Jpn. J. Cancer Res. 87, 1070–1077, October 1996

Metastatic Potential of Lymphoma/Leukemia Cell Lines in SCID Mice Is Closely Related to Expression of CD44

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To investigate whether the lymphocyte homing receptors, adhesion molecules regulating normal lymphocyte traffic, influence the dissemination of lymphoma cells, 24 lymphoma/leukemia cell lines were inoculated into SCID mice subcutaneously, and the correlation between the expression of the adhesion molecules and the metastatic potential of the cell lines was examined. Among the six adhesion molecules examined (LFA-1, ICAM-1, CLA, VLA-4, L-selectin and CD44), L-selectin increased the incidence of lymph node metastasis, and CD44 expression was related to both lymph node and organ (hematogenous) metastasis. A monoclonal antibody to the standard form of CD44 (CD44s), Hermes-3, inhibited the local growth and remote metastasis of CD44$^+$ cell lines. Thus, it is concluded that at least CD44s expression is important in both lymphatic and hematogenous metastasis.

Key words: Malignant lymphoma — Adhesion molecule — Hermes-3 — Metastasis — Animal model

Most mature lymphocytes recirculate continuously between the blood and the lymphoid organs via high endothelial venules (HEVs).[1] This step is mediated by interactions between the lymphocyte homing receptors expressed on the lymphocyte cell surface and the endothelial ligands.[2–6] Recently, several homing receptors have been identified and characterized.[6–8]

VLA-4, which belongs to the β1-integrin family, is involved in the strong adhesion of lymphocytes to the endothelial cell surface through VCAM-1.[8–10] It is also involved in the adhesion of B cells to follicular dendritic cells in the germinal centers.[11] LFA-1 (CD11a/CD18), which belongs to the β2-integrin family, has also been implicated in strong lymphocyte adhesion to HEVs through ICAM-1 and ICAM-2, which belong to the immunoglobulin (Ig) superfamily.[6–8, 10] ICAM-1 is expressed not only on endothelial cells, but also on leukocytes, fibroblasts and epithelial cells.[8] L-Selectin (LECAM-1, CD62L) belongs to the selectin family and binds to the sialyl Lewis^x,[12] MAdCAM-1,[13] CD34[14] and GlyCAM-1.[15] L-Selectin participates in the lymphocyte homing to HEVs of the peripheral lymph nodes through CD34 and GlyCAM-1 and that of the mucosal lymphoid apparatus through MAdCAM-1.[8, 10] CLA (cutaneous lymphocyte-associated antigen) is thought to be a skin lymphocyte homing receptor expressed on memory T cells and to bind to the vascular adhesion molecule E-selectin preferentially expressed at inflammatory skin sites.[16] CD44 is a widely distributed cell surface glycoprotein with proposed multifunctions in cell-cell and cell-extracellular matrix interactions that are involved in hematopoiesis, cell migration and lymphocyte homing.[17, 18] CD44 is known to bind to hyaluronic acid, collagen and fibronectin, but the ligand on endothelial cells remains to be clarified.[17, 18] CD44 isoforms are generated by alternative splicing of at least 10 variant exons.[19] The major CD44 isoform on lymphocytes is the smallest standard molecule (CD44s). Different, larger variant isoforms of CD44 are expressed widely throughout the body, especially on epithelial cells. The variant exon 6 and exon 9 (CD44-6v and CD44-9v) are also transiently upregulated on T cells after mitogenic stimulation.[19] Recently, CD44-6v was reported to be related with metastatic or aggressive behavior of malignant tumors including non-Hodgkin's lymphoma.[20–22)]

Because non-Hodgkin's lymphomas represent the malignant counterparts of normal lymphocytes, the above-mentioned adhesion molecules regulating normal lymphocyte traffic may also, at least to a certain extent, be involved in the dissemination of these malignancies. In the present work, we have explored the correlation between the expression of these adhesion molecules and the dissemination of lymphoma/leukemia cells transplanted into C.B.17 scid/scid mice (SCID mice) using human hematopoietic cell lines.

MATERIALS AND METHODS

Animals SCID mice were purchased from Japan CLEA Co., Tokyo and were maintained under pathogen-free

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Table I. Expression of Adhesion Molecules in the Cell Lines (%)

