Differentiation-Dependent Expression of Sialyl Stage-Specific Embryonic Antigen-1 and I-Antigens on Human Lymphoid Cells and Its Implications for Carbohydrate-Mediated Adhesion to Vascular Endothelium

By Katsuyuki Ohmori, Akiko Takada, Tomoya Yoneda, Yumiko Buma, Kunimi Hirashima, Kiyotaka Tsuyuoka, Akira Hasegawa, and Reiji Kannagi

Expression of two developmentally regulated carbohydrate antigens, the sialyl stage-specific embryonic antigen-1 (SSEA-1) and I-antigens, in human lymphocytes and lymphocytic leukemia cells was investigated using specific monoclonal antibodies. Sialyl SSEA-1 was expressed only on natural killer (NK) cells, and was essentially absent on resting mature T and B cells among normal peripheral lymphocytes. On the other hand, the I-antigen was strongly expressed on virtually all mature B cells, moderately expressed on most mature T cells, but not expressed on NK cells in normal donors. Expression of the two antigens on normal T and B cells was reversible; in vitro stimulation of normal lymphocytes with concanavalin A (Con A) resulted in the loss of I-antigen and appearance of sialyl SSEA-1 on CD3+ T blasts, whereas stimulation with pokeweed mitogen led to loss of I-antigen expression and appearance of sialyl SSEA-1 antigen on CD19+ B blasts. Among lymphocytic leukemia cells, sialyl SSEA-1 was detected primarily on leukemia cells having immature properties such as most common acute lymphocytic leukemia (CALL) blasts, while the I-antigen was frequently expressed on malignant cells having relatively mature properties, such as those found in adult T-cell leukemia or chronic lymphocytic leukemia, and only occasionally on CALL blasts. Among normal peripheral lymphocytes, the sialyl SSEA-1 ‘I-antigen’ NK cells selectively underwent E-selectin (ELAM-1, endothelial-leukocyte adhesion molecule-1)-dependent adhesion to endothelial cells, while the I-antigen ‘sialyl SSEA-1’ mature T and B cells did not, in line with the recent finding that sialyl SSEA-1 serves as a specific ligand for E-selectin. Con A blasts, which are sialyl SSEA-1 ‘I-antigen’, also exhibited significant E-selectin-dependent adhesion to endothelial cells. These results indicate that expression of the sialyl SSEA-1 and I-antigens varies alternately depending on the differentiation/activation status of the lymphocytes, and that this at least partly regulates the behavior of lymphocytes at the vessel wall.

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MATERIALS AND METHODS

MoAbs used for flow cytometric analysis. MoAbs FH-610,14 (specific to sialyl SSEA-1, supplied by Dr Sen-itiroh Hakomori, Biomedical Institute, Seattle, WA) and C615 (specific to I-antigen, supplied by Dr Bruce A. Fenderson, Washington University, Seattle) are both murine IgM and were purified from ascitic fluids as described previously.1113-15 The carbohydrate structures of the sialyl SSEA-1 and I-antigens and the specificities of the MoAbs are summarized in Table 1. The FH-6 antibody reacts specifically to the carbohydrate sequence carrying sialyl Lea terminus that resides on the i-antigenic structure; hence, the antigenic epitope is called sialyl Lea.14

Among the antibodies used for the cell surface marker analysis, Leu 4 (CD23) for T cells, Leu 12 (CD19), Leu 16 (CD20) for B cells, Leu 11 (CD16), Leu 19 (CD56) for NK cells, and the antibodies against IL-2 receptor, HLA-DR, and HPCA-1 were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA). The antibody J5 was supplied from the Coulter Immunology Division (Hialeah, FL), and the anti-μ, γ, δ, κ, λ antibodies were from Tago Immunonochemicals (Burlingame, CA).

From the Department of Laboratory Medicine, Kyoto University, School of Medicine, Kyoto; the Laboratory of Experimental Pathology, Aichi Cancer Center Research Institute, Nagoya; and the Department of Applied Bio-organic Chemistry, Gifu University, School of Agriculture, Gifu, Japan.

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Address reprint requests to Reiji Kannagi, MD, PhD, Laboratory of Experimental Pathology, Research Institute, Aichi Cancer Center, I-1 Kanokoden, Chikusa, Nagoya, 464, Japan.

