 Contribution of Carbohydrate Antigens Sialyl Lewis A and Sialyl Lewis X to Adhesion of Human Cancer Cells to Vascular Endothelium

Akiko Takada, Katsuyuki Ohmori, Tomoya Yoneda, Kiyotaka Tsuyuoka, Akira Hasegawa, Makoto Kiso, and Reiji Kannagi

Laboratory of Experimental Pathology, Research Institute, Aichi Cancer Center, Nagoya, 464 [A. K., K. T., R. K.]; Department of Laboratory Medicine, Kyoto University, School of Medicine, Kyoto, 606 [K. O., T. Y.]; and Department of Applied Bio-organic Chemistry, Gifu University, Faculty of Agriculture, Gifu, 501-11 [A. H., M. K.], Japan

ABSTRACT

The carbohydrate antigen, sialyl Le$^a$, is known to be a ligand for the cell adhesion molecule called ELAM-1 (E-selectin, endothelial cell leukocyte adhesion molecule-1), which is present on cytokine-activated human endothelial cells. Recently, we reported that another carbohydrate antigen, sialyl Le$^b$, can also serve as a ligand for ELAM-1 (A. Takada, K. Ohmori, N. Takahashi, K. Tsuyuoka, K. Yago, K. Zenita, A. Hasegawa, and R. Kannagi, Biochem. Biophys. Res. Commun., 179: 713-719, 1991). Both sialyl Le$^a$ and sialyl Le$^b$ are expressed in many human malignant cells. In order to assess the contribution of these carbohydrate antigens to the adhesion of human malignant cells to vascular endothelium, we selected a panel of 12 cultured human epithelial cancer cell lines and a panel of 12 human leukemia cell lines which express sialyl Le$^a$ and/or sialyl Le$^b$ antigens. All 12 epithelial cancer cell lines exhibited a clearly ELAM-1-dependent adhesion to cytokine-activated human umbilical vein endothelial cells, while only 3 of the 12 leukemia cell lines exhibited significant participation of ELAM-1 in the adhesion. With regard to epithelial cancer cells, the adhesion of 6 cancer cell lines, mostly of colon and pancreas origin, was dependent almost exclusively on sialyl Le$^a$. A significant contribution of the sialyl Le$^a$ antigen was noted in the adhesion of the other 6 cell lines, including cancers of lung and liver origin. These results imply that the sialyl Le$^a$/ELAM-1 adhesion system, as well as the sialyl Le$^b$/ELAM-1 adhesion system, plays an important role in the adhesion of human cancer cells to human umbilical vein endothelial cells. With regard to leukemia cells, on the other hand, adhesion of the 3 leukemia cell lines that showed ELAM-1-dependent adhesion was mediated by the sialyl Le$^b$ antigen, and none of these leukemia cell lines exhibited sialyl Le$^a$ or exhibited sialyl Le$^b$-dependent adhesion.

INTRODUCTION

The lectin-like domain of ELAM-1$^1$ is known to recognize an oncocyteal carbohydrate antigen, sialyl Le$^a$ (1-4). Recently, other investigators and our group (5-7) found that another cancer-associated carbohydrate antigen, sialyl Le$^b$, can also serve as a ligand for ELAM-1. Findings in support of this include: (a) several cultured human colon cancer cell lines, for instance, Colo205, exhibit a clearly ELAM-1-dependent adhesion to rIL1/β-activated HUVECs (5, 7); (b) this adhesion is significantly inhibited by treatment with several anti-sialyl Le$^a$ antibodies but not by anti-sialyl Le$^b$ antibody treatment (5, 7); (c) pretreatment of HUVECs with either sialyl Le$^a$ or sialyl Le$^b$ glycolipid results in nearly complete inhibition of adhesion (5); and (d) synthetic sialyl Le$^a$-protein conjugate can bind to ELAM-1 (6, 8). The antigen sialyl Le$^a$, as well as sialyl Le$^b$, is known to be expressed frequently on human cancer cells, (9-12), and we thought it worthy to study the contribution of these carbohydrate antigens to the adhesion of human malignant cells.

