E-Selectin and Vascular Cell Adhesion Molecule-1 Mediate Adult T-Cell Leukemia Cell Adhesion to Endothelial Cells

By Takayuki Ishikawa, Akihiro Imura, Kyoko Tanaka, Hirohumi Shirane, Minoru Okuma, and Takashi Uchiyama

We studied the adhesion properties of peripheral blood leukemic cells from 10 patients with adult T-cell leukemia (ATL) to endothelial cells to better understand the mechanism of leukemic cell infiltration. ATL cells expressed lymphocyte function-associated antigen-1 (LFA-1), but the expression of very late antigen-4 (VLA-4) and sialyl-Lewisα (SLex) was marked. They did not express sialyl-Lewisβ (SLeα). Cell adhesion assays, which were performed in nine patients, showed marked adhesion of ATL cells to intercellular [IL]-1–activated human umbilical vein endothelial cells (HUVEC). A monoclonal antibody (MoAb) against E-selectin consistently inhibited ATL cell adhesion, and an MoAb against vascular cell adhesion molecule-1 (VCAM-1) or an MoAb against VLA-4 sometimes diminished it. In contrast, an MoAb against LFA-1 had a minor effect on freshly isolated ATL cell adhesion to HUVEC. The percentage of SLex+ cells in the cell population adherent to IL-1–activated HUVEC was slightly higher than that in unseparated cells. These results, together with the detection of E-selectin expression on the endothelium at ATL skin lesions, indicate that E-selectin–mediated adhesion is the major pathway for the adherence of ATL cells to endothelial cells. In addition, the ligand for E-selectin on ATL cells appears to differ from that on neutrophils.

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MATERIALS AND METHODS

Monoclonal antibodies (MoAbs). Purified IgG MoAbs, anti-Tac (anti-CD25) and 2R-B (anti-interleukin [IL]-2 receptor β chain), were prepared as reported previously.12,13 OKT4 (anti-CD4), OKT8 (anti-CD8), OKM1 (anti-CD11b), OK1a (anti-HLA class-2), and X63 (control IgG) were obtained from the American Type Tissue Culture Collection (Rockville, MD). 3G8 (anti-CD16) was a gift from Dr J.C. Unkeles14 (Mount Sinai Medical Center, New York, NY). HP2/1 (anti-CD49d, the α chain of VLA-4), 84H10 (anti-CD54, ICAM-1), and J-173 (anti-CD44) were purchased from Immunotech (Marseille, France). 3B7, 7A9 (anti-E-selectin) and 2G7 (anti-VCAM-1) were kindly provided by Dr W. Newman15 (Otsuka America Pharmaceutical, Rockville, MD). 3B7 and 7A9 were shown to be equally effective in blocking E-selectin–mediated leukocyte adhesion to HUVEC. Leu-8 (anti-LECAM-1), Leu-18 (anti-CD45RA), UCHL1 (anti-CD45RO), and B1 (anti-CD20) were obtained from Becton-Dickinson Immunocytochemistry Systems (Mountain View, CA), Nichirei (Tokyo, Japan), and Coulter Immunology (Hialeah, FL), respectively. All of the MoAbs listed above were purified IgG antibodies, MMH23 (anti-CD18, the β chain of LFA-1 and Mac-1), MMH24 (anti-CD11a, the α chain of LFA-1), and TUK4 (anti-CD14) were purchased from Dakopatts (Glostrup, Denmark) as hybridoma culture supernatants. Two anti-sialyl Lewisα MoAbs (2D3 [a purified IgM antibody] and 1H4 [a purified IgG antibody]), as well as two anti-sialyl Lewisα MoAbs (FH6 [a purified IgM antibody] and SNH3 [an IgM antibody, hybridoma culture supernatant]) were gifts from Dr R. Kannagi (Aichi Cancer Research Institute, Nagoya, Japan). Another anti-sialyl Lewisα mAb (CSLEX-1 [an IgM antibody, ascitic fluid]) were ob-
E-SELECTIN IN ATL CELL ADHESION