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Preparation of cells and fluorescence-activated flow cytometry. Peripheral blood samples were obtained from healthy donors and patients with nonhematologic disorders or patients with lymphoid malignancies at Kyoto University Hospital. Lymphocytes were isolated from Ficoll/Paque (Pharmacia, Uppsala, Sweden) by standard methods. For stimulation of peripheral lymphocytes, lymphocytes were cultured at 1 × 10^6/mL in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) containing 5 pg/mL of concanavalin A (Con A; Pharmacia) for 96 hours, or containing 1% of the pokeweed mitogen (PWM) solution supplied by Life Technologies, Inc (Grand Island, NY) for 96 hours.

Cultured human lymphoid cell lines (Raji, FL-318, NALM-6, YT, MOLT-3, and ATL-2) were obtained from the First Division of Department and Internal Medicine, Kyoto University, and MOLT-15 and P12/Ichikawa were kindly supplied by Dr Jun Minowada (Fujiwaki Cell Center, Hayashibara Biological Research Institute, Okayama, Japan). These cells were cultured in RPMI 1640 medium supplemented with 10% FCS.

Flow cytometric analysis of lymphocytes was performed using FACScan (Becton Dickinson). The indirect immunofluorescence method was applied for staining of lymphocytes with the antibodies C6 and FH-6, using a fluorescein isothiocyanate (FITC)-labeled rabbit antimurine IgM (μ-chain specific antibody) as the secondary antibody (Cappel Inc, Malvern, CA). Contamination of monocytes in the lymphocyte preparation in the flow cytometric analysis was checked for by staining with an anti-CD14 antibody (Leu M3), and was less than 1% in all analyses described in this report.

Monolayer cell adhesion assay using human umbilical vein endothelial cells (HUVECs). HUVECs (2 to 6 passages after isolation; obtained from Kurabo Co Ltd, Osaka, Japan) were stimulated with 10 ng/mL of recombinant (r) IL-1β for 4 hours in 24-well plates. To these plates, lymphocytes prepared from peripheral blood (5 × 10^5/0.5 mL/well) or Con A blasts (8 × 10^5/0.5 mL/well) were added and incubated for 20 minutes at room temperature with rotation (100 rpm). Flat-bottomed 96-well plates were used in the case of leukemia cells (1 × 10^6/60 µL/well) and incubation was performed for 30 minutes at room temperature with rotation (120 rpm). The number of attached cells was counted directly under a microscope.

MoAbs used for inhibition of cell adhesion. Monoclonal anti-E-selectin antibody (ICAM-1, 022A-1 and BRA4, both murine IgG1) were obtained from British Biotechnology Ltd (Abington, Oxon, UK), and these antibodies, when used, were preincubated with HUVECs at 50 µg/mL for 30 minutes at room temperature before the adhesion experiments with lymphocytes.

Table 1. Carbohydrate Structures of Immunodominant Epitopes of the Sialyl SSEA-1 and I-Antigens, and Specific MoAbs Used in This Study

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Structure of Immunodominant Epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialyl</td>
<td>FH-6</td>
<td>NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3R</td>
</tr>
<tr>
<td>SSEA-1</td>
<td>(μM)</td>
<td>Fucα1-3*</td>
</tr>
<tr>
<td>I-antigen</td>
<td>C6</td>
<td>Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3R</td>
</tr>
<tr>
<td></td>
<td>(μM)</td>
<td>Galβ1-4GlcNAcβ1-3R</td>
</tr>
</tbody>
</table>

* The symbol ± indicates that the second fucose residue is not strictly required for the antibody to react with the antigen. The antibody FH-6 was initially raised against the sialyl Le⁺ carried by polylactosamine with α1 → 3 internal fucosyl substitution, but later turned out to react also with sialyl Le⁺ carried by a polylactosamine with at least two repeating N-acetyllactosamine units.

RESULTS

Expression of the sialyl SSEA-1 and I-antigens among peripheral lymphocytes in normal individuals. The carbohydrate antigen sialyl SSEA-1 was expressed only in a very minor population (7.6% ± 4.6% [n = 60]) of peripheral lymphocytes of healthy individuals, when the FH-6 antibody was used for the flow cytometry. These sialyl SSEA-1⁺ cells were confirmed to be essentially NK cells (Fig 1A), as reported by us earlier.

The antigen was absent on normal resting T cells (both CD4⁺ and CD8⁺ cells) and B cells.