ELAM-1 has been recognized as a molecule involved in the adhesion of leukocytes to vascular endothelium, and several cultured human leukemia cell lines have been frequently used in experimental studies to demonstrate this. It is well documented that, in addition to ELAM-1, ICAM-1 and VCAM-1 are also involved in the adhesion of leukocytes and leukemia cells to endothelial cells (13-15). However, the molecular mechanism involved in the adhesion of human epithelial cancer cells to endothelial cells has not been studied extensively.

It has been reported that some melanoma cells interact with endothelial cells via the very-late antigen-4/VCAM-1 system, while some colon cancer cells involve ELAM-1 (16, 17). This study was undertaken to evaluate the role played by the sialyl Le$^a$ and sialyl Le$^b$ antigens in the adhesion of human leukemia and cancer cells. For this purpose, we chose a panel of 12 cultured human epithelial cancer cell lines, which express sialyl Le$^a$ and/or sialyl Le$^b$ antigens, and looked at the role of these carbohydrate antigens in the adhesion to rIL1β-activated HUVECs. A panel of 12 cultured leukemia cell lines was also chosen, to serve as a reference.

MATERIALS AND METHODS

Human Cancer and Leukemia Cells

Cultured human cancer cell lines, Capan-2, WiDr, and CoR-1, were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan), and SW1116 was obtained from American Type Culture Collection (Rockville, MD); QG90 and QG56 were gifts from the Kyushu Cancer Center; MKN74 and Colo205 were gifts from Dr. Shiochi Ooshi, Niigata University; C-1 was a gift from Dr. Yoshihiro Hayata, Tokyo Medical University. All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Cultured human leukemia cell lines, HPB-ALL, U937, NALM-6, MOLT-3, CMK, TK1B and THP1-0, were obtained from the First Division of the Department of Internal Medicine, Kyoto University. Jurkat, P12/Ichikawa, and MOLT-15 were kindly supplied by Dr. Jun Minowada, Fujisaki Cell Center, Hayashibara Biology Research Institute, Okayama, Japan. These cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (18).

Monolayer Cell Adhesion Assay Using HUVECs

HUVECs (2-6 passages after isolation, obtained from Kurabou Co., Ltd., Osaka, Japan) were stimulated with 1.0 ng/ml of rIL1β for 4 h in 96-well plates (5, 19, 20). To these plates the $^{3}$H-labeled cultured human cancer cells or leukemia cells (1 x 10$^6$/60 µl/well) were added and incubated for 30 min at room temperature with rotation (120 rpm) (5). A short incubation time and continuous rotation were applied to minimize possible nonspecific bindings of epithelial cancer cells to the plates. In preliminary experiments, these conditions were shown to be suitable for the evaluation of the initial phase of cell adhesion, in which adhesion molecules of the selectin family are known to play important roles, and thus allowed the detection of the maximum contribution of the selectin family adhesion molecules. After the adherent cells were washed three times, they were treated with 1% Triton X-100. The lysate was collected using a Skatron supernatant collection system (Skatron As, Lier, Norway), and the radioactivity in each well was measured with a gamma-counter. The number of attached cells was calculated from the specific radioactivity of the labeled cells. Adherence of cancer or leukemia cells was corrected for binding to unstimulated HUVECs. The rIL1β was obtained from the...
Central Research Laboratory of Otsuka Pharmaceutical Co., Tokushima, Japan, and the recombinant basic fibroblast growth factor used for the in vitro culture of HUVECs was from the Central Research Laboratory of Takeda Pharmaceutical Co., Juso, Japan.

