Sialyl Lewis<sup>x</sup> Expression on Human Langerhans Cells

Nobuko Tabata,* Setsuya Aiba,* Satoshi Nakagawa,* Haruo Ohtani,† and Hachiro Tagami*

*Department of Dermatology and †Department of Pathology, Tohoku University School of Medicine, Sendai, Japan.

Recently, it has been demonstrated that the skin-infiltrating T cells express cutaneous lymphocyte-associated antigen, which is the ligand of E-selectin or endothelial-leukocyte adhesion molecule, suggesting that cutaneous lymphocyte-associated antigen functions as the homing receptor of the skin infiltrating T cells. In contrast, the mechanism for the migration of Langerhans cells from the bone marrow to the skin has not been clarified. Sialyl Lewis<sup>x</sup> acts as a ligand for endothelial-leukocyte adhesion molecule and granule membrane protein 140. We examined the expression of sialyl Lewis<sup>x</sup> in epidermal dendritic cells in human skin. Two-color immunofluorescence study on an epithelial sheet revealed that human leukocyte antigen DR<sup>+</sup> or CD1a<sup>+</sup> epidermal dendritic cells were partially sialyl Lewis<sup>x</sup>-positive, although all of the sialyl Lewis<sup>x</sup>-positive dendritic cells were human leukocyte antigen DR<sup>+</sup> and CD1a<sup>+</sup>. Further analysis of these dendritic cells by flow cytometry demonstrated that most of the human leukocyte antigen DR<sup>+</sup> and CD1a<sup>+</sup> epidermal cells expressed sialyl Lewis<sup>x</sup> on their cell surface, although the magnitude of its expression was more variable than that of CD1a expression, and that some of human leukocyte antigen DR<sup>+</sup> cells were clearly sialyl Lewis<sup>x</sup>-positive. Immunoperoxidase study of normal skin showed the presence of sialyl Lewis<sup>x</sup>-positive dendritic cells not only in the epidermis but also in the upper dermis. These data demonstrating the heterogeneity of the expression of sialyl Lewis<sup>x</sup> by epidermal Langerhans cells suggest their possible relationship to the stage of maturation as well as to the migration of Langerhans cells from the bone marrow to the skin. Key words: adhesion molecule/ELAM-1/flow cytometry

In this study, we have performed immunofluorescent staining for epithelial sheet preparations, and flow cytometry for epidermal cell suspensions as well as immunoperoxidase staining for skin cryostat sections. We have found weak to strong expression of SLe<sup>x</sup> by most of the human leukocyte antigen (HLA)-DR<sup>+</sup> epidermal LCs and its expression by some dermal cells.

MATERIALS AND METHODS

Antibodies

The antibodies we used were the following: FH6 specific for SLe<sup>x</sup> (a gift from Dr. Y. Fukushi, Department of Urology, Tohoku University School of Medicine, Sendai, Japan), purified or biotinylated anti-HLA-DR antibody (Becton Dickinson, Mountain View, CA), purified or fluorescein isothiocyanate (FITC)--conjugated OX6 (anti-CD1a antibody) (Ortho Diagnostics Systems, Raritan, NJ), FITC-conjugated goat F(ab')<sub>2</sub> fraction of anti-mouse immunoglobulin (IgG) (7 and light chains), biotinylated goat anti-mouse IgG and IgM, Texas red-conjugated goat F(ab')<sub>2</sub> fraction of anti-mouse IgG and IgM, Texas red-conjugated avidin, phycoerythrin (PE)--conjugated avidin (TAGO, Burlingame, CA). Relevant isotype-matched mouse monoclonal antibodies from mouse immunoglobulin standards (PharMingen, San Diego, CA) were used as negative controls.

Epidermal Sheets

Epidermal sheets were prepared from unaffected skin of two adult vitiligo patients and from normal skin from three healthy volunteers with the suction blister (epidermal grafting) method as described previously [18]. All subjects gave informed consent before the examination. The epidermal sheets from vitiligo patients were obtained from a part of uninvolved epidermal sheets during epidermal grafting. Briefly, we raised blisters, approximately 2 cm in diameter, applying a negative pressure of 200 to 400 mm Hg from 20-ml disposable plastic syringes placed on the skin of the abdomen for 1 to 2 h. The edge of the blister was washed in phosphate-buffered saline (PBS), fixed in acetone at room temperature for 20 min, and stored in 1% bovine serum albumin in PBS.