On the other hand, a majority of peripheral blood lymphocytes in healthy individuals expressed the I-antigen as detected using the antibody, C6. The mean ± SD of the percentages of the I-antigen⁺ cells in healthy donors were 67.9% ± 6.9% (n = 52). As shown in a typical cytofluorogram depicted in Fig 2A, the I-antigen⁺ cells consist of two peaks; one small population, indicated by the arrow A, exhibited a very high antigen density, while the other population, as indicated by the arrow B, displayed a relatively low antigen density. In normal individuals, the I-antigenⁿ⁺ cells comprised 12.6% ± 3.5% and the I-antigenⁿ⁻⁺ cells comprised 56.2% ± 8.2% on average (n = 52).

Two-color analysis indicated that the I-antigenⁿ⁺ cells were B cells that were CD19⁺ (Fig 2A). In fact, more than 95% of B cells in all of the tested healthy individuals strongly expressed the I-antigen. On the other hand, the I-antigenⁿ⁻⁺ cells were T cells (CD3⁺ and CD4⁺) and NK cells (CD16⁺ and CD56⁺) (Fig 3B).
Fig 1. Expression of sialyl SSEA-1 in normal peripheral lymphocytes (a), peripheral lymphocytes containing activated T cells (b), and Con A-stimulated lymphocytes (c). In each panel, the uppermost panel shows a cytofluorogram of the sialyl SSEA-1+ cells, and the lower three panels show the results of two-color analysis. Lymphocytes were prepared from the peripheral blood of a healthy donor in panels a and c, and from a patient with a nonhematologic disorder in panel b. Sialyl SSEA-1 was detected with the FH-6 antibody, which is specific to sialyl Le	extsuperscript{b} hapten, by an indirect immunofluorescence method with FITC-labeled rabbit antimurine IgM (μ-chain specific) antibody. In two-color analysis, lymphocytes were double-stained with phycoerythrin (PE)-labeled MoAbs against Leu 4 (CD3), Leu 12 (CD19), or Leu 19 (CD56) after staining with FH-6.

Expression of the I-antigen was not specific to any particular subset of T cells; 85.6% ± 6.7% of CD4+ cells and 50.7% ± 14.1% of CD8+ cells expressed the antigen (n = 30). The NK cells in healthy individuals, as far as monitored with CD16 and CD56, were entirely I-antigen negative. In healthy individuals, the percentages of I-antigen	extsuperscript{high} and I-antigen	extsuperscript{dim+} cells were essentially dependent on the percentages of mature B and T cells; the percentages of I-antigen	extsuperscript{−} cells was a function of the percentage of NK cells.

Expression of the sialyl SSEA-1 and I-antigens among peripheral lymphocytes in patients with nonhematologic disorders. When the peripheral lymphocytes from patients with various nonhematologic disorders were tested for expression of the sialyl SSEA-1 and I-antigens ad libitum, some patients with chronic infection or malignancies of organs other than the hematopoietic system were occasionally found to have a relatively low frequency of I-antigen	extsuperscript{dim+} cells. A typical example is shown in Fig 2b. The results of detailed surface marker analysis indicated that these patients had a relatively high percentage of activated T cells, which was reflected by the increased number of HLA-DR	extsuperscript{dim+} T cells. In patients with nonhematologic disorders, a good negative correlation (r = −.704) was observed between the percentage of the I-antigen	extsuperscript{+}CD3+ cells and the percentage of the HLA-DR	extsuperscript{dim+}CD3+ cells among CD3+ cells (Fig 3a). The expression of I-antigen on T cells is concluded to be highly dependent on their activation status.
A considerable proportion of these I-antigen-negative activated T cells expressed sialyl SSEA-1. A typical example of such a patient is shown in Fig 1b, indicating that an activated population of CD3⁺ T cells expresses sialyl SSEA-1. Again a significant negative correlation ($r = .528$) was observed between the proportion of the I-antigen⁺CD3⁺ cells and the proportion of the sialyl SSEA-1⁺CD3⁺ cells of these patients (Fig 3b). The relative percentages of I-antigen⁺dim⁺ cells and sialyl SSEA-1⁺ cells among peripheral T cells seemed to reflect the activation status of T cells in the respective patient.

