Sialyl SSEA-1 Antigen as a Carbohydrate Marker of Human Natural Killer Cells and Immature Lymphoid Cells

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The distribution of a carbohydrate antigen, the sialyl SSEA-1 (sialyl Le^x-i), in human lymphoid cells was investigated by flow cytometry with a specific monoclonal antibody, MoAb FH-6. We concluded that the lymphocytes positive for the sialyl SSEA-1 antigen present in normal peripheral blood (PB) are natural killer (NK) cells since the positive cells had an NK activity toward K562 cells, and most of the sialyl SSEA-1$^+$ cells were simultaneously positive for Leu-11 (CD-16) and Leu-19. Essentially, no T and B cells, defined by Leu-4 (CD3) and Leu-16 (CD20), were positive for the sialyl SSEA-1 antigen in PB samples taken from healthy donors and patients with disorders unrelated to lymphoid malignancies. Among the malignant lymphoid cells, many sialylated SSEA-1$^+$ cells were observed in large granular lymphocyte (LGL) leukemia cells and some acute lymphoblastic leukemia (ALL) blasts, but not in CLL cells or malignant lymphoma cells. Sialyl SSEA-1 was also positive in some cultured human lymphoid cell lines. We conclude that expression of the sialyl SSEA-1 antigen is strictly limited to a distinct population of NK cells among the mature lymphocytes in normal PB, but the antigen is present in a wide range of immature lymphoblasts of T- and B-cell lineages as well as the NK-cell lineage. The sialyl SSEA-1 antigen disappears from the surface of immature lymphocytes of T- and B-cell lineages during the course of maturation.

SSEA-1 ANTIGEN was first described by Solter and Knowles as an embryonic antigen that appears in the murine preimplantation embryo in a stage-specific manner.[1] The antigenic determinant was carbohydrate in nature, and subsequent biochemical studies showed that the carbohydrate structure of the antigenic epitope was a Le^x-hapten which resided at the terminus of the i-antigenic core structure.[2] Many of the monoclonal antibodies (MoAbs) directed to human leukemia cells and granulocytes have been shown to react with the Le^x-hapten,[4-6] and the physiologic significance of this antigen in human hepatopoietic cells has been extensively studied.[7-11] Among the mature peripheral blood (PB) cells, the antigen was present specifically in granulocytes and monocytes and absent in lymphocytes and erythrocytes. However, when immature hematopoietic cells were examined, the antigen was present in a much wider range of cells[12-16]; the immature lymphoid and erythroid cells contained the antigen as well as immature cells of granulocytic and monocytic lineages.

We studied the distribution of the sialylated form of SSEA-1 antigen, which was specifically recognized by the murine MoAb FH-6, among the cells of lymphocytic lineage, including normal peripheral lymphocytes and malignant lymphoid cells.

MATERIALS AND METHODS

Preparation of MoAb and FACS analysis. The MoAb directed to the sialylated form of the SSEA-1 antigen (FH-6, murine IgM) was prepared as described previously.[17-19] The carbohydrate structure of the antigen recognized by FH-6 and its relation to the SSEA-1 antigen are summarized in Table 1. Some MoAbs, includ-

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Table 1. Carbohydrate Structure of the Sialyl SSEA-1 Defined by MoAb FH-6 Used in This Study and Its Relation to SSEA-1

Antigen	Hapten	Antibody	Carbohydrate Structure
			[—Le^x antigen—]
			[———— i antigen ————]
Sialyl SSEA-1	Sialyl Le^x-i	FH-6	NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3R Fucα1↗3 ±(Fucα1↗3)*
			[—Le^x antigen—]
			[———— i antigen ————]
SSEA-1	Le^x-i	Anti-SSEA-1	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3R Fucα1↗3 ±(Fucα1↗3)*

SSEA-1 has the Le^x-hapten and i-antigen structures; both structures are necessary for the anti-SSEA-1 antibody to react with the antigen.[3] Therefore, the antigen is designated as Le^x-i in hapten symbols. Similarly, the presence of NeuAc2-3 residue, Le^x-hapten, and i-antigen structures are all necessary for the antibody FH-6 to react with the antigen, and the sialylated SSEA-1 antigen is designated as sialyl Le^x-i in hapten symbols.[18,19]

*The symbol ± indicates that the second fucose residue is not strictly required for the antibody to react with the antigen, but the difucosylated structure is more commonly found in cell surface glycoconjugates.