Lymphocyte preparation. Peripheral blood leukemic cells from 10 ATL patients were studied. The diagnosis of ATL was made based on the clinical features, hematologic findings (including the characteristic surface expression of CD25; Tac antigen, serum antibodies against HTLV-1, and, in some cases, monoclonal HTLV-1 provirus integration into leukemic DNA. Peripheral blood mononuclear cells were isolated by Ficoll-Conray density gradient centrifugation. In all cases, the percentage of leukemic cells determined by both CD4+ and CD25+ cells and used for each assay was greater than 90%. In cases where the initial purity of the isolated leukemic cells was low, negative immunoselection with magnetic beads was performed. To purify the ATL cells, peripheral blood mononuclear cells were incubated for 30 minutes at 4°C with a cocktail of MoAbs against CD8, CD11b, CD14, CD16, and CD20. After two washes, immunomagnetic beads (Dynabeads M-450, Dynal, Oslo, Norway) were added and incubated for 1 hour at 4°C to remove non-ATL cells, which were then removed with a Dynal magnetic particle concentrator. The proportion of the CD4+ cells and CD25+ cells in the negatively selected cells was greater than 99% and 95%, respectively. Since the ATL cells from case no. 9 were positive for CD8, as well as CD4, no MoAb against CD8 was used when purifying cells from this patient and the purity of ATL cells obtained also exceeded 95%. In some experiments, cryopreserved ATL cells, thawed just before each assay, were used. Resting CD4+CD45RO+ T cells from healthy volunteers were purified by the same negative immunoselection technique using additional MoAbs against HLA-DR and CD45RA. The percentage of the resting CD4+CD45RO+ T cells was approximately 90%. Lymphocytes prepared in this manner were labeled with 51Cr and used for the adhesion assays. To obtain acutely activated T cells, 10 ng/mL of IL-1-β for 4 hours at 37°C. After washing out the IL-1, lymphocytes (1 x 10^5 cells in a final volume of 10 mL) were added and incubated with the HUVEC for 20 minutes at 37°C. The nonadherent cells were then removed by gentle washing with prewarmed RPMI 10% FCS, and the remaining adherent cells were harvested by rinsing twice with PBS containing 5 mMol/L EDTA. After the last rinse with PBS/EDTA, few lymphocytes were detected microscopically.

Flow cytometric analysis. Cells (2 x 10^5 in 20 μL) were stained with a saturating amount of unconjugated MoAb followed by incubation with the fluorescein isothiocyanate (FITC)-conjugated (Fab')2 fraction of goat antimouse IgG (Tago, Burlingame, CA) or rabbit antimouse IgM (Zymed, San Francisco, CA). When contaminating non-ATL cells were thought likely to influence the results, further incubation was performed with a phycoerythrin-conjugated IL-2 receptor antibody (CD25, Becton-Dickinson Immunocytochemistry Systems) after all unoccupied binding sites of the secondary antibodies were blocked with normal mouse serum. The stained cells were analyzed using a FACScan (Becton-Dickinson Immunocytochemistry Systems), and when dual-color analysis was performed, lymphocytes were gated based on the expression of CD25. The quantitation of the cell populations described in this report was based on cutoff points designed to exclude 95% or more of the negative control population.

Immunohistochemistry. Skin tissue specimens were obtained from cases no. 1 and 10 for immunohistochemical examination. The specimens were divided into two pieces, one fixed in formalin and the other frozen at –80°C. Then, serial, acetone-fixed, air-dried cryostat sections (3 to 5 μm) were prepared from the snap-frozen tissue specimens and stained using a HISTOSET Immunoperoxidase Staining Kit (Ortho Diagnostic Systems, Raritan, NJ) according to the manufacturer’s directions.

RESULTS

Expression of adhesion molecules on ATL cells. Table 1 shows the clinical profiles of the patients examined. Nine of 10 patients had evidence of organ involvement, which was diagnosed mainly from the clinical findings and was histologically confirmed in cases no. 1 and 10 (skin), as well as case no. 7 (lung). ATL cells were positive for CD4, CD25, and the β chain of the IL-2 receptor in all patients (data not shown). CD45RO was also positive, except in case no. 8, in which 70% of the ATL cells were CD45RO+. Therefore, we compared the ATL cells with normal CD4+CD45RO+ memory T cells in the following experiments. ATL cells from all