Expression of the sialyl SSEA-1 and I-antigens in lymphocytes undergoing blastogenesis. To further test the hypothesis that activation of T cells results in a decrease of I-antigen⁺ cells and increase of sialyl SSEA-1⁺ cells, peripheral lymphocytes from normal individuals were cultured and stimulated with Con A. This resulted in the rapid loss of I-antigen expression (Fig 2c), and appearance of sialyl SSEA-1 (Fig 1c). After 4 days of culture, the I-antigen⁺ cells almost entirely disappeared, as shown in Fig 2c. More than 85% of the Con A blasts strongly expressed sialyl SSEA-1, which were identified as CD3⁺ T cells undergoing blastogenesis (Fig 1c).

Similarly, when normal peripheral lymphocytes were stimulated with PWM, B cells, as well as T cells, gradually lost the I-antigen from the cell surface (Fig 4, right panel), whereas CD19⁺ B blasts acquired sialyl SSEA-1 (Fig 4, left panel).
Expression of the sialyl SSEA-1 and I-antigens in malignant cells of various types of lymphocytic leukemia. Expression of the sialyl SSEA-1 and I-antigens appears to be differentiation dependent in lymphocytic leukemia cells. Figure 5a shows the frequencies of sialyl SSEA-1+ cells in leukemic cells of various human lymphoid malignancies. The antigen is preferentially expressed on blasts having immature characteristics such as cALL blasts, and is essentially absent on leukemia cells in patients with chronic lymphocytic leukemia (CLL) and adult T-cell leukemia (ATL) (see also ref 8). This distribution pattern contrasts with that of the I-antigen (Fig 5b). The leukemia cells in patients with CLL and ATL, which have relatively well-differentiated properties such as surface expression of IgM or CD4, exhibited a very high frequency of I-antigen, whereas the antigen was less frequently positive on blasts in cALL that have immature characters.

When the ratio of the two antigens, ie, I-antigen+ leukemia cells (%) / sialyl SSEA-1+ leukemia cells (%), was plotted for these patients, it was usually over 10 in patients with CLL and ATL, and mostly below 1.0 in patients with cALL. The ratio showed a wide range of values, from 0.04 to 2.1, in patients with CALL.

From the results described above, the I-antigen was concluded to be a marker for well-differentiated leukemia cells, and sialyl SSEA-1 a marker for leukemia cells having relatively undifferentiated properties. Table 2 displays the results on the cultured human lymphocytic leukemia cells, which also generally support this conclusion. These carbohydrate markers were distinct and did not parallel other B-cell markers.

Figure 3. Expression of the sialyl SSEA-1 and I-antigens on peripheral T cells in patients with nonhematologic disorders. (a) The correlation of the percentage of I-antigen+ cells in total T cells versus the percentage of HLA-DR+ cells in total T cells. (b) The correlation of the percentage of sialyl SSEA-1+ cells in total T cells versus the percentage of I-antigen+ cells in T cells. Sialyl SSEA-1 was stained with the FH-6 antibody, which is specific to the sialyl Lea/i hapten, and the I-antigen was stained with the C6 antibody.

Figure 4. Expression of the sialyl SSEA-1 and I-antigens in normal peripheral CD19+ lymphocytes (upper panel) and PWM-stimulated CD19+ lymphocytes (lower panel). Sialyl SSEA-1 was stained with the FH-6 antibody that is specific to the sialyl Lea/i hapten, and the I-antigen was stained with the C6 antibody.
Table 2. Expression of the Sialyl SSEA-1 and I-Antigens in Cultured Human Lymphocytic Leukemia and Lymphoma Cells (%)