Cell line	Origin	Adhesion molecule						
		VLA-4	L-selectin	CLA	LFA-1α	LFA-1β	ICAM-1	CD44
TALL-1	T-ALL	90.9	23	0	100	79.5	25.5	100
KE-37	T-ALL	45.5	0	0	23	33.2	56.5	100
HPB-ALL	T-ALL	95.2	0	0	50	53.9	0	100
HSB-2	T-ALL	88	63.5	1	100	100	100	94
MOLT-4	T-ALL	99.1	21.5	0	96	89.8	5	100
KOPT-K1	T-Lymphoma	100	4.3	0	0	0	0	90
JURKAT	T-Lymphoma	96.1	5.5	0	0	15.3	39.4	0
MAT	T-Lymphoma	62.6	8.8	0	100	18	2.5	100
DL-40	T-Lymphoma	50.5	5.5	1	1	1	0	1
HUT-102	Mycosis fungoides	0	0	66.9	96.5	95.5	99	100
MT-1	ATL	0	34	24.6	94.5	91	0	5
SALT-3	ATL	65.3	0	0	21.4	13.9	100	100
ED-S	ATL	80.8	71	78	93.5	81.9	76.7	72.8
MT-2	HTLV-1 transformed T	72	3.5	5.5	9.5	5.5	100	73
MT-4	HTLV-1 transformed T	69.3	89.3	65.3	85	50.4	82.4	85.2
BALL-1	B-ALL	98	3	0	1	4	94	85
BALM-1	B-ALL	88.2	14.9	0	72.4	77.5	92	22.7
SU-DHL-4	B-Lymphoma	75.8	0	0	79	81.3	95.7	0
SCOTT	B-Lymphoma	85	2.5	2	6	3.5	14.5	1
KW	B-Lymphoma	84.5	25	0	9	4.5	94.5	1
KCA	B-Lymphoblastoid	4	0	1	2.5	9.5	100	100
LBF	B-Lymphoblastoid	89	87	62.5	100	95.5	88	100
LB-25	B-Lymphoblastoid	90	90.5	100	100	100	100	5
NALL-1	Non-B, non-T, ALL	92	4.8	55	1.5	3.8	39	92

conditions at the Animal Center for Medical Research of Okayama University Medical School.

Cell lines The cell lines used in this study included T-ALL, T-lymphoma, ATL, mycosis fungoides, HTLV-I-transformed T-cell, B-ALL, B-lymphoma, B-lymphoblastoid and non-B non-T-ALL lines and are listed in Table I. The KW, KCA, LBF, and LB-25 cell lines were kindly provided by Dr. E. C. Butcher (Stanford University, California). The other cell lines were maintained in our laboratory or were provided by the Fujisaki Cell Bank Center at Hayashibara Biochemical Laboratories (Okayama). All cell lines were cultivated in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 U/ml penicillin, 50 μg/ml streptomycin and 4 m*M* L-glutamine and incubated in a 5% CO_2-humidified atmosphere at 37°C.

Antibodies Monoclonal antibodies (mAbs) to adhesion molecules included anti-VLA-4 mouse mAb (Immunotech S. A.; Marseille, France), two kinds of mouse mAbs against LFA-1 (DAKO, Glostrup, Denmark), and anti-ICAM-1 mouse mAb (BBA-3) (British Biotechnology Ltd., Oxon, UK). Anti L-selectin mouse mAb (Dreg56), anti-CD44 mouse mAb (Hermes-3), and anti-CLA rat mAb (Heca452), which were kindly provided by Dr. Butcher, were also used. These mAbs were used at a dilution of 1 : 20–1 : 40. Abs against CD1 (Leu6), CD3 (Leu4), CD4 (Leu3a), CD5 (Leu1), CD8 (Leu2a), CD19 (Leu12), CD20 (Leu16), CD22 (Leu14) and Leu7 were purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA). Abs against pan B cells (L26), CD45RO (OPD4), CD30/Ki-1 (Ber-H2), CD45 (LCA) and EMA (E26) were purchased from DAKO (Glostrup), MT-1 was from Bio-Science Products AG (Emmenbrucke, Switzerland), and mAbs against Cdw75 (LN-1), CD74 (LN-2) and HLA-DR (LN-3) were from Techniclone International Corporation (Santa Ana, CA). Rabbit antisera to the kappa light chain of human Ig and the lambda light chain of human Ig were purchased from Becton Dickinson Immunocytometry Systems.

Immunofluorescence Expression of the adhesion molecules was examined by the indirect immunofluorescence method using six kinds of mouse mAbs and one kind of rat mAb against various adhesion molecules (VLA-4, LFA-1, ICAM-1, L-selectin, CD44 and CLA). Cells (1×10^5) were incubated on ice for 1 h with each mAb and then incubated with FITC-conjugated anti-mouse Ig goat serum or FITC-conjugated anti-rat Ig goat serum diluted 1 : 20 (TAGO, Burlingame, CA) for 30 min on ice. The stained cells were observed with a fluorescence microscope, and the ratio of positive cells was calculated.

Immunocytochemistry Immunostaining was performed

by an indirect avidin-biotin complex method (SensiTek universal kit, ScyTek Laboratories, Logan, UT) as follows. Cell smears were fixed in cold acetone for 10 min and then incubated with bovine serum diluted 1 : 10 for 10 min to block nonspecific staining. They were washed, and incubated with appropriate dilutions of each antibody at room temperature for 1 h, with biotin-conjugated anti-mouse Ig goat IgG for 30 min, and then with peroxidase-labeled streptavidin. The reaction was visualized by incubating with 0.3 mg/ml diaminobenzidine for 5–8 min in the presence of hydrogen peroxide in 0.1 *M* phosphate-buffered saline (pH 7.4).