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cases expressed CD11a (LFA-1), and its fluorescence intensity was equivalent to that of normal memory T cells. The percentage of CD49d+ (VLA-4) cells and its fluorescence intensity were not different between normal memory T cells and ATL cells from six patients (cases no. 2, 3, 4, 6, 7, and 9). VLA-4 was not expressed in two patients (cases no. 8 and 9). In case no. 4, the addition of anti-E-selectin MoAb (3B7 and/or 7A9) also reduced the adhesion of ATL cells to activated HUVEC, which appeared to be the reason for the failure of adhesion inhibition by the anti-VLA-4 or anti-VCAM-1 MoAbs. The addition of anti-E-selectin MoAbs (3B7 and/or 7A9) also reduced the adhesion of ATL cells to activated HUVEC in six patients (cases no. 2, 3, 5, 6, 7, and 8). In case no. 4, the combination of an anti-VCAM-1 MoAb and an anti-E-selectin MoAb inhibited ATL cell adhesion more strongly than addition of the anti-VCAM-1 MoAb alone, indicating that an E-selectin-mediated adhesion pathway was also present. In case no. 9, the addition of an anti-E-selectin MoAb to the combination of anti-LFA-1 and anti-VLA-4 MoAbs dramatically decreased ATL cell adhesion to IL-1-activated HUVEC. Thus, an E-selectin-mediated pathway was detected in all patients tested. The degree of adhesion inhibition by anti-VCAM-1 or anti-VLA-4 MoAbs and that by anti-E-selectin MoAbs were not consistent in each case. In other words, the inhibition by anti-VCAM-1 or anti-VLA-4 MoAbs was more marked than by anti-E-selectin MoAb in cases no. 4, 6, and 7, and vice versa in cases no. 2, 3, 5, and 8.

In contrast, the addition of an anti-LFA-1 MoAb (MHM23) alone had no effect on ATL cell adhesion to resting or IL-1-activated HUVEC. In cases no. 6 and 7, the combination of anti-VCAM-1 and anti-E-selectin MoAbs inhibited ATL cell adhesion with IL-1-activated HUVEC almost completely, and the addition of the anti-LFA-1 MoAb to this combination of MoAbs had no effect on ATL

### Table 1. Clinical Features of the 10 ATL Patients and Adhesion Molecule Expression on Their ATL Cells

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Clinical Stage</th>
<th>Lung</th>
<th>Skin</th>
<th>Other</th>
<th>CD11a (M2)</th>
<th>CD49d (HP2/1)</th>
<th>LECAM-1 (Leu-8)</th>
<th>SLex</th>
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<tbody>
<tr>
<td>1</td>
<td>Crisis*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>99.9</td>
<td>30.3t</td>
<td>95.5</td>
<td>10.9</td>
</tr>
<tr>
<td>2</td>
<td>Chronic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>97.9</td>
<td>6.4</td>
<td>42.3</td>
</tr>
<tr>
<td>3</td>
<td>Chronic</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>99.9</td>
<td>98.6</td>
<td>97.7</td>
<td>70.6t</td>
</tr>
<tr>
<td>4</td>
<td>Acute</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>99.9</td>
<td>99.9</td>
<td>98.7</td>
<td>3.2</td>
</tr>
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<td>+</td>
<td>+</td>
<td>99.9</td>
<td>8.3t</td>
<td>58.51</td>
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</tr>
<tr>
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<td>Lymphoma</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>98.1</td>
<td>82.3</td>
<td>87.9</td>
<td>34.3t</td>
</tr>
<tr>
<td>7</td>
<td>Chronic</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>99.5</td>
<td>98.4</td>
<td>96.7</td>
<td>8.5</td>
</tr>
<tr>
<td>8</td>
<td>Chronic</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>99.9</td>
<td>5.6</td>
<td>61.11</td>
<td>65.8t</td>
</tr>
<tr>
<td>9</td>
<td>Crisis</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>99.7</td>
<td>87.9</td>
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<td>+</td>
<td>99.6</td>
<td>1.8</td>
<td>61.81</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.
* Crisis means acute transformation from chronic ATL.
† Mean fluorescence intensity of positive cells was lower than that of healthy control cells.
‡ Strongly positive cells exceeded 20%.

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cell adhesion (Table 2). In case no. 8, there was moderate inhibition of adhesion by the anti-E-selectin MoAb, while the combination of anti-LFA-1, anti-VLA-4, and anti-E-selectin MoAbs resulted in a profound decrease of cell adhesion (Table 2). Considering that VLA-4 expression was hardly detectable in this patient, LFA-1-mediated adhesion appeared to occur. In case no. 9, although anti-LFA-1 or anti-VLA-4 MoAbs did not diminish cell adhesion when either one was added alone, the combination of these two MoAbs decreased cell adhesion (Table 2).