<table>
<thead>
<tr>
<th>Cells</th>
<th>Origin</th>
<th>Sialyl SSEA-1</th>
<th>I-Antigen</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD25</th>
<th>CD7</th>
<th>CD10</th>
<th>CD19</th>
<th>CD20</th>
<th>SmIg</th>
<th>IL2R</th>
<th>HLA-DR</th>
<th>HPCA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL-318</td>
<td>B-NHL</td>
<td>0.1</td>
<td>64.3</td>
<td>1.3</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
<td>99.1</td>
<td>99.9</td>
<td>99.9</td>
<td>98.2</td>
<td>NT</td>
<td>98.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Raji</td>
<td>Burkitt</td>
<td>0.3</td>
<td>98.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.7</td>
<td>0.2</td>
<td>0.5</td>
<td>99.9</td>
<td>100.0</td>
<td>100.0</td>
<td>0.7</td>
<td>0.7</td>
<td>98.8</td>
<td>0.2</td>
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<tr>
<td>ATL-2</td>
<td>ATL</td>
<td>1.1</td>
<td>89.1</td>
<td>2.0</td>
<td>99.7</td>
<td>2.0</td>
<td>98.9</td>
<td>1.4</td>
<td>2.0</td>
<td>1.4</td>
<td>0.9</td>
<td>99.9</td>
<td>99.9</td>
<td>0.7</td>
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<td>NALM-6</td>
<td>ALL</td>
<td>79.9</td>
<td>41.4</td>
<td>1.1</td>
<td>0.2</td>
<td>0.7</td>
<td>0.2</td>
<td>0.6</td>
<td>99.9</td>
<td>100.0</td>
<td>19.1</td>
<td>0.9</td>
<td>99.9</td>
<td>99.9</td>
<td>0.7</td>
</tr>
<tr>
<td>P12/chikawa</td>
<td>ALL</td>
<td>70.7</td>
<td>15.4</td>
<td>6.4</td>
<td>29.2</td>
<td>2.6</td>
<td>99.1</td>
<td>97.3</td>
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<td>5.4</td>
<td>1.9</td>
<td>1.8</td>
<td>0.8</td>
<td>6.0</td>
<td>1.2</td>
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<tr>
<td>MOLT-15</td>
<td>ALL</td>
<td>55.1</td>
<td>11.7</td>
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<td>0.4</td>
<td>1.6</td>
<td>0.4</td>
<td>71.3</td>
<td>1.9</td>
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<td>0.6</td>
<td>1.3</td>
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<tr>
<td>MOLT-3</td>
<td>ALL</td>
<td>92.7</td>
<td>1.9</td>
<td>1.4</td>
<td>0.8</td>
<td>1.2</td>
<td>0.6</td>
<td>63.6</td>
<td>2.0</td>
<td>0.6</td>
<td>1.1</td>
<td>0.6</td>
<td>0.4</td>
<td>98.3</td>
<td>0.8</td>
</tr>
<tr>
<td>YT</td>
<td>LGLL</td>
<td>90.1</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>N.T.</td>
<td>1.6</td>
<td>0.1</td>
<td>1.8</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
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</tr>
</tbody>
</table>

Abbreviations: NT, not tested; B-NHL, B-cell type non-Hodgkin’s lymphoma; LGLL, large granular lymphocytic leukemia; ALL, acute lymphocytic leukemia.
lymphocytes were recovered from the nonadherent fraction. On the other hand, the lymphocyte population that adhered to the addition of the antibodies directed to the components of the adherent and nonadherent fractions of lymphocytes was pure sialyl Le" glycolipid carrying the same hapten structure to be mediated not only by E-selectin/sialyl SSEA-1, but also adherent sialyl SSEA-1+ I-antigen- cells, as expected, were cells (indicated by arrowheads), and essentially all I-antigen+ cells (indicated by an arrow), while nonadherent cells as indicated by an arrow, while nonadherent cells also showed a significant E-selectin-dependent adhesion to the rIL-1β-activated HUVECs. The inhibition pattern obtained by the addition of antibodies against these molecules. When analyzed by flow cytometry (Fig 7b), the cells adherent to HUVECs were again remarkably enriched with sialyl SSEA-1^high+ cells as indicated by an arrow, while nonadherent cells were negative or only weakly positive for sialyl SSEA-1. The I-antigen was not analyzed, because it was not expressed on these Con A blasts from the beginning of the adhesion experiments. The adherent cells were shown to be mainly CD3+ T blasts (data not shown).

Adhesion of Con A blasts to endothelial cells and its relation to the expression of carbohydrate antigens. Con A blasts, which strongly express sialyl SSEA-1, also exhibited significant E-selectin–dependent adhesion to the rIL-1β-activated HUVECs (Fig 6b). The number of adherent cells far exceeded that observed in unstimulated peripheral lymphocytes from normal individuals, even that observed in case 1. The role played by the sialyl SSEA-1/E-selectin adhesion system was significant, as is shown by liposome inhibition experiments. The other two cell adhesion systems, ie, ICAM-1/LFA1 and VCAM-1/VLA4, also seemed to participate in the reaction, judging from the further increase in the inhibition of adhesion by the addition of antibodies against these molecules. When analyzed by flow cytometry (Fig 7b), the cells adherent to HUVECs were again remarkably enriched with sialyl SSEA-1^high+ cells as indicated by an arrow, while nonadherent cells were negative or only weakly positive for sialyl SSEA-1. The I-antigen was not analyzed, because it was not expressed on these Con A blasts from the beginning of the adhesion experiments. The adherent cells were shown to be mainly CD3+ T blasts (data not shown).