Reverse transcription-polymerase chain reaction (RT-PCR) Total cellular RNA was isolated from 10^6 cells of each cell line using RNA sol B (CINNA/BIOTECX Laboratories, Inc., Houston, TX) according to the manufacturer's instructions. cDNA was synthesized from 5 μg of total cellular RNA by reverse transcription with viral reverse transcriptase. The procedure was performed using the Ready·To·Go T-Primed First-Strand Kit (Pharmacia Biotech, Tokyo) following the kit manual. Semi-nested and nested PCR for CD44 was carried out as follows. The primers, designed according to the reported sequence of human CD44,[23] were 5′-TTACACCTTTTCTACTGTACACCCC (F5), 5′-CCAGGCAACTCCTAGTAGTACAAC (F10), 5′-TTTGGGGTGTCCTTATAGGACCAG (R16), 5′-TCAGATCCATGAGTGGTATGGGAC (R15), and 5′-GAATGGGAGTCTTCTCTGG-GTGTT (R10). First, 30 cycles of PCR were performed with two primers, F5 and R16. To detect the standard form of CD44 (CD44s), 30 cycles of PCR were performed with primers F5 and R15. Primers F10 and R10 were used for PCR of the second step to detect the variant exon 6 of CD44 (CD44-v6). PCR reactions were performed as follows: 100 ng of cDNA was used as the template in a 50 μl reaction mixture that contained 10 m*M* Tris-HCl (pH 8.0), 80 m*M* KCl, 500 μg/ml bovine serum albumin, 0.1% sodium cholate, 0.1% Triton X-100, 200 μM of each NTP, the appropriate primers at 60 p*M*, and 0.8 units of Tth DNA polymerase. Each PCR cycle consisted of 1 min at 95°C, 2 min at 62°C, and 3 min at 72°C. PCR performed for 30 cycles at each amplification.

Heterotransplantation SCID mice (6-week-old males) were inoculated subcutaneously with 2.0×10^7 cells from each cell line to their left back. Four animals were used for each cell line. All of them were kept under pathogen-free conditions for 12 weeks. During this period, all visceral organs and enlarged lymph nodes were removed from mice found dead and from those killed after having been found in a moribund state. The rest of the mice were killed at the end of the observation period and examined in the same manner. Frozen tissue blocks of the primary subcutaneous tumors and some metastatic tumors were made from the mice killed at the end of the experiment. Tissues from the brain, salivery glands, lungs, heart, liver, kidneys, spleen, pancreas, stomach, large intestine, seminal vesicles and testicles of all animals were immediately fixed in buffered formalin, stained with hematoxylin and eosin, and observed microscopically. The numbers of metastatic foci in the organs and the lymph nodes were evaluated. To discriminate transplanted human cells from SCID mouse cells, tissues were immunostained with anti-human CD45 mouse mAb and rat mAbs, L3/T4 and Ly5 (B220), which react with murine T and B cells, respectively.

Effect of anti-CD44 mAb on growth and metastasis of heterotransplanted leukemia cells To evaluate the effect of anti-CD44 mAb on the progression of CD44-positive lymphoma cells, the tumor-bearing SCID mice were treated with anti-CD44 mAb. We used a T-ALL line, HSB-2, which is CD44-positive and highly metastatic. First, 2×10^7 HSB-2 cells per mouse were inoculated subcutaneously into 30 male SCID mice, 5 weeks old, in their left back. Beginning 1, 3, 7, or 10 days after the inoculation, 100 μg of anti-CD44 mAb, Hermes-3, was given intraperitoneally to 6 mice of each group twice a week. Tumor size was calculated by measuring the lengths of three dimensions of the tumors. Dead or moribund mice were autopsied to examine metastatic foci in the visceral organs and lymph nodes. The rest of the mice were killed 6 weeks after the first inoculation, and observed in the same way. As a negative control, a class-matched anti-human CD3 mouse mAb, OKT-3, was successively injected into the other six mice beginning one day after the transplantation.

Cell proliferation assay The cytotoxic effect of mouse mAbs on HSB-2 cells was examined by keeping HSB-2 cells (1×10^4/well) with each mAb (100 μg/ml) added to the medium in 96-well culture plates (Iwaki glass, Chiba). The cell number of each well was counted 3 days later.

Statistical analysis The χ square test was used to compare the numbers of mice with metastatic tumors. The paired *t* test was also used to compare the numbers of organs or lymph nodes in each group. A *P* value below 0.05 was considered significant.

RESULTS

Adhesion molecule profiles of each cell line Expression of adhesion molecules was examined by immunofluoroscopy (Table I). When over 20% of the cells reacted intensely with a given mAb, a cell line was evaluated as positive. Among the 24 human lymphoma/leukemia cell lines examined, 21 were VLA-4-positive, 9 were L-selectin-positive, 7 were CLA-positive, 15 were LFA-1α-positive, 13 were LFA-1β-positive, 17 were ICAM-1-positive, and 17 were CD44-positive.