Monoclonal Antibodies Used for Inhibition of Cell Adhesion

Monoclonal anti-ELAM-1, anti-ICAM-1, and anti-VCAM-1 antibodies (BBA2, BBA4, and BBA6, all murine IgG1) were obtained from British Biotechnology Ltd., Abington, Oxon, United Kingdom. These antibodies were preincubated with HUVECs at 50 µg/ml for 30 min at 37°C prior to the adhesion experiments with cancer or leukemia cells for inhibition experiments (5). When a mixture of these monoclonal antibodies was used for preincubation of HUVECs, the final concentration of each antibody in the preincubation period was adjusted to 50 µg/ml. Monoclonal anti-LFA1/3 (CBL5, IgG1) was obtained from Immunotech S.A. (Marseille, France), and this antibody was preincubated with cultured leukemia cells at 50 µg/ml for 30 min at room temperature prior to application to the monolayer of HUVECs.

Monoclonal antibodies SNH3 (specific to sialyl Lewis X, supplied by Dr. Sen-itiroh Hakomori, Biomembrane Institute, Seattle, WA) and 2D3 (specific to sialyl Lewis A, established in our laboratory) are both murine IgM and were purified from ascitic fluids as described previously (2, 5). The carbohydrate structures of the sialyl Lewis X and sialyl Lewis A antigens and the specificities of the monoclonal antibodies are summarized in Table 1.

For inhibition of cell adhesion, these monoclonal antibodies were preincubated with cultured cancer cells or leukemia cells at 25-100 µg/ml for 30 min at room temperature prior to application to the monolayer of HUVECs (5).

Inhibition of Cell Adhesion with Liposomes containing the Ligand Glycolipid

The pure synthetic sialyl Le^a glycolipid used for the inhibition experiment had the structure, NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAcβ1→3Galβ1→4Glcβ1→1Cer (5, 7, 21).

The anomeric structures and linkages of the terminal four sugar residues of this synthetic glycolipid are identical with those in the sialyl SSEA-1 epitope as shown in Table 1. The sialyl Le^a liposome contained 40 µg/well of the glycolipid, 20 µg/well of cholesterol, and 40 µg/well of phosphatidylcholine (5). The control liposome contained the same amount of cholesterol and phosphatidylcholine but no glycolipid. The liposome suspensions were preincubated with HUVECs for 30 min at room temperature prior to the addition of lymphocytes.

Fluorescence-activated Flow Cytometry

Flow cytometric analysis was performed using FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The indirect immunofluorescence method was applied for staining of cultured cells with the antibodies SNH3 and 2D3 as the first antibody (stained for 30 min at room temperature at the concentration of 1.0 µg/ml/1 × 10⁶ cells), followed by the addition of the fluorescein isothiocyanate-labeled rabbit anti-murine IgM (µ chain specific) antibody as the second antibody (Cappel Inc., Malvern, CA), as was described previously (22).

RESULTS

Adhesion of Human Cancer and Leukemia Cells to rIL1β-activated HUVECs

When human cancer cell lines were added to the rIL1β-activated HUVECs in 96-well plates, a variable number of cells adhered to HUVECs. As shown in Fig. 1a, the number of adherent cells ranged from 1.1 × 10³ (CoR-1) to 8.5 × 10⁴ (Colo201), and this range was comparable to, or even greater than, the range of adhered human leukemia cells, which was from 1.1 × 10³ to 4.3 × 10⁴. rIL1β had a clear stimulatory effect on the adhesion; far fewer cancer or leukemia cells were adherent to HUVECs in the absence of preincubation with rIL1β. The stimulation index for rIL1β for adhesion, i.e., the number of adherent cancer cells to rIL1β-activated HUVECs divided by the number of adherent cells to nonstimulated HUVECs, ranged from 1.5 to 6.4. The stimulation index for leukemia cells (1.7–9.3).