Table 2. Characterization of Sialyl SSEA-1$^+$ Lymphocytes Prepared From PB of Healthy Donors and Patients With Disorders Not Related to Lymphoid Malignancy by Two-Color Flow Cytometric Analyses

	Positive Cells (%)						
Case	Sialyl SSEA-1$^+$	Sialyl SSEA-1$^+$ and Leu-4$^+$	Sialyl SSEA-1$^+$ and Leu-16$^+$	Sialyl SSEA-1$^+$ and Leu-11$^+$	Sialyl SSEA-1$^+$ and Leu-11$^-$	Sialyl SSEA-1$^+$ and Leu-19$^+$	Sialyl SSEA-1$^+$ and Leu-19$^-$
1	4.5	0.6	0.1	3.0	1.5	3.8	0.7
2	15.3	0.3	0.1	11.2	4.1	10.8	4.5
3	8.6	1.0	0.2	6.0	2.6	5.8	2.8
4	7.8	0.4	0.2	5.2	2.6	5.1	2.7
5	6.5	0.7	0.2	4.0	2.5	5.0	1.5
6	11.3	0.9	0.1	7.7	3.6	9.0	2.3
7	28.3	0.5	0.1	19.2	9.1	26.0	2.3
8	20.5	0.2	0.1	16.5	4.0	18.5	2.0
9	7.2	0.7	0.1	5.5	1.7	5.6	1.6
10	20.6	0.3	0.2	11.1	9.5	13.0	7.6
11	20.7	0.4	0.3	18.1	2.6	16.3	4.4
12	6.3	1.0	0.2	4.0	2.3	3.8	2.5
13	7.6	0.6	0.2	5.4	2.2	5.7	1.9
14	16.4	0.8	0.1	13.2	3.2	15.1	1.3
15	28.3	0.3	0.2	18.6	9.7	21.3	7.0
Mean ± SD	14.0 ± 8.1	0.6 ± 0.3	0.2 ± 0.1	9.9 ± 5.9	4.1 ± 2.9	11.0 ± 7.1	3.0 ± 2.0

ing Leu4 (CD3) (for T cell), Leu16 (CD20) (for B cell), Leu7, Leu11 (CD16), and Leu19 (for NK cell),[20-22] were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, CA); others were obtained from Coulter Immunology Division (Hialeah, FL).

Human PB was obtained from healthy donors and also from patients with lymphoid malignancies. Lymphocytes were isolated from Ficoll/Hypaque by standard methods.[21] Two LGL leukemia patients were included. Case 13, a 39-year-old woman with a chief complaint of hepatosplenomegaly had a WBC count of 71,000 at onset and moderate NK activity of peripheral lymphoid cells; case 14, a 51-year-old woman had a chief complaint of arthralgia, a WBC count of 18,900 at onset, and high NK activity of peripheral lymphoid cells.

Cultured human lymphoid cell lines (YT, INC, MOLT-3, HPB-ALL, T-34, ATL-2, and FL18) were obtained from the First