Tumorigenesis and metastatic potential of the cell lines in

Table II. Transplantability and Organ or Lymph Node Metastases of the Cell Lines

Cell line	No. of mice with tumor (%)	No. of mice with lymph node metastases (%)	No. of mice with organ metastases (%)	Metastasized organ	
				No.	Site
TALL-1	4 (100)	3 (75)	4 (100)	13	BRN LNG LIV SPL PAN KID
KE-37	4 (100)	0	0	0	
HPB-ALL	4 (100)	2 (50)	3 (75)	8	LIV SPL PAN KID
HSB-2	3 (75)	3 (75)	3 (75)	11	BRN LIV SPL PAN KID
MOLT-4	3 (75)	2 (50)	2 (50)	4	LIV PAN KID
KOPT-K1	1 (25)	0	0	0	
MAT	2 (50)	2 (50)	2 (50)	5	HRT LNG LIV
DL-40	4 (100)	0	0	0	
MT-1	3 (75)	1 (25)	0	0	
BALL-1	4 (100)	3 (75)	3 (75)	8	LNG LIV SPL PAN KID TES SML
BALM-1	4 (100)	4 (100)	2 (50)	6	BRN LNG LIV PAN KID TES
SCOTT	3 (75)	0	1 (25)	1	BRN
KW	3 (75)	2 (50)	0	0	
KCA	4 (100)	2 (50)	3 (75)	8	BRN LNG LIV SPL PAN TES SML
LBF	4 (100)	4 (100)	3 (75)	8	BRN LNG LIV TES SML
NALL-1	3 (75)	1 (25)	2 (50)	3	LIV KID

BRN, brain; SAL, salivary gland; HRT, heart; LNG, lung; Liv, liver; SPL, spleen; PAN, pancreas; KID, kidney; TES, testis; SML, seminal gland.

Table III. Transplantability and Metastatic Potential of the Cell Lines Which Are Positive or Negative for Various Adhesion Molecules

Adhesion molecules	No. of cell lines	No. of mice inoculated	No. of mice with		
			Tumor (%)	Meta (Organ)[a] (%)[b]	Meta (Lymph node)[c] (%)[b]
VLA-4	Positive 21	84	44 (52)	25 (57)	26 (59)
	Negative 3	12	7 (58)	3 (43)	3 (43)
L-Selectin	Positive 9	36	20 (56)	12 (60)	15[d] (75)
	Negative 15	60	31 (55)	16 (48)	14 (45)
CLA	Positive 7	28	10 (35)	5 (50)	6 (60)
	Negative 17	68	41 (60)	23 (56)	23 (56)
LFA-1α	Positive 15	60	29 (48)	19 (66)	21 (72)
	Negative 9	36	22 (61)	9 (40)	8 (36)
LFA-1β	Positive 13	52	29 (56)	19 (66)	17 (59)
	Negative 11	44	22 (50)	9 (41)	11 (50)
ICAM-1	Positive 17	68	36 (53)	21 (58)	22 (61)
	Negative 7	28	15 (54)	7 (47)	7 (47)
CD44	Positive 17	68	38 (56)	27[e] (71)	26[e] (68)
	Negative 7	28	13 (46)	1 (8)	3 (23)

a) Organ metastases.
b) Number of mice with metastases per number of mice with tumors (%).
c) Lymph node metastases.
d) $P<0.05$. *e*) $P<0.01$.

SCID mice Each human lymphoma/leukemia cell line was transplanted subcutaneously into 4 SCID mice. Among the 24 cell lines, 15 cell lines formed primary tumors at the inoculated site on the left back. The metastatic potential of these 15 cell lines is presented in Table II. Among the 15 cell lines which formed a subcutaneous tumor at the inoculation site, 9 produced both organ and lymph node metastases, 1 showed only organ metastases, and 2 showed only lymph node metastases. MAT cells did not form primary subcutaneous tumors, but several organs and lymph nodes were involved by tumor cells in two mice.

Expression of the adhesion molecules and its correlation with the metastatic potential Transplantability and

metastatic potential of the adhesion molecule-positive or -negative cell lines are presented in Table III. The number of mice which showed tumor growth at the inoculation site did not correlate with the surface expression of any adhesion molecule. On the other hand, organ and lymph node metastasis correlated with the expression of some adhesion molecules. L-Selectin tended to confer a potential of lymph node metastasis. L-Selectin-positive cell lines (TALL-1, MOLT-4, HSB-2, MT-1, ED-S, MT-4, KW, LBF and LB-25) were successfully transplanted into 20 mice among the 36 mice inoculated and metastasized into the lymph nodes in 15 mice, while L-selectin-negative cell lines (KE-37, HPB-ALL, DL-40, JURKAT, KOPT-K1, MAT, SALT-3, HUT-102, MT-2, BALL-1, BALM-1, SU-DHL-4, SCOTT, KCA and NALL-1) showed tumor growth in 31 mice out of 60 mice inoculated, and generated lymph node metastasis in 14 mice. This difference in lymph node metastases was significant ($P<0.05$). CD44 tended to confer a potential of organ and lymph node metastasis. CD44-positive cell lines (TALL-1, KE-37, MOLT-4, HSB-2, HPB-ALL, KOPT-K1, MAT, SALT-3, ED-S, HUT-102, MT-2, MT-4, BALL-1, BLM-1, KCA, LBF and NALL-1) produced tumors in 38 mice out of 68 mice inoculated, and metastasized into the organs of 27 mice and into the lymph nodes of 26 mice, while CD44-negative cell lines (DL-40, JURKAT, MT-1, SU-DHL-4, SCOTT, KW and LB-25) developed tumors in 13 mice out of 28 mice inoculated and showed metastatic foci only in the organs of one mouse and in the lymph nodes of three mice. These differences in both organ and lymph node metastasis were statistically significant ($P<0.01$).