Table 1 Carbohydrate structures of immunodominant epitopes of the sialyl Lewis X and sialyl Lewis A antigens and specific monoclonal antibodies 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 Lewis X</td>
<td>SNH3 (IgM)</td>
<td>NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAcβ1→3Galβ1→4Glcβ1→1Cer</td>
</tr>
<tr>
<td>Sialyl Lewis A</td>
<td>2D3 (IgM)</td>
<td>NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAcβ1→3Galβ1→4Glcβ1→1Cer</td>
</tr>
</tbody>
</table>

Fig. 1. Adhesion of cultured human cancer cells and leukemia cells to rIL1β-activated HUVECs. a, number of adherent cells to activated HUVECs; b, stimulation index by rIL1β treatment. Cancer cells: O, colon cancer; ■, stomach cancer; O, pancreas cancer; ▲, hepatocellular cancer; A, lung cancer; D, teratocarcinoma. Leukemia cells: ●, non-lymphocytic leukemia; ○, lymphocytic leukemia.

Contribution of Cell Adhesion Molecules to the Adhesion of Human Cancer Cells to HUVECs

To evaluate the contribution of various cell adhesion molecules to the adhesion of human cancer cells, rIL1β-activated HUVECs were preincubated with monoclonal antibodies directed to ELAM-1, ICAM-1, or VCAM-1, to specify the involved adhesion molecule. Typical inhibition patterns are shown in Fig. 2, which shows that only anti-ELAM-1 antibody had a clear inhibitory effect on the adhesion of...
4 representative cultured human epithelial cancer cell lines to rIL1β-activated HUVECs.

Surprisingly, the adhesion of all 12 cancer cell lines tested was significantly inhibited by the treatment with anti-ELAM-1 antibody as shown in Fig. 3. The effect of anti-ICAM-1 and anti-VCAM-1 antibodies was much less prominent. The anti-ICAM-1 antibody inhibited the adhesion of only one of the 12 cell lines, and the anti-VCAM-1 treatment had no significant effect on the adhesion of any of the epithelial cancer cell lines. The significant contribution of ELAM-1 was further confirmed by showing that saturation of the lectin domain of ELAM-1 by sialyl Le⁺ liposomes prior to the addition of cancer cells clearly inhibited the cell adhesion (Fig. 4). In this experiment, inhibition was due to the saturation of the lectin domain of ELAM-1 on HUVECs by sialyl Le⁺ liposomes, and the results do not necessarily indicate that all cancer cells adhered to HUVECs via their sialyl Le⁺ expressed at the cell surface.

![Figure 2](image-url)  
**Fig. 2.** Typical inhibition patterns of the adhesion of human cancer cells to rIL1β-activated HUVECs by the treatment with anti-ICAM-1, anti-ELAM-1, and anti-VCAM-1 antibodies. HUVECs were treated with the respective antibodies (50 μg/ml) prior to the adhesion experiment for 30 min. The results of QG-56 (lung cancer), Colo201 (colon cancer), MKN-74 (stomach cancer), and SW116 (colon cancer) cells are shown.

![Figure 3](image-url)  
**Fig. 3.** Contribution of three cell adhesion molecules, ELAM-1, VCAM-1, and ICAM-1, to the adhesion of cultured human cancer cells to rIL1β-activated HUVECs. All cells exhibited a significant inhibition of adhesion by treatment with the anti-ELAM-1 antibody. Bars, SD. * difference from control values is statistically significant at P < 0.05; ** at P < 0.01; and *** at P < 0.001 by Student’s t test.
CANCER CELL ADHESION MEDIATED BY SIALYLATED LEWIS A ANTIGEN

Fig. 4. Effect of treatment of HUVECs with sialyl Le<sup>x</sup>-liposome on the adhesion of cultured human cancer cells. For liposome inhibition, HUVECs were pretreated with a liposome suspension containing 40 μg of synthetic sialyl Le<sup>x</sup> glycolipid for 30 min at 37°C prior to the incubation with cancer cells. Colon cancer; stomach cancer; pancreas cancer; teratocarcinoma.