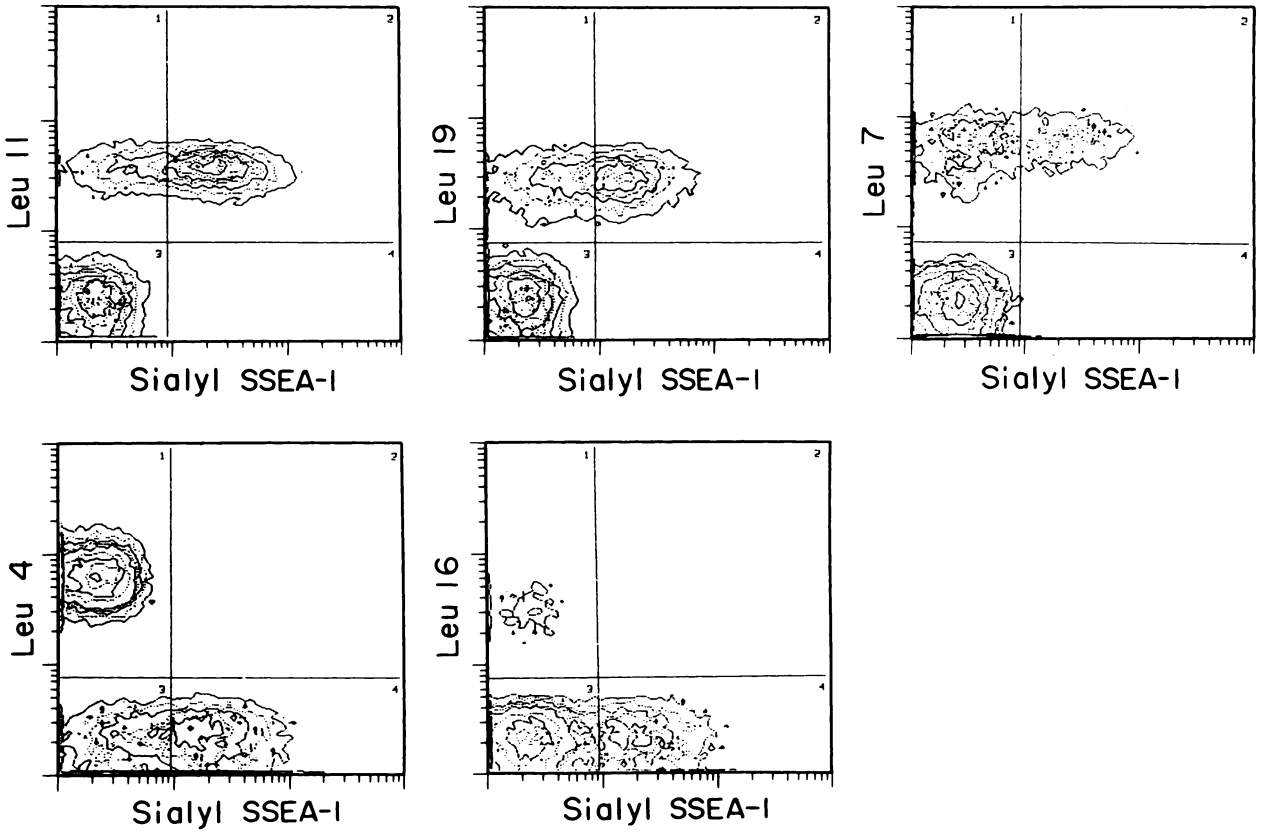

Fig 1. Two-color flow cytometric analysis of the sialyl SSEA-1$^+$ lymphocytes double-stained with Leu-11 (CD16), Leu-19, Leu-7, Leu-4 (CD3), and Leu-16 (CD20). Lymphocytes were prepared from the PB of a healthy donor. Sialyl SSEA-1 was detected with MoAb FH-6 by an indirect immunofluorescence method with FITC-labeled rabbit anti-murine IgM (μ chain specific) antibody. Other antigens were visualized with PE-labeled MoAbs by a direct immunofluorescence method.

Division of Internal Medicine, Kyoto University, Japan, and cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS).[23-25]

Analysis and sorting of lymphocytes were performed by flow cytometry with a FACS analyzer and FACS 440 (Becton Dickinson). Contamination of monocytes in the lymphocyte fraction in FACS analysis was checked by staining with Leu-M3 (CD14) antibody, and was <1% in all analyses in this study. The indirect immunofluorescense method was applied for staining of lymphocytes with the antibody FH-6 by using FITC-labeled rabbit anti-murine IgM (μ chain specific) antibody as a second antibody; MoAbs FH-2 and AH-6 (both murine IgM), with specificities different from that of FH-6, were used as negative isotype-matched controls throughout the study.

Fractionation of human peripheral lymphocytes. In some experiments, isolated peripheral lymphocytes were further fractionated by a nylon-wool column, and nonadherent cells were subjected to discontinuous density-gradient centrifugation with 47.5% to 27.5% Percoll solution (Pharmacia, Uppsala, Sweden) in RPMI medium.[20]

Assay of NK cell cytotoxicity. NK cell activity was determined by using, as effector cells, the Leu-11 (CD16)$^+$ sialyl SSEA-1$^+$ and Leu-11 (CD16)$^+$ sialyl SSEA-1$^-$ peripheral lymphocytes obtained from healthy donors and enriched by FACS (FACS 440 Becton Dickinson). A NK-sensitive human erythroleukemia cell line, K562, was labeled with ^{51}Cr-sodium chromate, and the labeled cells were used as target cells in a six-hour radioisotope release assay conducted in triplicate with varying effector/target ratios.[26]