To investigate further the role of LFA-1-mediated ATL cell adhesion to HUVEC, freshly isolated ATL cells were modified by short-term culture or by exposure to PMA. In case no. 5, the adhesion of cultured ATL cells to IL-1-activated HUVEC was clearly inhibited by the addition of anti-LFA-1 MoAb, although this did not occur with freshly isolated cells (Fig 1). In case no. 7, PMA-stimulated ATL cells showed adhesion to resting HUVEC and this adhesion was almost completely inhibited by the anti-LFA-1 MoAb. PMA-stimulated ATL cells also adhered well to IL-1-activated HUVEC, and the sole addition of the anti-LFA-1 MoAb slightly inhibited this adhesion (Fig 1). These results suggested that the LFA-1-mediated adhesion pathway was not fully functional in freshly isolated ATL cells.

As described above, the ATL patients displayed considerable variability in the adhesion characteristics. Therefore, it seemed important to know whether reproducible results could be obtained from the same patient when examined at different times. In case no. 5, we obtained blood samples at two different times with a 1-month interval before the administration of anticancer drugs, and found that the surface expression of adhesion molecules did not differ between these two samples (data not shown). The ATL cells were cryopreserved and thawed just before assay, but the freezing and thawing procedures did not affect cell viability or surface phenotype (data not shown). As shown in Fig 2, the frozen and thawed cells displayed a somewhat increased adhesion to resting HUVEC, which could not be blocked by the anti-LFA-1 MoAb. Adhesion to IL-1-activated HUVEC occurred via E-selectin-mediated pathway on both occasions, and VLA-4- or LFA-1-mediated adhesion were not observed despite different levels of cell adhesion, which still remained after the blocking by any combination of MoAbs tested.

**Table 2. Effects of Antibodies Against Adhesion Molecules on the Adhesion of Freshly Isolated ATL Cells to HUVEC**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Control</th>
<th>LFA-1</th>
<th>Control</th>
<th>LFA-1</th>
<th>VLA-4</th>
<th>E-selectin</th>
<th>VLA-4 + E-selectin</th>
<th>LFA-1 + VLA-4</th>
<th>LFA-1 + E-selectin</th>
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<td>1</td>
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<td>ND</td>
<td>19.6</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>ND</td>
<td>17.4</td>
<td>ND</td>
<td>ND</td>
<td>11.8*</td>
<td>7.4</td>
<td>5.9*</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>6.8</td>
<td>ND</td>
<td>38.4</td>
<td>ND</td>
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<td>21.2</td>
<td>14.6*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>30.8</td>
<td>ND</td>
<td>ND</td>
<td>25.1*</td>
<td>27.5</td>
<td>19.9*</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>ND</td>
<td>74.3</td>
<td>74.1</td>
<td>ND</td>
<td>73.8*</td>
<td>24.6</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>6</td>
<td>4.2</td>
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<td>31.8</td>
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<td>6.4*</td>
<td>ND</td>
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<tr>
<td>7</td>
<td>7.4</td>
<td>7.9</td>
<td>48.8</td>
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<td>8.3*</td>
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<td>9.3</td>
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<td>ND</td>
<td>31.1</td>
<td>12.6</td>
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Data are the mean values of duplicate or triplicate wells, and show percentage adherence to HUVEC. Abbreviation: ND, not determined.

* An anti-VCAM-1 MoAb was used instead of an anti-VLA-4 MoAb.

![Fig 1. Effects of short-term culture or activation with PMA on the adhesion of ATL cells to HUVEC. Freshly Isolated ATL cells were cultured with growth medium for 48 hours at 37°C (case no. 5), or were stimulated by 10 ng/mL PMA for 30 minutes during 51Cr labeling (case no. 7). The adhesion of ATL cells to HUVEC was assayed in the presence of mAbs against various adhesion molecules at a concentration of 10 μg/mL, except for the control MoAb (30 μg/mL). Data show the mean percentage of adherent cells in duplicate wells. The MoAbs used were MHM 23 (anti-LFA-1), 2G7 (anti-VCAM-1), 3B7 and/or 7A9 (both anti-E-selectin), and X63 (control).](image-url)
with or without adherence to HUVEC. In case no.
there was a slight increase of SLex+ cells among the adher-
ent population of both patients, although a significant pro-
portion of the adherent cells were SLex-. The expression of
LFA-1 was uniformly observed and there were no differ-
ences in the expression of LFA-1 between the cells with or
without adherence to IL-1-activated HUVEC (data not shown).