CD44 variant exon 6 (v6) expression In order to decide which of CD44s and CD44v correlates better with metastatic potential, we examined the mRNA expression of CD44 v6, which is known to be related to metastasis or tumor progression in various human tumors, including non-Hodgkin's lymphomas,[21, 22] in 17 CD44-positive cell lines. All of the cell lines, which were reacted with Hermes-3 mAb recognizing CD44s, expressed mRNA of CD44s as evidenced by RT-PCR. m-RNA of CD44 v6 was also detected in 15 cell lines, but not KOPT-K1 or BALL-1 (data not shown).

Inhibition of growth and metastasis formation of a CD44-positive cell line, HSB-2 We examined the suppressive effect of an anti-human CD44s mAb, Hermes-3, on growth and metastasis formation of a CD44-positive human leukemia cell line, HSB-2. In the OKT-3-treated control group, 5 of 6 mice inoculated with HSB-2 cells developed subcutaneous primary tumors on their left back, all of which had multiple metastatic foci in 2–5 visceral organs (liver, kidney, lungs, adrenal glands, spleen and meninges) and the intrathoracic lymph nodes. The size of the primary tumors was 18.5 cm^3 on average.

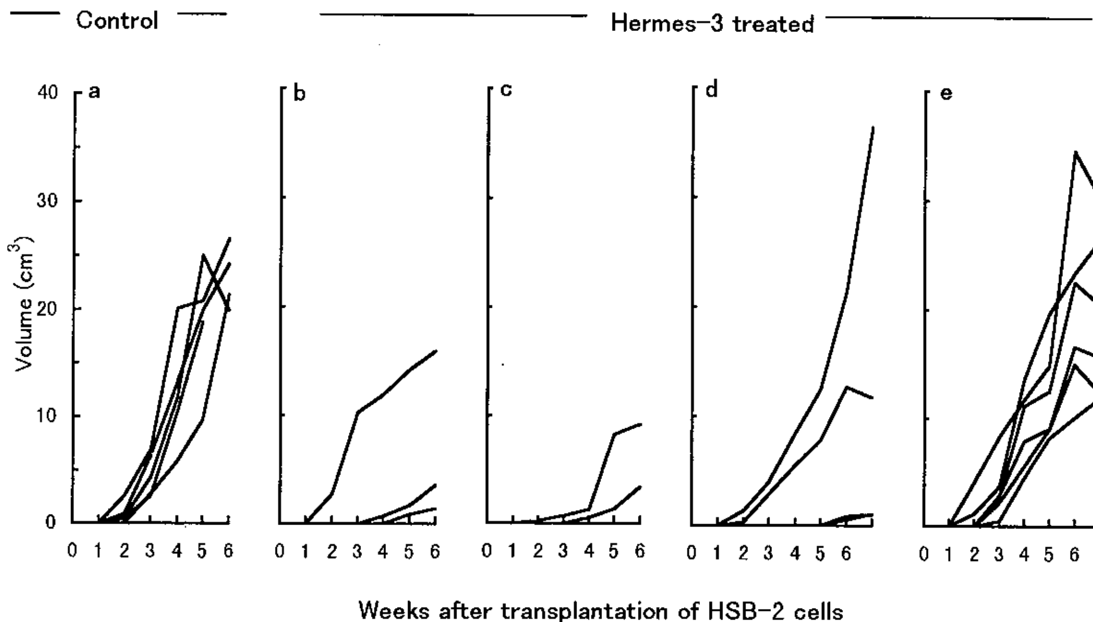

Fig. 1. The inhibitory effect of anti-CD44 mAb, Hermes-3, on the growth of transplanted HSB-2 cells. SCID mice were inoculated with 2.0×10^7 HSB-2 cells subcutaneously and then given OKT-3 control mAb (a) or Hermes-3 (b–e) intraperitoneally twice a week. Treatment was begun on the 1st (a, b), 3rd (c), 7th (d), and 10th (e) posttransplantation day. Each group consisted of six animals.