Immunofluorescence Staining of Epidermal Sheet and Enumeration of SLe<sup>x</sup>-Positive Langerhans Cells

Epidermal sheets prepared by the suction blister method from the five subjects were incubated with the above-mentioned different primary antibodies for 3 h at room temperature, washed three times with PBS, and then incubated with FITC-conjugated goat anti-mouse IgG for 1 h at room temperature. After washing three times with PBS...
PBS, the specimens were mounted with PermaFluor (Lipshaw Immunon, Pittsburgh, PA). For two-color immunofluorescence staining, the following combinations of immunofluorescence staining were performed: 1) purified monoclonal antibody (FH6 or isotype-matched control IgM antibody) followed by the incubation with FITC-conjugated goat anti-mouse IgG and biotinylated monoclonal antibody (biotinylated anti-HLA-DR antibody or biotinylated isotype-matched control) followed by reaction with Texas red-conjugated avidin; 2) purified monoclonal antibody (FH6 or isotype-matched control) followed by the incubation with Texas red-conjugated anti-mouse IgG and FITC-conjugated monoclonal antibody (FITC-conjugated anti-CD1a or FITC-conjugated isotype-matched control); and 3) FITC-conjugated monoclonal antibody (FITC-conjugated anti-CD1a or FITC-conjugated isotype-matched control) and biotinylated monoclonal antibody (biotinylated anti-HLA-DR antibody or biotinylated isotype-matched control) followed by reaction with Texas red-conjugated avidin. The specimens were incubated in normal mouse serum for 30 min between each immunofluorescence staining. Finally, we took pictures of three randomly selected different fields at 50X for each two-color immunofluorescence staining and counted more than 50 cells for each field. The data were expressed as the mean ± SD of percent of SLEX+ cells among HLA-DR+ or CD1a+ dendritic epidermal cells.

**Langerhans Cells–Enriched Epidermal Cell Suspensions** A fragment of fresh normal human skin was obtained from an edge of a split-thickness skin graft taken from the normal buttock skin of two patients who had undergone skin surgery. An epidermal sheet was separated by treating it with 0.5% trypsin in PBS at 4°C overnight, and epidermal cell suspensions were obtained after treatment with DNase [19]. They were applied on Ficol-paque density gradient and the interface cells were used as LC-enriched epidermal cell suspensions.

**Two-Color Flow Cytometry Analysis of Langerhans Cells** The LC-enriched epidermal cell suspension was first incubated with FH6, anti-CD1a, or appropriate isotype-matched control monoclonal antibodies on ice for 30 min, washed three times with PBS supplemented with 1% FCS and 0.02% NaN3, and followed by FITC-conjugated goat anti-mouse IgG and IgM. After washing three times with PBS, aliquots were incubated in normal mouse serum and then labeled with biotin-conjugated anti-HLA-DR antibody, followed by phycoerythrin-conjugated avidin. Immunolabeling was performed at 4°C. Fluorescence profiles were generated using a fluorescence-activated cell sorter scan and Consort 30 software (Becton Dickinson).

**Immunoperoxidase Staining of the Skin** Normal human skin was obtained from the surrounding portion of pigmented nevus in two healthy subjects embedded in OCT compound (Miles, Elkhart, IN) and snap frozen in acetone with dry ice. Six-micron cryostat sections prepared from it were fixed in cold acetone for 10 min, washed in PBS, and stained with an avidin-biotin peroxidase method [20]. Briefly, after blocking with 1% horse serum diluted in PBS, sections were serially treated with 1 μg/ml of FH6, anti-CD1a, anti–HLA-DR antibodies or isotype-matched control mouse monoclonal antibodies at 4°C overnight, thereafter with biotinylated anti-mouse IgG for 30 min, and with peroxidase conjugated avidin for 30 min. Each step was followed by washing three times with PBS. The reagents for avidin-biotin peroxidase technique (Histofine SAB-PO(M) kit) was pur-

**Table 1.** Percentage of SLEX+ Cell in Epidermal HLA-DR+ Cells or CD1a+ Cells

<table>
<thead>
<tr>
<th>Case</th>
<th>SLEX+ Cells/DR+ Cells</th>
<th>SLEX+ Cells/CD1a+ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77 ± 17*</td>
<td>73 ± 18</td>
</tr>
<tr>
<td>2</td>
<td>40 ± 28</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>89 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>64 ± 10</td>
<td>ND*</td>
</tr>
</tbody>
</table>

* Mean ± SD.
* ND, Not done
chased from Nichirei, Tokyo, Japan. Finally, the sections were developed with diaminobenzidine solution and 1% hydrogen peroxide, and counterstained with Mayer's hematoxylin.