Contribution of Cell Adhesion Molecules to the Adhesion of Human Leukemia Cells to HUVECs

In contrast, the inhibitory effect of anti-ELAM-1 antibody on the adhesion of human leukemia cells to HUVECs was less remarkable. As shown in Fig. 5, the treatment with anti-VCAM-1 antibody most prominently inhibited the adhesion of human leukemia cells to the activated HUVECs; the adhesion of as many as 8 of the 12 human leukemia cell lines was clearly and significantly inhibited by pretreating HUVECs with anti-VCAM-1 antibody. The adhesion of 6 of them was purely VCAM-1 dependent, while that of the other 2 cell lines (TK1B and MOLT-15) was also inhibited by the anti-ELAM-1.

A significant inhibition by anti-ELAM-1 antibody on adhesion was observed with 5 cell lines; two of them were dependent also on VCAM-1 as mentioned above. The 3 cell lines (HL60, U937, and FMC10) showed a significant inhibition of adhesion only with the anti-ELAM-1 antibody, and no appreciable inhibition was seen by the anti-ICAM-1 or anti-VCAM-1 treatments. However, the inhibition of adhesion of these three cell lines by the treatment with anti-ELAM-1 antibody alone was only partial. Significant increases in the inhibition of the adhesion of these three cell lines were noted with pretreatment with various mixtures of antibodies, such as the mixture of anti-ELAM-1, anti-VCAM-1, and anti-ICAM-1, and anti-LFA1, as compared with pretreatment with anti-ELAM-1 alone, indicating the complicated synergistic action of these molecules in the adhesion phenomenon.

The treatment with antibodies against the LFA-1/ICAM-1 adhesion system exerted an inhibitory effect on the adhesion of only one cell line (THP1-0). Probably, under the conditions applied for these experiments, the contribution of the LFA1/ICAM-1 system is underestimated, because the adhesion through the LFA1/ICAM-1 system requires a longer incubation time, and the continuous rotation has an inhibitory effect on this cell adhesion system.

Contribution of Sialyl Le<sup>a</sup> and Sialyl Le<sup>x</sup> to the ELAM-1-mediated Adhesion of Human Cancer Cells to HUVECs

When cancer cells were pretreated with monoclonal anti-sialyl Le<sup>a</sup> or anti-sialyl Le<sup>x</sup> antibody prior to the addition to HUVECs, a clear inhibition of adhesion was observed in most cases, as shown in Fig. 6. Adhesion of two cancer cell lines (QG56 and MKN74) was completely inhibited by pretreating the cells with anti-sialyl Le<sup>a</sup> antibody.
and was not affected by the anti-sialyl Le\(^a\) treatment. On the other hand, adhesion of the other two cancer cell lines (Colo201 and SW1116) was completely inhibited by pretreating the cells with anti-sialyl Le\(^a\) antibody and was not affected by the anti-sialyl Le\(^a\) treatment.

In total, the pretreatment with anti-sialyl Le\(^a\) inhibited the adhesion of 6 of the 12 tested human cancer cell lines (Fig. 7a). The cancer cell lines in which adhesion was clearly inhibited by treatment with anti-sialyl Le\(^a\) included 3 colon cancer cell lines, 1 pancreas cancer cell line, 1 lung cancer cell line, and 1 teratocarcinoma cell line.

As shown in Fig. 8a, a flow cytometric analysis of these cell lines indicated that these cells strongly expressed sialyl Le\(^a\), which is compatible with the observed sialyl Le\(^a\)-dependent adhesion. The expression of sialyl Le\(^a\) was less significant in 2 of them, but the remaining 4 cell lines strongly expressed sialyl Le\(^a\) at their surface (Fig. 8a). It is curious that these 4 cell lines express both sialyl Le\(^a\) and sialyl Le\(^a\) strongly at their surface, but adhesion of these cells to HUVECs is almost exclusively dependent on sialyl Le\(^a\) and not on sialyl Le\(^a\), because only the anti-sialyl Le\(^a\) antibody inhibited the adhesion and the anti-sialyl Le\(^a\) antibody did not. These cells express both antigens, but it was concluded that sialyl Le\(^a\) is preferentially involved in the cell adhesion.