**Immunohistochemical examination.** In cases no. 1 and
10, biopsy samples of skin lesions were examined to deter-
mine the expression of adhesion molecules on the endotho-
eelial cells. Sections from formalin-fixed specimens were
stained with hematoxylin/eosin, which showed the diffuse
infiltration of atypical lymphocytes into the upper dermis
and focal infiltration into the deep dermis in both patients
data not shown). Immunohistochemical staining of cryo-
stat sections showed that the infiltrating lymphocytes were
CD4+ (data not shown) and CD25+ (Fig SA and D), indicat-
ing that they were ATL cells. Some of the small vessels in
the dermis were strongly stained with an anti-E-selectin mAb,
BBA 1 (Fig 5C and F), and more of the vessels were also
stained with the anti-ICAM-1 MoAb, 84H10 (Fig 5B and
E). The infiltrating lymphocytes were also stained with
84H10 in both patients, which was compatible with the flow
cytometric findings (data not shown). In contrast, none of
the vessels were reactive with the anti-VCAM-1 MoAb,
BBA 5, in either patient (data not shown).

**DISCUSSION**

The adhesion of ATL cells to endothelial cells is appar-
ently one of the critical events in leukemic cell infiltration

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**Fig 2. Adhesion to HUVEC of ATL cells from the same patient at
different times. Peripheral blood leukemic cells from case no. 5
were isolated and frozen in liquid nitrogen at two different times
with a 1-month interval. The cells were thawed just before assay,
and their viability exceeded 95%. The adhesion of 51Cr-labeled cells
was examined in the presence of various MoAbs. Data show the
mean percentage of adherent cells in triplicate wells, and indicate SD.
HP2/1 was used for an anti-VLA-4 MoAb.

**Expression of adhesion molecules on ATL cells with or
without adherence to HUVEC.** In cases no. 1, 2, and 5, we
compared the cell surface expression of adhesion molecules
in the cells that did and did not adhere to IL-1-activated
HUVEC (Fig 4). In case no. 1, the adhesion blocking study
with cryopreserved and thawed ATL cells showed that the
E-selectin-mediated adhesion pathway was predominant,
and that LFA-1- and VLA-4-mediated adhesions did not
occur (data not shown). However, we could detect only
weak expression of SLex on the cells adherent to HUVEC.
There was no difference in VLA-4 expression between cells
with or without adherence to HUVEC. In case no. 2, the
VCAM-1-mediated pathway played an important role in
the ATL cell adhesion to activated HUVEC, and in case no.
5, the E-selectin-mediated pathway was predominant.
There were a slight increase of SLex+ cells among the adher-
ent population of both patients, although a significant pro-
portion of the adherent cells were SLex-.
into various organs. We used HUVEC monolayers to investigate the molecules involved in ATL cell-endothelial cell interactions, because such monolayers can be prepared easily and the adhesion molecules on HUVEC have been intensively studied.

The ATL cells from nine patients showed adhesion to IL-1-activated HUVEC, but adhesion to resting HUVEC was seen in only two cases. The blocking studies were performed using freshly isolated ATL cells from eight patients, and showed that VLA-4 (or VCAM-1) and E-selectin mediated the adhesion of ATL cells to IL-1-activated HUVEC (Table 2). Despite its uniform expression on ATL cells, the contribution of LFA-1 to cell adhesion was slight or nonexistent. Although the adhesion characteristics of ATL cells, as well as their expression of adhesion molecules, differed considerably from patient to patient, the assay results were shown to be almost identical in the same patient at different times (Fig 2). E-selectin-mediated adhesion was detected in all of the assessable patients, which suggests that E-selectin plays a pivotal role in ATL cell/endothelial cell interactions. The finding that E-selectin was expressed on the endothelial cells in cutaneous ATL lesions also supports this notion (Fig 5).

It seems unlikely that VLA-4 plays a major role in the adhesion of ATL cells to endothelial cells in vivo because of the following findings, even though some VLA-4- or VCAM-1-mediated adhesion to IL-1-activated HUVEC was detectable in many patients. First, VLA-4 was not expressed consistently on ATL cells. In fact, organ infiltration was detected in all four patients in whom the expression of VLA-4 was not demonstrated or was observed in only a small proportion of the ATL cells (Table 1). Second, VLA-4+ cells were not concentrated in the cell population adherent to HUVEC (Fig 4). Finally, the immunohistochemical studies of cases no. 1 and 10 did not detect VCAM-1 molecules on the endothelial cells at the skin where ATL cells infiltrated.