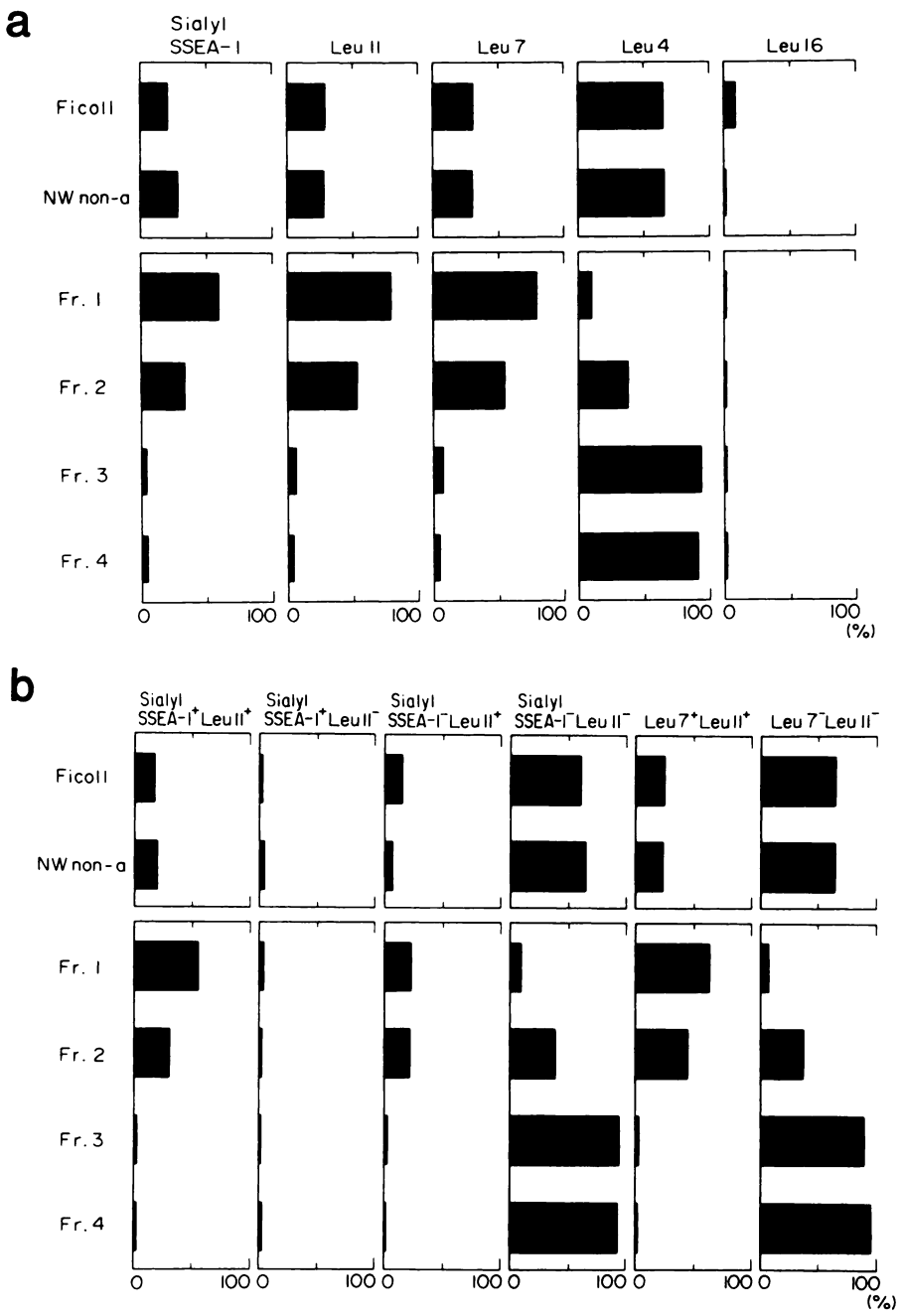

Fig 2. Flow-cytometric analysis of the peripheral lymphocytes separated by nylon-wool column and density-gradient centrifugation. Pooled lymphocytes prepared from the PB of healthy donors were used for the starting material. The concentrations of the Percoll solution used in the discontinuous density-gradient system were 27.5%, 32.5%, 37.5%, 42.5%, and 47.5% from the top of the tube. The fraction was numbered from the low-density fraction. One-color flow cytometric analysis of each fraction (a); results of two-color analysis performed on the same fraction (b).

RESULTS

Characterization of sialyl SSEA-1$^+$ lymphocytes in the PB of healthy donors. The percentage of sialyl SSEA-1$^+$ lymphocytes in the peripheral lymphocytes of 60 healthy donors ranged from 2% to 21% (mean ± SD, 7.6% ± 4.6%). When two-color FACS analyses were performed on the peripheral lymphocytes obtained from healthy donors and patients with nonmalignant diseases with >5% of sialyl SSEA-1$^+$ lymphocytes (Table 2), most sialyl SSEA-1$^+$ cells overlapped with Leu-11 (CD16)$^+$ or Leu-19$^+$ lymphocytes, both of which are markers for NK cells. No significant number of sialyl SSEA-1$^+$ cells (<1%) were detected in T- and B-cell populations defined by Leu-4 (CD3) and Leu-16 (CD20) antibodies, respectively.

Figure 1 shows the two-color cytometric patterns of sialyl SSEA-1$^+$ cells with the antibodies of the peripheral lymphocytes prepared from a healthy donor. Most of the sialyl SSEA-1$^+$ cells were simultaneously positive for Leu-7, Leu-11 (CD16), and Leu-19, and essentially negative for Leu-4 (CD3), and Leu-16 (CD20). Leu-7$^+$, Leu-11 (CD16)$^+$ and Leu-19$^+$ cells showed various degrees of sialyl SSEA-1 expression; ie, not all of the Leu-11 (CD16)$^+$ and Leu-19$^+$ cells were positive for the sialyl SSEA-1 antigen. In the case of the donor analysis shown in Fig 1, 42.9% of Leu-7$^+$ cells, 51.8% of Leu-11 (CD16)$^+$ cells, and 51.8% of Leu-19$^+$ cells were positive for the sialyl SSEA-1 antigen, while only 3.2% of Leu-4 (CD3)$^+$ T cells and 1.9% of Leu-16 (CD20)$^+$ B cells were positive for the sialyl SSEA-1 antigen.