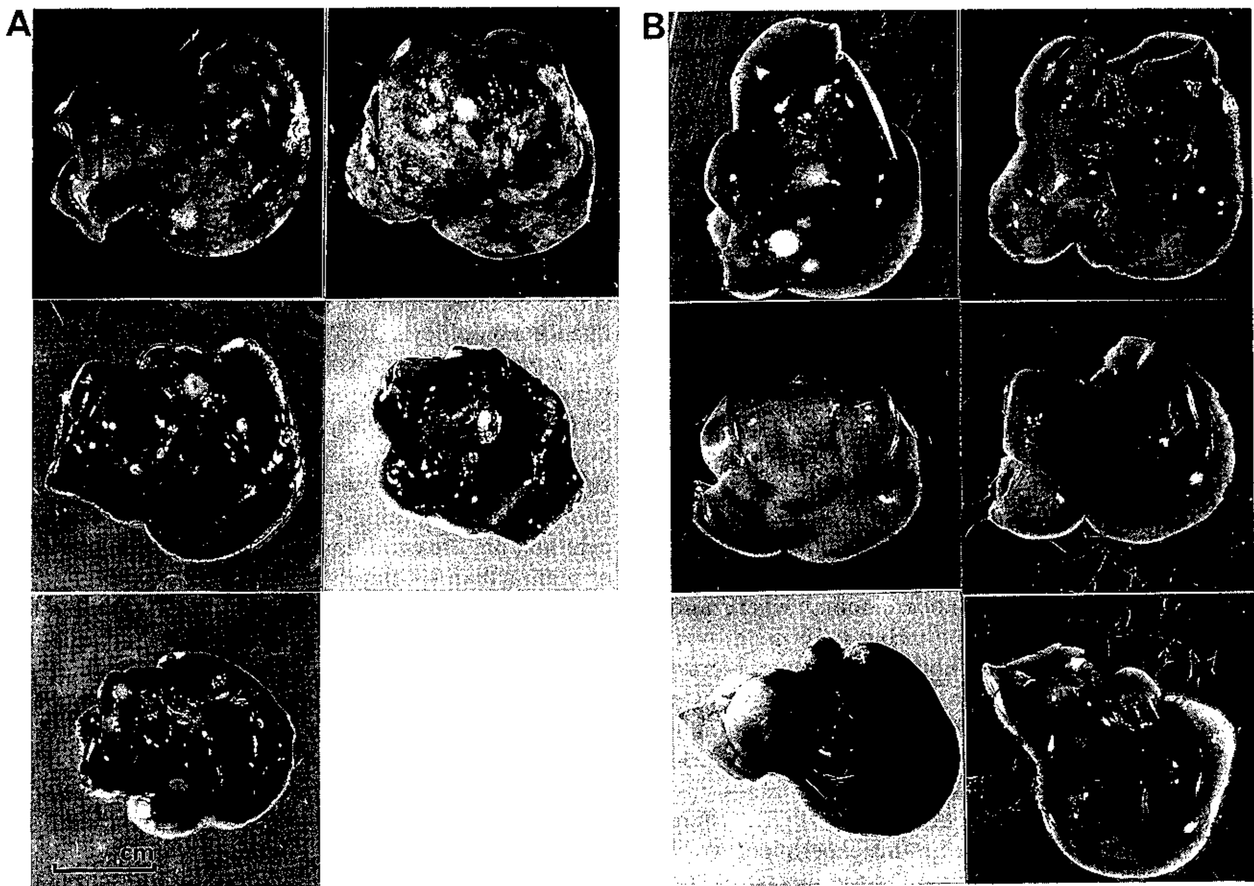

Fig. 2. Metastatic foci in the liver in the SCID mice transplanted subcutaneously with HSB-2 cells. A, Treated with OKT-3 control mAb. Five of six mice developed subcutaneous tumor at the inoculation site and showed multiple metastases in the liver. B, Treated with the anti-CD44 mAb, Hermes-3. All 6 mice developed subcutaneous tumor, but only 1 of 6 mice developed metastasis.

In contrast, in the experimental groups treated with Hermes-3 beginning 1–7 days after the inoculation, growth of the tumor cells at the inoculation site was remarkably inhibited (Fig. 1). Average sizes of the primary tumors in these groups were 3.5, 2.1, and 8.4 cm^3, respectively. No metastatic foci were observed in these animals. All six mice treated with Hermes-3 beginning 10 days after the transplantation developed tumors on their left back of almost the same size (19.6 cm^3 in average) as the OKT-3-treated ones, but only one mouse had metastatic foci in its right lung, liver, and right kidney (Fig. 2). The average number of metastasized organs was 0.5 in the Hermes-3-treated mice and 3.8 in the control mice. This difference was statistically significant ($P<0.01$).

Effect of antibodies on HSB-2 cell culture Anti-human CD44 mAb, Hermes-3 and anti-human CD3 mAb, OKT-3 did not inhibit the proliferation of HSB-2 cells in short-term cultures (data not shown).

DISCUSSION

The effect of six adhesion molecules (VLA-4, L-selectin, CLA, LFA-1, ICAM-1 and CD44) on transplantability and metastatic potential of lymphoma/leukemia cell lines was examined using a transplantation system into SCID mice. Among these adhesion molecules, two homing receptors, L-selectin and CD44, were demonstrated to increase lymphoma/leukemia metastases. L-Selectin expression had some augmenting effect on the lymph node metastases. L-Selectin is involved in the lymphocyte homing to HEVs of the peripheral lymph nodes and the mucosal lymphoid tissues.[8, 10] Considering this function of L-selectin, it is reasonable to assume that L-selectin helps lymphoma/leukemia cells home to HEVs of the peripheral lymph nodes.