The treatment with the anti-sialyl Le\(^a\) antibody significantly inhibited the adhesion of 6 cell lines, including 2 lung cancer cell lines, 1 stomach cancer cell line, 1 hepatocellular cancer cell line, and 2 colon cancer cell lines (Fig. 7b). The inhibition was nearly complete in 4 cell lines. The adhesion of the remaining 2 cell lines was completely inhibited by pretreating HUVECs with anti-ELAM-1 but was less sensitive to the pretreatment of cells with either antibody, suggesting the involvement of an other third unknown carbohydrate ligand in the adhesion of these cell lines.

**Fig. 6.** Typical inhibition patterns of the adhesion of human cancer cells to rIL1β-activated HUVECs by treatment with anti-sialyl Le\(^a\) and anti-sialyl Le\(^a\) antibodies. Cancer cells were treated with the respective antibodies (25 μg/ml) prior to the adhesion experiment for 30 min. The results of QG-56 (lung cancer), Colo201 (colon cancer), MKN-74 (stomach cancer), and SW1116 (colon cancer) cells are shown.

**Fig. 7.** Contribution of the two carbohydrate antigens, sialyl Le\(^a\) and sialyl Le\(^a\), to the adhesion of cultured human cancer cells to rIL1β-activated HUVECs. a, results of cancer cells which showed a sialyl Le\(^a\)-dependent adhesion. b, results of cancer cells which showed a sialyl Le\(^a\)-dependent adhesion. The results obtained by treatment with the mixture of anti-sialyl Le\(^a\) and anti-sialyl Le\(^a\) antibodies are also shown. A synergistic action of the two carbohydrate antigens had been expected, but, in fact, no such action was detected. Bars, SD. *p < 0.05; **p < 0.01; ***p < 0.001, by Student’s t test.
CANCER CELL ADHESION MEDIATED BY SIALYL LEWIS A ANTIGEN

Fig. 8. Expression of the two carbohydrate antigens, sialyl Le\textsuperscript{a} and sialyl Le\textsuperscript{x}, at the surface of the cultured human cancer cells as ascertained by flow cytometry. a, results of cancer cells which showed a sialyl Le\textsuperscript{a}-dependent adhesion. Note that 2 cell lines (Capan2 and 2102Ep) express only sialyl Le\textsuperscript{a} antigen, while the other 4 cell lines express both sialyl Le\textsuperscript{a} and sialyl Le\textsuperscript{x} antigens strongly. b, results of cancer cells which showed a sialyl Le\textsuperscript{x}-dependent adhesion, which express only sialyl Le\textsuperscript{x}, and do not (or only slightly) express the sialyl Le\textsuperscript{a} antigen.

As shown in Fig. 8b, these cell lines strongly expressed sialyl Le\textsuperscript{x}, while the expression of sialyl Le\textsuperscript{a} was weak or undetectable. It was quite reasonable that they underwent the sialyl Le\textsuperscript{x}-dependent adhesion.

**Contribution of Sialyl Le\textsuperscript{x} and Sialyl Le\textsuperscript{a} to the Adhesion of Human Leukemia Cells to HUVECs**

In similar inhibition experiments, the adhesion of only 3 leukemia cell lines (HL60, U937, and FMC10) of 12 was mediated by the sialyl Le\textsuperscript{x} antigen as shown in Table 2. It is noteworthy that the 12 human leukemia cell lines tested in this study all expressed sialyl Le\textsuperscript{x} at their cell surface as ascertained by flow cytometric analysis, but most of these cell lines, except the 3 leukemia cell lines, did not show a clear ELAM-1 dependency. These findings indicate that the surface expression of sialyl Le\textsuperscript{x} antigen is not necessarily indicative of which cells undergo the ELAM-1-dependent adhesion, when the cells are heavily equipped with other adhesion molecules.