The LFA-1 expressed on the freshly isolated ATL cells did not contribute to adhesion to HUVEC in cases no. 6 and 7, although a slight to moderate contribution was observed in cases no. 8 and 9 (Table 2). The results obtained in cases no. 5 and 7 (Fig 1) indicated that the LFA-1 molecules expressed on freshly isolated ATL cells did not function properly and that some stimulus is needed for them to fully function as ligands of ICAMs.

E-selectin was first recognized as an endothelial cell surface antigen induced by cytokines and endotoxins, and its expression is known to be limited to the venous endothelium at sites of inflammation. In contrast to its wide distribution in acute inflammation, the expression of E-selectin is normally limited to the venous endothelium of the skin in chronic inflammation. Short-term skin organ culture has shown that E-selectin is readily expressed on endothelial cells in vitro, which suggests that its expression on the venous endothelium is easily inducible in the skin. It was reported that ATL cells produce several kinds of cytokines, including IL-1, Thus, the frequent skin involvement in ATL might be partly explained through the production of
cytokines by ATL cells, which then induce the E-selectin expression on the venous endothelium in the skin and subsequent E-selectin-mediated adhesion of ATL cells. Several sialylated carbohydrates are known to be ligands for E-selectin. The glycoproteins containing SLex are thought to be the ligands of E-selectin in neutrophils. SLex and SLex have been reported to be the counter-receptors for E-selectin binding in some cancer cell lines that adhere to IL-1-activated HUVEC via an E-selectin-mediated pathway. The expression of SLex on ATL cells was not detected in our nine patients, and SLex was not consistently detectable on the ATL cells (Table 1). Significant E-selectin-mediated adhesion to HUVEC was observed in case no. 4, although only a low percentage of ATL cells was positive for SLex (Tables 1 and 2). The population of SLex+ cells was not increased among the ATL cells adherent to HUVEC in cases no. 1 and 5, despite the predominant role of the E-selectin-mediated pathway in ATL cell-HUVEC adhesion (Table 2 and Fig 4). There seems to be two possibilities regarding the controversial results: (1) SLex expressed on ATL cells was not fully recognized by the MoAbs used in this study. A previous study has demonstrated the different reactivity of anti-SLex MoAbs with leukemic cell lines. Examination using other MoAbs may detect SLex epitope on ATL cells. (2) A carbohydrate other than SLex or SLex is the ligand for E-selectin on ATL cells. Recent reports have indicated that the cutaneous lymphocyte antigen defined by HECA-452 MoAb, which is another sialylated carbohydrate antigen and closely associated with SLex, is E-selectin ligand for memory T cells. The E-selectin ligand for ATL cells may be this cutaneous lymphocyte antigen, since the majority of ATL cells have the same phenotype as memory T cells. In any case, the ligand for E-selectin appears to be different from that of neutrophils.

The majority of ATL cells express CD25 (Tac antigen) and HLA-DR. In addition, the ATL cells from 20% to 30% of patients proliferate in response to IL-2 and/or IL-4 without any other stimuli. These observations indicate that ATL cells share some of the characteristics of activated T cells. Because the adhesion of ATL cells to IL-1-activated HUVEC was mostly mediated through E-selectin and partly through VLA-4, they resembled resting CD4+CD45RO+ memory T cells, rather than PMA-stimulated memory T cells in this respect. The major difference in the adhesion properties between ATL cells and PMA-stimulated memory T cells was the inability of the LFA-1 molecules on ATL cells to effectively bind with ICAMs. Thus, the activated status of ATL cells appears to be different from that of PMA-stimulated memory T cells.

In neutrophils, the interaction of SLex/E-selectin is thought to be responsible for leukocyte trapping, and the interaction of LFA-1 and Mac-1/ICAMs are considered to be the key events in their firm attachment and transendothelial migration. In T cells, the molecules responsible for
the transmigration have been reported to be LFA-1 and VLA-4. One can then ask what molecules are responsible for the transmigration of ATL cells, especially those which do not express VLA-4 molecules. There seem three possibilities on this issue. First, LFA-1 molecules on ATL cells would acquire the ability to bind to ICAMs in the course of cell adhesion through a mechanism that was not reproduced using HUVEC as the endothelial cells. The induction of ICAM-1, as well as E-selectin expression, on the endothelial cells in ATL skin lesions supports this idea. Second, E-selectin may play a predominant role in both the adhesion and transendothelial migration of ATL cells. Third, there may be other as yet unidentified molecules that are responsible for ATL cell transmigration. Further studies in this area should help to elucidate the mechanism of ATL cell infiltration and perhaps allow the development of new therapeutic approaches to prevent it.

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