It was shown that expression of the CD44 molecule was closely associated with the metastatic potential of

lymphoma/leukemia cell lines to both lymph nodes and organs. CD44 binds not only to the extracellular matrix, but also to the endothelial cell surface, and thus functions as a lymphocyte homing receptor.[4, 17, 18] Because the anti-CD44 mAb Hermes-3 inhibited lymphocyte-binding to mucosal HEVs, CD44 is thought to be especially involved in lymphocyte homing to the mucosal lymphoid tissues.[4] CD44 expression in non-Hodgkin's lymphomas is closely related to the clinical stage of the disease; patients with lymphoma exhibiting higher CD44 expression more often showed tumor spread beyond stage II and had a poorer prognosis with more frequent hematogenous dissemination than those with lymphoma showing no or weak expression of the CD44 antigen.[24–26] To confirm the relationship between CD44 and increased metastatic potential of lymphoma cells, we examined whether Hermes-3 could inhibit the metastases of HSB-2 cells. Hermes-3 markedly interfered with both hematogenous and lymphatic spread of HSB-2 cells, as seen in a system of rat pancreatic adenocarcinoma, where anti-CD44v prevented metastasis.[27] Hermes-3 is a mAb against CD44s and is able to inhibit lymphocyte homing to HEVs.[28] The major isoform of CD44 on lymphocytes is CD44s, but overexpression of CD44-6v has been found in some aggressive non-Hodgkin's lymphomas.[21] CD44-6v expression was detected in almost all the CD44-positive lymphoma/leukemia cell lines by RT-PCR. Therefore, it could not be decided from this experiment which CD44v was primarily responsible for the metastatic potential conferred by CD44 molecules. Considering the result obtained in the blocking test with Hermes-3, however, the CD44s molecules are at least involved in conferring metastatic potential upon lymphoma cells. In addition to the inhibitory effect on metastasis, Hermes-3 showed a suppressive effect on lymphoma growth at the inoculation site when it was administered early after the transplantation of lymphoma cells. The CD44 molecule has a domain participating in adhesion to extracellular matrices, which are also involved in the invasion and growth of tumor cells.[17, 18] Anti-CD44 mAb may inhibit the local growth of lymphoma cells by interfering with their adhesion to extracellular matrices.

In this experiment, the homing of human lymphoma/leukemia cells was examined *in vivo* using a SCID mouse system. Therefore, it is critically important that the human lymphocyte homing receptors can bind to the mouse vascular addressins. It is possible that evolutional changes in the molecular mechanism of cell adhesion have taken place in different species although many adhesion molecules show a high degree of amino acid sequence homology.[18, 29, 30] If so, a progressive decline in the compatibility of lymphocyte-HEV recognition systems might have occurred. However, the functional association of adhesion molecules may be conserved if evolutionary alteration affects only noncritical domains. In fact, it has been shown that tissue-specific lymphocyte-endothelial recognition mechanisms involved in lymphocyte homing are evolutionally conserved across a variety of mammalian species.[31, 32]

Expression of other adhesion molecules (VLA-4, CLA, LFA-1 and ICAM-1) did not correlate with the metastatic potential of the lymphoma/leukemia cell lines examined. In accordance with this result, there has been no report of a correlation between overexpression of these molecules and advanced clinical stages or metastatic potential of lymphomas, although expression of the CLA antigen was related to epidermotropism of T-cell lymphomas.[33]

We evaluated six molecules which might be related to lymphoma metastasis. Among these molecules, two lymphocyte homing receptors, L-selectin and CD44, were demonstrated to be important factors which conferred metastatic potential. This indicates that the behavior of lymphocyte malignancies is regulated to some extent by the molecules which control the traffic of normal lymphocytes. These findings provide some insight into the mechanism of lymphoma development and into possible therapeutic strategies.

(Received May 7, 1996/Accepted July 17, 1996)

REFERENCES

1) Butcher, E. C. The regulation of lymphocyte traffic. *Curr. Top. Microbiol. Immunol.*, **128**, 85–122 (1986).
2) Jalkanen, S., Reichert, R. A., Gallatin, W. M., Bargatze, R. F., Weissman, I. L. and Butcher, E. C. Homing receptors and the control of lymphocyte migration. *Immunol. Rev.*, **91**, 39–60 (1986).
3) Pals, S. T., Horst, E., Scheper, R. J. and Meijer, C. J. L. M. Mechanisms of human lymphocyte migration and their role in the pathogenesis of disease. *Immunol. Rev.*, **108**, 111–133 (1989).
4) Berg, E. L., Goldstein, L. A., Jutina, M. A., Nakache, M., Picker, L. J., Streeter, P. R., Wu, N. W., Zohou, D. and Butcher, E. C. Homing receptors and vascular addressins: cell adhesion molecules that direct lymphocyte traffic. *Immunol. Rev.*, **108**, 5–18 (1989).
5) Stoolman, L. M. Adhesion molecules controlling lymphocyte migration. *Cell*, **56**, 907–910 (1989).
6) Mackay, C. R. and Imhof, B. A. Cell adhesion in the immune system. *Immunol. Today*, **14**, 99–102 (1993).
7) Springer, T. A. Adhesion receptors of the immune system. *Nature*, **346**, 425–434 (1990).
8) Carlos, T. M. and Harlan, J. M. Leukocyte endothelial

adhesion molecules. *Blood,* **84,** 2068–2101 (1994).

