determination of the percentage of HECA-452\(^+\) LC (Table II). In the case of contact eczema and parapsoriasis two and three biopsies, respectively, were obtained from patients other than those used in Table II.

* CD30\(^+\) secondary large cell anaplastic lymphoma.

* Results are means ± SD.
metric analysis compared to fluorescence microscopy, ii) an unmasking of the reactive epitope by the enzyme treatment used to prepare the single cell suspension, and/or iii) an upregulation of this antigen during cell preparation. However, because treatment of cryostat sections from normal skin with trypsin prior to staining did not lead to enhanced reactivity of MoAb HECA-452 with LC and because there was no significant variation in HECA-452 reactivity on LC in epidermal sheets prepared with either trypsin, dispase, or NH₄SCN (data not shown), unmasking of HECA-452-reactive epitopes by trypsin does not seem to be the reason for the detection of higher percentages of HECA-452⁺ cells upon flow cytometry compared to in situ immunohistochemistry. This conclusion is further supported by the observation that an epidermal single cell suspension prepared with dispase and ethylene diamine tetraacetic acid showed a similar staining pattern as the trypsin-treated sample of the same specimen (data not shown). The third possibility, namely, upregulation of the HECA-452 antigen during cell preparation, also seems to be a rather unlikely explanation, because ex vivo culture of whole skin specimens up to 72 h did not result in increased HECA-452 binding to LC (data not shown). Due to theoretical considerations (such as the higher amount of antigen present on single cells compared to sectioned cells and the higher sensitivity of photomultipliers in the flow cytometer compared to the investigators’ eyes examining tissue specimens by fluorescence microscopy), we believe that the main reason for the discrepancy between the in situ and the flow-cytometry results is the different sensitivity of the two readout systems used.

The structure recognized by the MoAb HECA-452 has been reported to be serologically identical to sLeᵩ on neutrophils [11] but to differ from sLeᵩ on established memory/effector T cells [7,10,11]. Although the molecular configuration detected by HECA-452 on monocytes [8], dendritic cells [21], and high endothelial venules [17,22] has not been studied in detail, it seems to involve sLeᵩ because these cells react with the sLeᵩ-specific MoAb CSLEX1 [23-26]. To define the molecular configuration of the HECA-452-reactive epitope on LC we performed two series of experiments. First, double-labeling of epidermal single cell suspensions with CSLEX1 and anti-CD1a MoAbs revealed that all LC express sLeᵩ. This result confirms a recent publication showing that CD1a⁺ cells of buccal mucosa and normal skin label with the anti-sLeᵩ MoAb FH6 [27]. In further experiments, we found that treatment of skin sections with neuraminidase leads to the loss of HECA-452 binding and to the unmasking of CD15 (Leᵩ) [19], similar to what has been reported for T cells [11]. These experiments show that binding of HECA-452 is dependent on the expression of Leᵩ carbohydrates decorated with sialic acid. Although these data strongly suggest that the HECA-452-reactive structure on LC is sLeᵩ, the expression of other HECA-452-reactive oligosaccharides cannot be excluded. In addition to HECA-452 recognizing different carbohydrate molecules on different cells, the protein backbone to which the carbohydrates are attached also differs between various leukocyte subsets [8]. Proteinbiochemical studies would be needed to fully characterize the HECA-452-reactive antigens on LC.

Although Symington et al. [28] recently reported that trypsin-treated keratinocytes react with an anti-sLeᵩ MoAb, we could not detect binding of either HECA-452 or CSLEX1 to trypsin-treated keratinocytes. Because Symington et al. [28] used different MoAbs, one explanation for the contradictory result might be that the MoAbs employed by us do not bind to keratinocytes because of lower affinity or/and steric hindrance. How is the expression of the HECA-452-reactive molecule on LC regulated? It is known that T cells acquire CLA expression during the naive to memory cell transition in skin-associated peripheral lymph nodes [7] and that CLA expression can be induced in vitro by mitogen stimulation of T cells in combination with interleukin-6 or transforming growth factor-β [7]. In analogy one might presume that the HECA-452-reactive structure on LC may also be induced by the presence of antigen and cytokines. Compatible with this assumption are our data that LC residing in the epidermis of lesional skin from inflammatory or neoplastic lymphocytic disorders show a markedly enhanced expression of HECA-452-reactive molecules. Both the percentage of LC expressing detectable levels of HECA-452-reactive molecules (71 ± 17% versus 20 ± 18%, p < 0.001) and the intensity of staining with this MoAb on a single cell level were increased in diseased versus normal skin. In contrast, in seborrhoeic keratosis, a hyperproliferative disorder of keratinocytes not accompanied by T-cell infiltration, only small numbers of HECA-452⁺ LC were seen. Thus, it is possible that cytokines released from the infiltrating/neoplastic T cells may induce the expression of HECA-452-reactive antigens on resident epidermal LC. On the other hand, it is equally conceivable that LC precursors...
enter the skin in a HECA-452+ state. Once establishing residence in the epidermis, the cytokine level they encounter determines the degree to which HECA-452-antigen expression is maintained.

CONCLUSION
At the moment, it is unclear which, if either, of these concepts is valid. The assumption that the functional significance of HECA-452-antigen expression by a given cell is to allow its binding to E-selectin provides us with a logical argument favoring the latter hypothesis. First, there is no evidence for a role of HECA-452-antigen expression on resident LC, especially as keratinocytes do not express E-selectin [14,29] and adhesion of LC to keratinocytes has been shown to be mediated via E-cadherins [30]. Second, the possibility that resident LC upregulate HECA-452-antigen expression when they leave the epidermis to gain access to the lymphatics [31] is unlikely, because i) we have not seen E-selectin expression on vascular structures consistent with lymphatics (L. Picker, unpublished observation) and ii) we are not aware of data indicating that receptor-ligand interactions play a role in lymphocyte recruitment into lymphatic vessels. Conversely, there is circumstantial evidence supporting the hypothesis that LC precursors express HECA-452 antigens, which might enable these precursors to bind to the dermal microvasculature and, thus, facilitate their extravasation into the skin. Our observation of markedly enhanced numbers of HECA-452+/CD1a+ cells in the dermis of most dermatoes studied may be taken as an indication of an increased immigration of LC-precursors into lesional skin. The upregulation of E-selectin expression by the dermal microvasculature in inflammatory skin [14] and in neoplastic lymphocytic disorders [32] may provide the necessary basis for this process. After LC (precursors) reach the epidermis and take residence between the keratinocytes their HECA-452-antigen expression would then gradually decrease either due to a lack of appropriate stimuli or due to active downregulation. This might explain our finding that in normal skin, where recruitment of LC would be expected to be at relatively low levels, most LC are HECA-452+/dim. The observation that developing monocytes upregulate HECA-452 reactivity in the bone marrow, maintain high levels in the blood, and rapidly lose expression of these determinants during differentiation into tissue macrophages (both in vitro and in vivo, L. Picker, unpublished), is in keeping with this concept. The identification of cells in the peripheral blood that react with HECA-452 or CSLEX1 and do not express either T-cell or neutrophil markers may verify this hypothesis and perhaps even lead to the discovery of the still elusive LC-precursor.

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