**Table 2 Expression of carbohydrate antigens on human leukemia cells**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Origin</th>
<th>Sialyl Le\textsuperscript{a} (%)</th>
<th>Sialyl Le\textsuperscript{x} (%)</th>
<th>Inhibition of adhesion</th>
<th>Binding through sialyl Le\textsuperscript{x}</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPB-ALL</td>
<td>Lymphocytic (T)</td>
<td>68.4</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>Jurkat</td>
<td>Lymphocytic (T)</td>
<td>92.0</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>MOLT-3</td>
<td>Lymphocytic (T)</td>
<td>84.8</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>NALM-6</td>
<td>Lymphocytic (B)</td>
<td>99.6</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>CMK</td>
<td>Myeloid</td>
<td>98.7</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>P28chikawa</td>
<td>Lymphocytic (T)</td>
<td>70.7</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>HL60</td>
<td>Myeloid</td>
<td>96.8</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>U937</td>
<td>Myeloid (mono)</td>
<td>96.3</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>FMC10</td>
<td>Myeloid (mono)</td>
<td>96.8</td>
<td>&lt;2.0</td>
<td>VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>TK1B</td>
<td>Myeloid (mono)</td>
<td>99.7</td>
<td>&lt;2.0</td>
<td>ELAM-1</td>
<td>+</td>
</tr>
<tr>
<td>MOLT-15</td>
<td>Lymphocytic (T)</td>
<td>55.1</td>
<td>&lt;2.0</td>
<td>ELAM-1 + VCAM-1</td>
<td>-</td>
</tr>
<tr>
<td>THPT-0</td>
<td>Myeloid (mono)</td>
<td>97.8</td>
<td>&lt;2.0</td>
<td>LFA1</td>
<td>-</td>
</tr>
</tbody>
</table>

a Expression of carbohydrate antigens was ascertained by flow cytometric analysis.
b Ascertained by the monolayer cell adhesion assay in the presence of antibodies against cell adhesion molecules.
c Ascertained by the monolayer cell adhesion assay in the presence of antibodies against sialyl Le\textsuperscript{x}.
None of the leukemia cells used in this study expressed sialyl Le\(^a\), as shown in Table 2. We analyzed 18 human leukemia cell lines in our preliminary experiments, but the sialyl Le\(^a\) antigen was not detected in any of them, and the treatment of cells with anti-sialyl Le\(^a\) antibody did not exert any significant effect on the adhesion of leukemia cells to HUVECs.

**DISCUSSION**

Our present study clearly indicated that epithelial cancer cells have an ability to adhere to endothelial cells and that their adhesion is enhanced by the activation of endothelial cells with cytokines such as rIL1, similar to the case of leukemia cells. This is in line with the results of other investigators (16, 17, 23). Our results also indicated that ELAM-1, first introduced as an adhesion molecule which mediates the adhesion of leukocytes to endothelial cells (19, 20), is of particular importance in the adhesion of human epithelial cancer cells to vascular endothelial cells. It is noteworthy that, among the three cell lines, similar to the case of leukemia cells. This is in line with the fact that the leukemia cell lines chosen also all express sialyl Le\(^a\). The sialyl Le\(^a\) antigen expressed at the surface does not always mediate the ELAM-1-dependent adhesion in leukemia cells. The leukemia cells seem to be equipped heavily with other adhesion molecules, and a synergistic action of the multiple adhesion molecules was frequently detected. The situation was much simpler with cancer cells, in which ELAM-1 appears to contribute almost solely to the adhesion to endothelial cells.