RESULTS

SLeX+ Cells in Epidermal Sheets The results of two-color immunofluorescent staining using various combinations of anti-HLA-DR, anti-CD1a, and FH6 antibodies performed in epidermal sheets were shown in Figs 1 and 2. All the HLA-DR+ cells or CD1a+ cells were not necessarily SLeX+, whereas all the SLeX+ cells were HLA-DR+ and CD1a+. Actual percentages of SLeX+ reactive cells among HLA-DR+ cells or CD1a+ cells were shown in Table I. The percentages of SLeX+ cells among HLA-DR+ cells and in CD1a+ cells varied from 40% to 77% and from 73% to 89%, respectively.

Flow Cytometry of LC Stained with Two-Color Immunofluorescence Two-color immunofluorescent staining using various combinations of anti-HLA-DR, anti-CD1a, and FH6 was further examined in the epidermal cell suspensions with flow cytometry. The data presented in Fig 3, which are representative data of three different experiments, showed that most of the HLA-DR+ epidermal cells, which were also CD1a+, expressed SLeX+. The level of SLeX+ expression by HLA-DR+ cells was more variable than that of CD1a (Fig 4). Furthermore, some of the HLA-DR+ cells were SLeX-.

SLeX+ Cells in the Skin Immunoperoxidase staining of the skin using FH6 revealed the presence of FH6-reactive (SLeX+) cells not only in the epidermis but also in the upper dermis (Fig 5a). Some keratinocytes also showed weak positive staining. The number of SLeX+ epidermal dendritic cells was fewer than that of HLA-DR+ cells or CD1a+ cells (Fig 5a,b,c). In the dermis, the number of SLeX+ cells was far less than that of HLA-DR+ but almost similar to that of CD1a+ cells (Fig 5a,b,c).

DISCUSSION

Recently, Picker et al [21,22] reported that the skin-infiltrating T cells express cutaneous lymphocyte-associated antigen (CLA) recognized by monoclonal antibody HECA-452, and that this antigen turns out to be the ligand of ELAM-1, suggesting that CLA func-

![Figure 3](image-url) Two-color immunofluorescence analysis of freshly prepared epidermal cell suspension by fluorescence-activated cell sorter. Epidermal cell suspensions were stained with FH6 (x) and anti-HLA-DR (y) (A), isotype-matched control (x) and anti-HLA-DR (y) (B), FH6 (x) and isotype-matched control (y) (C), isotype-matched control (x) and anti-HLA-DR (y) (D), anti-CD1a (x) and anti-HLA-DR (y) (E), isotype-matched control (x) and anti-HLA-DR (y) (F), anti-CD1a (x) and isotype-matched control (y) (G), and isotype-matched control (x) and (y) (H).

![Figure 4](image-url) Histogram of FH6+ cells or CD1a+ cells among HLA-DR+ epidermal cells. These histograms were obtained from the same samples used in Fig 3. A, SLeX+ cells stained with FH6. B, CD1a+ cells.
tions as a homing receptor of the skin-infiltrating T cells. Furthermore, it has been demonstrated that ELAM-1 is expressed on the vascular endothelial cells even in the normal skin, although the expression is weak or moderate [23]. These observations indicate that the interaction with ELAM-1 may play a crucial role in controlling the homing phenomenon of other leukocytes into the skin. Langerhans cells originate from the bone marrow [24]. However, the mechanisms underlying the migration of LCs or the precursors of LCs from bone marrow to the skin have not been clarified yet.

Sialyl Lewis\(^X\), whose expression was first reported in various tumors, has recently been demonstrated to be present on granulocytes, natural killer cells, and immature lymphocytes of T-cell and B-cell lineages [9]. Furthermore, it has been found that SLe\(^X\) functions as a ligand for vascular selectin families, ELAM-1 [10,11] and GMP-140 [12,13]. These observations suggest a role of SLe\(^X\) on leukocytes for their adhesion on vascular endothelial cells.