Analysis of sialyl SSEA-1$^+$ lymphocytes in the PB of healthy donors by nylon-wool column and density-gradient centrifugation. When the pooled peripheral lymphocytes prepared from healthy donors were subjected to nylon-wool-column chromatography, most of the sialyl SSEA-1$^+$ lymphocytes were recovered in the nonadherent fraction. The results of FACS analysis of these nylon-wool nonadherent lymphocytes that had been subjected to further fractionation on discontinuous density-gradient centrifugation is shown in Fig 2a. The sialyl SSEA-1$^+$ cells were enriched in fractions 1 and 2, which contained LGLs under microscopic examination, and these fractions contained most of the Leu-11 (CD16)$^+$ and Leu-7$^+$ cells. On the other hand, Leu-4 (CD3)$^+$ T cells were enriched in fractions 3 and 4, shown by microscopic examination to contain small lymphocytes but no significant number of sialyl SSEA-1$^+$, Leu-11 (CD16)$^+$, and Leu-7$^+$ cells. Two-color analyses of the same fractionated lymphocytes indicated that most of the sialyl SSEA-1$^+$ cells in fractions 1 and 2 were also positive for Leu-11 (CD16) (Fig 2b).

NK activity of sialyl SSEA-1$^+$ lymphocytes in the PB of healthy donors. Figure 3 shows the NK cytotoxic activity of Leu-11 (CD16)$^+$ sialyl SSEA-1$^+$ and Leu-11 (CD16)$^+$ sialyl SSEA-1$^-$ lymphocytes prepared by FACS from the PB of healthy donors. Both the Leu-11 (CD16)$^+$ sialyl SSEA-1$^+$ and Leu-11 (CD16)$^+$ sialyl SSEA-1$^-$ cells had a significant NK cell activity, whereas that of Leu-11 (CD16)$^-$ sialyl SSEA-1$^-$ cells was very low. A significant release of ^{51}Cr from the target cells by Leu-11$^+$ sialyl SSEA-1$^+$ and Leu-11$^+$ sialyl SSEA-1$^-$ cells was unequivocally observed [range

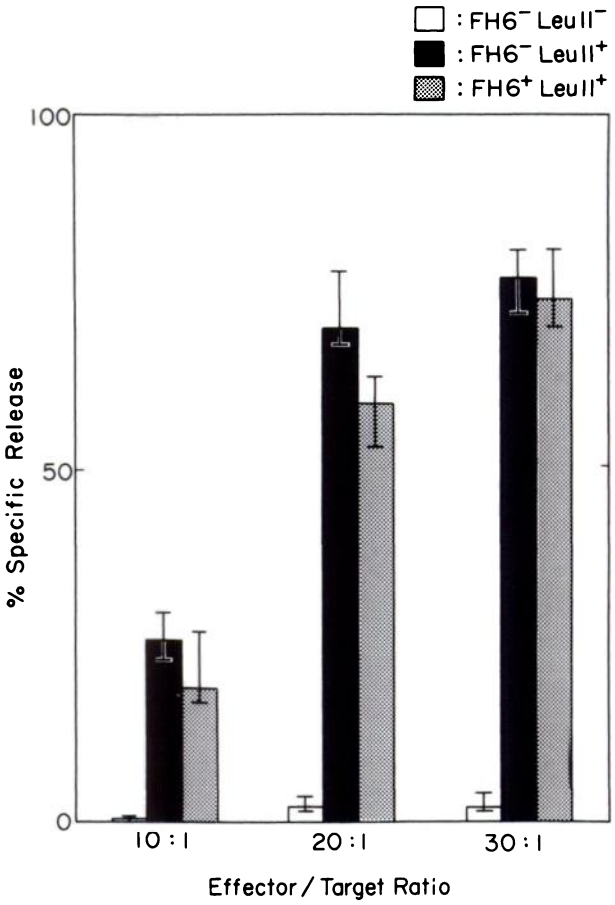

Fig 3. NK cytotoxic activities of Leu-11 (CD16)$^+$ sialyl SSEA-1$^+$ lymphocytes and Leu-11 (CD16)$^+$ sialyl SSEA-1$^-$ lymphocytes toward K562 cells. Both Leu-11 (CD16)$^+$ sialyl SSEA-1$^+$ lymphocytes and Leu-11 (CD16)$^+$ sialyl SSEA-1$^-$ lymphocytes were enriched by FACS of the lymphocytes prepared from the PB of healthy donors (dotted bars and closed bars, respectively). Leu-11 (CD16)$^-$ sialyl SSEA-1$^-$ cells similarly sorted served as controls (open bars).