Adhesion of cultured lymphocytic leukemia cells to endothelial cells. Several cultured human lymphocytic leukemia cells also showed a significant E-selectin–dependent adhesion to the rIL-1β-activated HUVECs (Fig 8). Liposome inhibition experiments clearly indicated the participation of E-selectin in the adhesion of leukemia cells to HUVECs. The inhibition pattern obtained by the addition of antibodies against cell adhesion molecules was very similar to the pattern observed with Con A blasts; ie, addition of any one antibody never yielded complete inhibition of adhesion, and a significant contribution of E-selectin was seen only when the appropriate combination of antibodies was used.

DISCUSSION

The I-antigen was first described as a differentiation antigen in human erythrocytes. It is well known that erythrocytes

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**Fig 6. Adhesion of normal peripheral lymphocytes (a) or Con A blasts (b) to rIL-1β-activated HUVECs, and effect of pretreatment with MoAbs directed to cell adhesion molecules or liposomes containing sialyl Le' determinant.** Anti-E-selectin antibody was used for the pretreatment of HUVECs at 50 μg/mL for 30 minutes at room temperature before the incubation with lymphocytes. Pretreatment with "Anti-E-selectin and other antibodies" indicates that HUVECs were pretreated with 50 μg/mL of anti-E-selectin and anti–ICAM-1 antibodies, while lymphocytes were pretreated with 50 μg/mL of anti-LFA1 and anti-VLA4 antibodies for 30 minutes at room temperature before the adhesion experiment. For liposome inhibition, HUVECs were pretreated with a liposome suspension containing 40 μg of synthetic sialyl Le' glycolipid for 30 minutes at room temperature before the incubation with lymphocytes. For cases 1 and 2, see text.
undergo a developmental change from i-antigenic cord erythrocytes to I-antigenic adult erythrocytes. Most leukocytes are known to express the i-antigen but not I-antigen, and it has been reported that some lymphocytes as well as CLL leukemia cells exceptionally express the I-antigen. Our present results confirmed and expanded the knowledge on the distribution of I-antigen in human lymphoid cells, using a specific murine MoAb instead of classical human sera containing cold agglutinins. The I-antigen is expressed preferentially by lymphoid cells having relatively well-differentiated characteristics. The distribution pattern of the I-antigen in lymphoid cells is in clear contrast to the distribution of sialyl SSEA-1, which is expressed in cells having immature properties. It is noteworthy that sialyl SSEA-1 is closely related to the i-antigen, i.e., SSEA-1 is the i-antigen that is modified by fucose residues in its carbohydrate structure.

Expression of the sialyl SSEA-1 and I-antigens in normal human lymphocytes is dependent on the cell lineage. Resting mature T and B cells are usually I-antigen+ sialyl SSEA-1−, while only NK cells are I-antigen− sialyl SSEA-1+. The expression of the I- and sialyl SSEA-1 antigens is not fixed in normal T and B cells, but is reversible depending on the activation status of the cells. Resting I-antigen+ sialyl SSEA-1− T and B cells become I-antigen− sialyl SSEA-1+ on activation or blastogenesis. It was also observed that Con A blasts convert gradually from I-antigen− sialyl SSEA-1− status to resting I-antigen+ sialyl SSEA-1− status on prolonged culture in vitro (data not shown).

Judging from the expression pattern in lymphocytic leukemia cells, lymphocytes of T- and B-cell lineages seem to undergo an antigenic shift generally from immature I-antigen− sialyl SSEA-1− status to I-antigen+ sialyl SSEA-1− status on differentiation, while NK cells always show the I-antigen− sialyl SSEA-1− pattern. The putative sequence of the differentiation-dependent expression of these antigens in normal lymphoid cells is illustrated in Fig 9. The ratio of the I-an-
The sialyl SSEA-1 and I-antigens both belong to the type 2 chain polylactosamines in their carbohydrate structure (Table 1). The differentiation-dependent change from I-antigen+ sialyl SSEA-1+ status to I-antigen+ sialyl SSEA-1- status is most probably mediated by alterations in the modifications of the polylactosamine backbones; loss of sialylation and fucosylation, followed by branch formation with GlcNAcβ1 → 6 linkage. Conversely, the restoration of I-antigen+ sialyl SSEA-1+ status from I-antigen+ sialyl SSEA-1- status on blastogenesis is mediated by reduced branch formation, accompanied by increased sialylation and fucosylation. Our results indicate that these metabolic changes are reversible, and frequently occur in either direction in human lymphocytes during the course of differentiation or activation. It will be interesting to see whether changes occur only in the carbohydrate side chains carried by glycoproteins and glycolipids, or are accompanied by altered synthesis of the entire glycoprotein and glycolipid molecules involving the core protein and lipid moieties.