In this study, we have demonstrated the expression of SLe\(^X\) on most of the CD1a\(^+\) and HLA-DR\(^+\) epidermal dendritic cells in the skin. Furthermore, subsequent flow cytometry also demonstrated that most of HLA-DR\(^+\) and CD1a\(^+\) epidermal dendritic cells expressed SLe\(^X\), although the magnitude of their SLe\(^X\) expression was more variable than that of their CD1a expression. These data suggest a possible mechanism by which LCs or their precursors migrate into the skin through the cutaneous vessels via the interaction between SLe\(^X\) and ELAM-1. Indeed, our subsequent immunoperoxidase staining of normal skin with FH6 showed the presence of the SLe\(^X\) cells not only in the epidermis but also in the upper dermis. Our data by two-color immunofluorescence study using epidermal sheets showed that some of HLA-DR\(^+\) and CD1a\(^+\) epidermal dendritic cells are SLe\(^X\). It is also demonstrated by flow cytometry that some of HLA-DR\(^+\) cells are SLe\(^X\), whereas all the HLA-DR\(^+\) cells are CD1a\(^+\). These data indicated that some of LC are SLe\(^X\)-. Sialyl Lewis\(^X\) has been reported to be expressed on immature lymphocytes of T- and B-cell lineages but to disappear during the process of maturation [9]; its expression on LCs also might be related to the stage of maturation. It is well-known that LCs take two different phenotypic and functional characteristics [25–28]: namely, freshly isolated LCs express weak class II antigen, show weak or minimal allogeneic and syngeneic T-cell stimulatory function and potent antigen processing capacity, whereas cultured LCs express more class II, major histocompatibility complex antigen show vigorous allogeneic and syngeneic T-cell stimulatory function and decreased antigen processing capacity. We have recently reported that even in vivo, epidermal LCs can take these phenotypic and functional characteristics similar to cultured LCs [25]. Thus, SLe\(^X\) expression on LC may be related to such different functional characteristics of LC.

Several authors have reported that keratinocytes also express SLe\(^X\) [17,29]. Indeed, our immunohistologic staining of cryostat sections sometimes revealed epidermal membrane staining of SLe\(^X\), although the magnitude of SLe\(^X\) expression by keratinocytes was much weaker than that by LC (data not shown). Symington et al [29] recently reported that the basal surface of an intact epidermal sheet was stained with anti-SLe\(^X\) antibody. From their paper, it is not clear how they prepared those specimens. By using the same anti-SLe\(^X\) antibody, however, our immunohistologic staining could not clearly reveal SLe\(^X\) expression by keratinocytes, whereas dendritic cells were brightly stained. Even in our flow cytometry study, we could not demonstrate the expression of SLe\(^X\) by keratinocytes, which should be HLA-DR–SLe\(^X\). However, we cannot rule out the possibility that the Ficol-paque density gradient of epidermal cell suspensions performed to enrich epidermal dendritic cells might lose SLe\(^X\)- keratinocytes.

**Figure 5.** Expression of SLe\(^X\), CD1a, and HLA-DR in normal skin. Cryostat skin sections were stained with each antibody for FHG (a), HLA-DR (b), CD1a (c), and with isotype-matched control (d). Bar, 100 \(\mu\)m.
REFERENCES


ANNOUNCEMENT

Cutaneous Malignancies: 1994 Skin Cancer Update will be held Friday to Sunday, January 21–23, 1994, sponsored by Scripps Clinic and Research Foundation, at the Sheraton Grande Torrey Pines Hotel, La Jolla, California.

This course is designed for dermatologists, oculoplastic surgeons and ophthalmologists, head and neck surgeons, plastic surgeons, family practice physicians, and other physicians with an interest in skin cancers. This course will provide an update on cutaneous neoplasms, melanoma, and facial reconstruction. The combination of lectures and optional hands on cadaver laboratory sessions will expose the attendee to the latest information and techniques. The course will offer 17 hours of category I CME credits.

For further information contact Department of Academic Affairs, 403C, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Road, La Jolla, CA 92037. Telephone: (619) 554-8556; FAX: (619) 554-6310.