33% to 89% at the effector/target (E/T) ratio of 20:1], and Fig 3 shows an example of such experiments.

Distribution of sialyl SSEA-1 antigen in the malignant cells of lymphoid lineage. The percentage of sialyl SSEA-1$^+$ cells in the peripheral lymphoid cells of patients with a lymphoid malignancy are shown in Fig 4, and the frequencies of other markers in these patients are summarized in Table 3. Most of the lymphoblastoid leukemia cells in patients with ALL contained a significant number of sialyl SSEA-1$^+$ cells, and the distribution pattern of positive cells was similar to the pattern of common-ALL (CALLA) antigen. Lymphoblastoid cells in some of these ALL patients expressed B-cell markers such as Leu-12 (CD19) or Leu-16 (CD20), and others expressed T-cell markers such as OKT11 (CD2), Leu-4 (CD3), or Leu-3a (CD4). These cells were still positive for sialyl SSEA-1 antigen (ascertained by two-color analyses, data not shown). This indicates that in malignant cells expression of sialyl SSEA-1 is not limited to the cells of NK lineage, but the antigen is present in the immature cells of T- and B-cell lineage. ALL cells having the

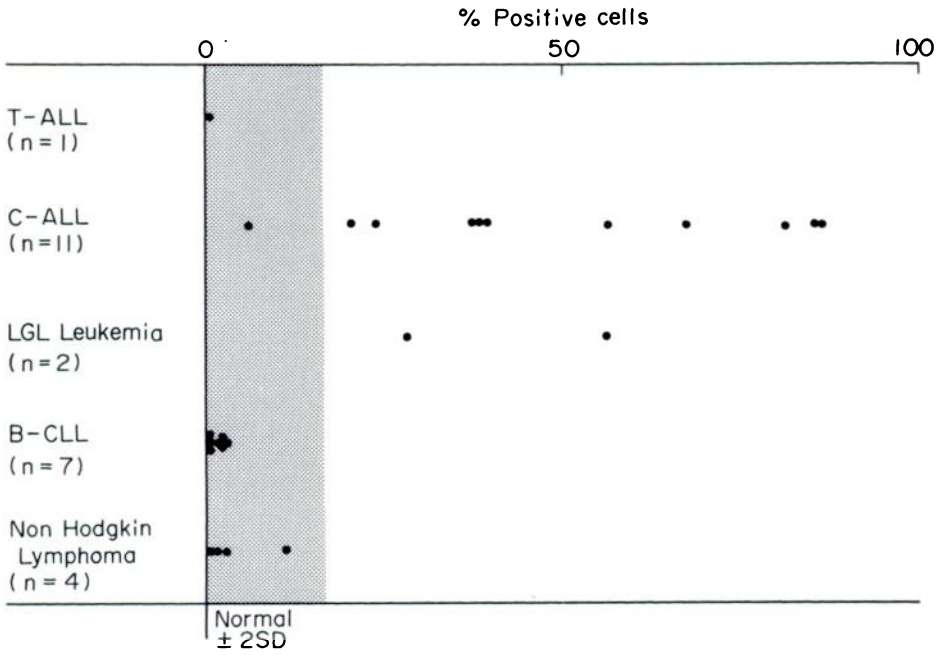

Fig 4. Occurrence of sialyl SSEA-1 in human peripheral lymphocytes and leukemic cells in patients with various lymphoid malignancies. Table 3 shows details of the cell-surface antigens of the individual patient.

overt T-cell characteristics such as in T-ALL tended to be negative for the antigen.

Besides ALL patients, lymphoid cells in the PB of two LGL leukemia patients showed a high frequency of sialyl SSEA-1$^+$ cells, which corresponded well with the high proportion of Leu-7$^+$ and Leu-11 (CD16)$^+$ cells in these patients. The percentage of sialyl SSEA-1$^+$ cells were within the normal range in all CLL and malignant lymphoma (leukemic stage) patients studied. Some established human lymphoid cell lines which are supposed to have originated

Table 3. Summary of Positive Frequencies of Sialyl SSEA-1 and Other Markers in Lymphoid Cells of Patients With Various Lymphoid Malignancies