Our results indicated that sialyl Le\(^a\) usually plays a major role in the adhesion to endothelial cells when the cancer cells express the sialyl Le\(^a\) antigen. The cancer cells, which express only sialyl Le\(^a\) antigen and no sialyl Le\(^a\) antigen, display sialyl Le\(^a\)-dependent adhesion to HUVECs. However, it was puzzling to see that the cancer cells that express both sialyl Le\(^a\) and sialyl Le\(^a\) antigens undergo almost exclusively sialyl Le\(^a\)-dependent adhesion. This observation was not exceptional, since the adhesion of all of the cancer cell lines that express both antigens was nearly exclusively dependent on sialyl Le\(^a\). This may be due to the difference in the surface density of the sialyl Le\(^a\) antigen, which may exceed that of sialyl Le\(^a\) antigen in these cells, or ELAM-1 may have a higher affinity for sialyl Le\(^a\) than for sialyl Le\(^a\). Our previous studies of the mucin carbohydrate chains produced by human epithelial cancer cells (12, 24) indicated that carbohydrate chains carrying sialyl Le\(^a\) determinant are, in general, much more well developed than carbohydrate chains carrying sialyl Le\(^a\) determinant. This may be related to the higher accessibility of sialyl Le\(^a\) at the cancer cell surface toward endothelial ELAM-1.

It is well known that the sialyl Le\(^a\) antigen is frequently expressed in cancers of the digestive tract, such as colon, pancreas, and biliary tract, with the exception of liver cell carcinoma (25, 26). The sialyl Le\(^a\) antigen is also known to appear in cancer cells of the digestive system, in addition to cancers of the lung and ovary (9–12, 24). Cancer cells originating in digestive organs other than the liver frequently express both antigens with a comparable strength, as was the case with the 4 colon cancer cell lines tested in this study. In such cases, sialyl Le\(^a\) plays a major role as a ligand in the ELAM-1-mediated adhesion of cancer cells to endothelium.

Sialyl Le\(^a\) is involved in the adhesion of cancer cells to ELAM-1 when the cancer cells express only sialyl Le\(^a\) antigen and no sialyl Le\(^a\) antigen or when the expression of the latter antigen is extremely weak. Clinical statistical studies indicate that sialyl Le\(^a\) is expressed more frequently than sialyl Le\(^a\) in cancers of the lung, liver, and ovary, while the frequencies of the two antigens are almost equal in stomach cancers (12, 24, 26). In this study, compatible with the clinical statistical findings, two of the three lung cancer cell lines, one liver cancer cell line, and one stomach cancer cell line expressed sialyl Le\(^a\) but much less sialyl Le\(^a\) antigen, and they adhered to endothelial cells via sialyl Le\(^a\).

It is postulated that sialyl Le\(^a\) expressed at the surface of cancer cells may promote hematogenous metastasis of cancers, since it is the ligand for the endothelial adhesion molecule. Some investigators (27, 28) have reported that metastatic lesions contain more sialyl Le\(^a\)-positive cells than the original tumor, which suggests a role of the carbohydrate antigen in metastasis. We speculate that sialyl Le\(^a\) may play a more important role than sialyl Le\(^a\) in the hematogenous metastasis of cancers of digestive organs other than the liver, while the metastasis of lung, liver, and ovarian cancers may well be mediated by sialyl Le\(^a\).

In the adhesion of leukemia cells to endothelial cells, on the other hand, carbohydrate antigens seem to play less significant roles than they played in the adhesion of cancer cells. A clear sialyl Le\(^a\)-ELAM-1-dependent adhesion was observed in only 3 of the 12 leukemia cell lines. Sialyl Le\(^a\) was not detected in any of the tested leukemia cell lines and was not involved in the adhesion of any leukemia cells to HUVECs, indicating that this antigen is much less frequently, if at all, expressed in leukemia cells. We conclude that the sialyl Le\(^a\)-ELAM-1 cell adhesion system plays an important role in the adhesion of epithelial cancer cells to endothelial cells, while its role in the adhesion of leukemia cells seems to be very minor.

**ACKNOWLEDGMENTS**

We thank Dr. Sen-itiroh Hakomori for the gift of the monoclonal antibodies. We also thank Masako Suzuki for her secretarial assistance in preparing the manuscript.

**REFERENCES**


Contribution of Carbohydrate Antigens Sialyl Lewis A and Sialyl Lewis X to Adhesion of Human Cancer Cells to Vascular Endothelium

Akiko Takada, Katsuyuki Ohmori, Tomoya Yoneda, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/2/354

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.