Sialyl SSEA-1 carries the terminal sialyl Le^a^-hapten structure, which has recently been identified as a specific ligand for E-selectin, the cell adhesion molecule that appears in cytotokine-activated endothelial cells. Therefore, the carbohydrate antigen status at the lymphocyte surface may affect the behavior of the cells toward the vascular endothelial cells in vivo. Our results clearly indicated that the sialyl SSEA-1/E-selectin cell adhesion system plays a significant role in the binding of human lymphocytes to rIL-1β-activated HUVECs, in addition to other well-known systems such as ICAM-1/LFA1 and VCAM-1/VLA4. Sialyl SSEA-1+ I-antigen+ lymphocytes preferentially bound to activated endothelial cells, while most I-antigen+ sialyl SSEA-1+ lymphocytes did not.

The differential expression of the two antigens in various lymphocyte subpopulations would be quite beneficial to host defense mechanisms in inflammatory reactions. Among peripheral lymphocytes, only NK cells are known to have an effector function in the absence of any specific antigenic stimulation, and our results suggest that these cells may be preferentially recruited to the vessel wall at the site of inflammation, since they are sialyl SSEA-1+ I-antigen-. On the other hand, most resting T and B cells, which do not usually have any direct effector functions in the absence of antigenic stimulation, are mostly I-antigen+ sialyl SSEA-1+ according to our analysis, and would remain in the circulating blood. Once activated by specific antigens, these cells undergo blastogenesis and become sialyl SSEA-1+ I-antigen-, and this would lead to the recruitment of these cells to the vessel wall at the inflammatory site. Therefore, the sialyl SSEA-1/E-selectin system seems to play a role as a precise cell selection system at the vessel wall, by which the lymphocyte populations, expected to exert beneficial effector functions in inflammatory reactions, are specifically sorted out from the vast majority of resting lymphocytes. The continuous change in the sialyl SSEA-1 and I-antigenic status at the surface of lymphocytes may serve as an important regulatory mechanism in this cell selection system, and contribute to host defenses.

Recently a distinct subset of the CD4+ memory T cells has been shown to express a carbohydrate antigen defined by the HECA-452 antibody, and this antigen is proposed to be one of the carbohydrate ligands for E-selectin. The specificity of the antibody HECA-452 seems to be broad, as it is reactive with both the sialyl Le^a^ and sialyl Le^a^- antigens. However, the anti-sialyl SSEA-1 antibody FH-6 used in this report did not react with resting T cells in healthy individuals (ref 11 and this report). The anti-sialyl Le^a^- antibody SNH3 was also not reactive with the memory T cells (data not shown). It is also reported that another anti-sialyl SSEA-1 antibody, CSLEX-1, is not reactive with memory T cells. Further studies are necessary to identify the HECA452-reactive carbohydrate antigen expressed on memory T cells.

The acquisition and constant expression of sialyl SSEA-1 on lymphocytic leukemic cells reflect the immature characteristics of the malignant cells, and must be also related to the behavior of the leukemic cells in blood vessels. I-antigen+ sialyl SSEA-1+ leukemia cells probably possess significant adhesive activity to E-selectin–positive endothelial cells, leading to enhanced extravascular infiltration of leukemia cells. It is ironic that this antigen, which is designed to serve as an important component of self-defense mechanisms under normal conditions, mediates the expansion and progression
of malignant disorders via the very same molecular mecha-

nism, once expressed on leukemic cells. In this sense, eval-
uation of the sialyl SSEA-1 and I-antigenic status of leukemic

cells, the “carbohydrate maturation index,” may serve as a pro-
gnostic marker in patients with lymphoid malignan-
cies.

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Differentiation-dependent expression of sialyl stage-specific embryonic antigen-1 and I-antigens on human lymphoid cells and its implications for carbohydrate-mediated adhesion to vascular endothelium

K Ohmori, A Takada, T Yoneda, Y Buma, K Hirashima, K Tsuyuoka, A Hasegawa and R Kannagi