Case	Diagnosis	Sialyl SSEA-1	Leu-11 (CD16)	Leu-7	OKT11 (CD2)	Leu-4 (CD3)	Leu-3a (CD4)	Leu-2a (CD8)	Leu-1 (CD5)	J 5 (CD10)	Leu-12 (CD19)	Leu-16 (CD20)	Smlg	HLA-DR
1	T-ALL	1.5(%)	0.9	1.2	94.9	32.7	47.9	52.9	78.8	2.9	1.1	2.6	2.6	1.7
2	C-ALL	81.9	1.5	4.6	13.7	9.8	4.6	7.5	6.9	84.8	33.1	67.3	0.0	81.7
3	C-ALL	35.3	NT	5.8	12.1	16.2	7.0	10.6	15.0	10.6	69.6	27.2	0.0	78.6
4	C-ALL	37.0	3.8	8.9	26.4	25.0	10.1	11.9	20.1	67.3	14.4	66.8	0.0	76.7
5	C-ALL	35.8	2.1	1.5	17.0	17.7	8.6	7.8	19.0	67.7	37.2	66.8	0.0	64.8
6	C-ALL	84.0	0.8	0.6	10.1	3.8	1.6	1.6	4.8	67.8	70.3	6.0	1.2	57.9
7	C-ALL	57.3	1.7	1.2	2.1	8.1	1.6	2.9	3.4	80.4	0.3	5.4	0.0	64.8
8	C-ALL	5.7	1.7	4.6	4.6	11.4	2.5	7.5	4.2	19.0	0.9	6.6	0.8	31.8
9	C-ALL	34.0	NT	NT	48.7	44.8	26.3	17.5	NT	32.5	10.4	35.3	6.9	41.9
10	C-ALL	81.0	NT	1.5	24.8	23.5	14.9	8.3	NT	76.3	70.5	72.9	3.3	74.6
11	C-ALL	22.2	0.5	5.0	1.1	1.8	0.9	1.0	1.7	25.3	16.7	2.6	0.8	86.5
12	C-ALL	60.8	0.7	3.4	9.1	7.9	2.0	6.1	6.1	2.5	69.6	23.6	2.6	93.8
13	LGL	28.2	92.0	NT	73.0	2.0	1.2	1.2	NT	NT	0.6	0.8	NT	97.0
14	LGL	56.3	46.3	18.8	84.5	23.0	17.5	16.6	21.2	4.5	5.0	8.5	3.2	60.6
15	B-CLL	0.8	0.6	4.0	12.7	12.8	8.0	2.2	23.0	1.1	67.1	44.1	G81.2 κ84.0	82.9
16	B-CLL	2.8	6.6	8.0	17.1	10.1	4.0	6.0	59.1	5.4	35.4	76.5	M73.4 κ72.8	62.0
17	B-CLL	1.8	3.3	9.8	8.4	5.0	2.1	3.0	23.4	3.8	6.1	54.3	M49.3 κ50.5	67.9
18	B-CLL	1.9	2.6	16.2	43.4	39.7	19.0	20.5	35.7	0.6	13.1	33.9	G37.2 κ38.0	36.3
19	B-CLL	2.2	4.3	7.4	10.8	6.4	4.0	2.9	22.5	2.3	58.1	65.9	G71.0 λ49.1	87.4
20	B-CLL	0.7	0.2	0.6	1.4	1.7	0.6	0.8	79.2	0.5	9.1	96.1	M89.1 λ90.3	97.4
21	B-CLL	2.6	0.4	7.1	1.9	1.4	1.0	0.9	1.5	4.6	19.7	66.0	M61.7 κ60.9	28.5
22	NHL	2.4	3.5	2.6	10.9	8.2	5.8	2.6	27.7	0.5	84.8	81.3	M82.6 κ84.0	83.7
23	NHL	1.0	1.1	0.9	73.7	87.5	81.2	21.0	88.1	2.6	10.9	15.4	1.0	70.2
24	NHL	0.3	0.2	0.3	35.9	28.7	17.9	7.9	29.1	1.2	0.6	8.7	1.0	20.1
25	NHL	11.2	0.8	4.9	16.3	13.2	13.4	2.6	12.9	64.2	24.0	73.7	M92.2 κ93.1	70.1

NT, not tested.

Table 4. Expression of Sialyl SSEA-1 in Cultured Human Lymphoid Cell Lines

Cells	Origin	Sialyl SSEA-1	OKT6 (CD1)	OKT11 (CD2)	Leu-4 (CD3)	Leu-3a (CD4)	Leu-2a (CD8)	Leu-1 (CD5)	J 5 (CD10)	Leu-16 (CD20)	Leu-11 (CD16)
YT	NK-like (LGL)	90.1	0.3	0.2	0.2	0.2	0.2	0.1	1.6	1.8	0.3
INC	C-ALL	65.3	1.6	0.8	1.2	0.8	0.2	0.2	55.6	50.1	0.7
MOLT-3	T-ALL	92.7	52.7	10.5	5.6	54.7	0.9	98.7	1.9	1.5	0.4
HPB-ALL	T-ALL	0.3	40.8	4.6	0.7	67.6	1.3	92.0	1.8	0.1	0.1
T-34	T-NHL	0.5	3.6	2.9	84.0	1.3	1.0	97.1	1.8	1.4	1.3
ATL-2	ATL	4.5	2.1	33.1	3.4	99.7	0.8	89.2	1.7	1.3	1.9
FL-18	B-NHL	0.5	0.3	0.4	0.2	0.1	0.3	0.1	72.3	100.0	0.2

from LGL cells and c-ALL cells were positive for the sialyl SSEA-1 as shown in Table 4.

DISCUSSION

Some mouse MoAbs directed toward normal and leukemic myeloid cells recognize the Le^x-hapten.[4-6] In early studies, this antigen was considered specific for myeloid (granulocytic and monocytic) cells,[9-11] and the MoAbs were used for differential diagnosis of lymphoblastic and nonlymphoblastic leukemia. Detection of Le^x antigen in leukemic cells has even been reported to have a prognostic value.[15] However, the antigen was later shown to be expressed at the surface of leukemic cells and normal cells, including lymphoid cells at very early stages of their differentiation.[8,10-12]

Some investigators studied the distribution of sialylated forms of Le^x antigen in human hepatopoietic cells by applying anti-Le^x MoAbs to cells that had been treated with neuraminidase[10-12,16] and reported that the sialylated forms of the antigen were also widely present on the surface of leukemic cells and normal cells, including lymphoid cells at very early stages of differentiation. However, by combining neuraminidase and anti-Le^x antibodies, all forms of the sialylated Le^x antigen can be detected, including NeuAc2-3Le^x, NeuAc2-6Le^x, NeuAc2-8NeuAc2-3Le^x, etc; therefore, the exact carbohydrate structure of the antigen cannot be specified. Moreover, preexisting Le^x antigens not sialylated but only sterically masked by unrelated sialic acid-containing glycoconjugates at the cell surface will also be exposed toward the anti-Le^x antibody by the treatment, and appearance of Le^x antigen after enzymatic treatment does not necessarily indicate the presence of the sialylated form of the antigen.[27] In this study, we avoided use of sialidase treatment and used a MoAb that specifically reacts with NeuAc2-3Le^x-i (sialyl SSEA-1) antigen, to characterize the antigen directly.

Our results clearly indicated that sialyl SSEA-1^+ cells in the mature peripheral lymphocytes are mostly NK cells, and the antigen can be used as a marker for NK cells as well as Leu-7, Leu-11 (CD16), and Leu-19. It is noteworthy that the Leu-7 antigen is also an acidic carbohydrate antigen with the type-2-chain polylactosamine structure (the repeating unit Galβ1-4GlcNAc) in the core chain, similar to the sialyl SSEA-1 antigen.[28] NK cells can be characterized as having unique acidic polylactosamine antigens at their surfaces as compared with lymphocytes of other lineages, and these cell surface carbohydrates may be related functionally to recognition of various target cells by the NK cells.

Not all Leu-11 $(CD16)^+$ or Leu-19^+ cells were positive for sialyl SSEA-1. Indeed, the sialyl SSEA-1 antigen divides the Leu-11 $(CD16)^+$ NK cells into two distinct populations, Leu-11 $(CD16)^+$ sialyl SSEA-1^+ cells and Leu-11 $(CD16)^+$ sialyl SSEA-1^- cells, both of which have an apparent NK cell activity. Besides NK cells, Leu-11 (CD16) is reported to be positive in a part of Leu-1 $(CD5)^+$ or Leu-4 $(CD3)^+$ T-cells,[21] and Leu-19 is positive in a part of Leu-4 $(CD3)^+$ cytotoxic T cells.[22] Because sialyl SSEA-1 is not detected in any Leu-4 $(CD3)^+$ T cells, the sialyl SSEA-1^+ cells probably represent the NK cell population without the overt T-cell markers such as Leu-1 (CD5) or Leu-4 (CD3).

Studies of the malignant lymphoid cells and cell line cells indicated that the sialyl SSEA-1 was expressed by a wider range of immature lymphoid cells, including NK-, T-, and B-cell lineages at very early stages of their differentiation. In cells at the very early stage of differentiation, the sialyl SSEA-1 appeared to be commonly present in the lymphoid cells of each lymphoid cell lineage. The antigen probably disappears during the course of differentiation in T- and B-cell lineages, and the antigen is persistently positive during the course of differentiation and even in the fully mature cells in the NK cell lineage.

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