9) Elices, M. J., Osborn, L., Takada, Y., Crouse, C., Luhowslyj, S., Hemler, M. E., and Lobb, R. R. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell,* **60,** 577–584 (1990).
10) Butcher, E. C. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell,* **67,** 1033–1036 (1991).
11) Freedman, A. S., Munro, J. M., Rice, G. E., Beirilacqua, M. P., Morimoto, C., McLntyre, B. W., Rhynhart, K., Pober, J. S. and Nadler, L. M. Adhesion of human B cells to germinal center *in vitro* involves VLA-4 and INCAM-110. *Science,* **249,** 1030–1033 (1990).
12) Berg, E. L., Magnani, J., Warnock, R. A., Robinson, M. K. and Butcher, E. C. Comparison of L-selectin and E-selectin ligand specificities: the L-selectin can bind the E-selectin ligands sialyl Le^x and sialyl Le^a. *Biochem. Biophys. Res. Commun.,* **184,** 1048–1055 (1992).
13) Berg, E. L., McEvoy, L. M., Berlin, C., Bargatze, R. F. and Butcher, E. C. L-Selectin-mediated lymphocyte rolling on MAdCAM-1. *Nature,* **366,** 695–698 (1993).
14) Baumhueter, S., Singer, M. S., Henzel, W., Hemmerich, S., Renz, M., Rosen, S. D. and Lasky, L. A. Binding of L-selectin to the vascular sialomucin CD34. *Science,* **262,** 436–438 (1993).
15) Lasky, L. A., Singer, M. S., Dowbenko, D., Imai, Y., Henzel, W. J., Grimbey, C., Fennie, C., Gillet, N., Watson, S. R. and Rosen, S. D. An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell,* **69,** 927–938 (1992).
16) Berg, E. L., Yoshino, T., Rott, L. S., Robinson, M. K., Warnock, R. A., Kishimoto, T. K., Picker, L. J. and Butcher, E. C. The cutaneous lymphocyte antigen CLA is a skin lymphocyte homing receptor for the vascular lectin ELAM-1. *J. Exp. Med.,* **174,** 1461–1466 (1991).
17) Günthert, U. CD44: a multitude of isoforms with diverse functions. *Curr. Top. Microbiol. Immunol.,* **184,** 47–63 (1993).
18) Lesley, J., Hyman, R. and Kincade, P. W. CD44 and its interaction with extracellular matrix. *Adv. Immunol.,* **54,** 271–335 (1993).
19) Makay, C. R., Terpe, H.-J., Sauder, R., Marston, W. L., Stark, H. and Günthert, U. Expression and modulation of CD44 variant isoforms in humans. *J. Cell Biol.,* **124,** 71–82 (1994).
20) Heider, K.-H., Hofmann, M., Hors, E., van den Berg, F., Ponta, H., Herrlich, P. and Pals, S. T. A human homologue of the rat metastasis-associated variant of CD44 is expressed in colorectal carcinomas and adenomatous polyps. *J. Cell Biol.,* **120,** 227–233 (1993).
21) Koopman, G., Heider, K-H., Horst, E., Adolf, G. R., van den Berg, F., Ponta, H., Herrlich, P. and Pals, S. T. Activated human lymphocytes and aggressive non-Hodgkin's lymphomas express a homologue of the rat metastasis-associated variant of CD44. *J. Exp. Med.,* **117,** 897–904 (1993).
22) Stauder, R., Eisterer, W., Thaler, J. and Günthert, U. CD44 variant isoforms in non-Hodgkin's lymphoma: a new independent prognostic factor. *Blood,* **85,** 2885–2899 (1995).
23) Screaton, G. R., Bell, M. V., Jackson, D. G., Cornelis, F. B., Gerth, U. and Bell, J. I. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc. Natl. Acad. Sci. USA,* **89,** 12160–12164 (1992).
24) Horst, E., Meijer, C. J. L. M., Badaszkiewicz, T., Ossekoppele, G. J., van Krieken, J. H. J. M. and Pals, S. T. Adhesion molecules in the prognosis of diffuse large-cell lymphoma: expression of a lymphocyte homing receptor (CD44), LFA-1 (CD11a/18), and ICAM-1 (CD54). *Leukemia,* **4,** 595–599 (1990).
25) Jalkanen, S., Joensuu, H., Söderström, K.-O. and Klemi, P. Lymphocyte homing and clinical behavior of non-Hodgkin's lymphoma. *J. Clin. Invest.,* **87,** 1835–1840 (1991).
26) Fujiwara, K., Yoshino, T., Miyako, K., Ohara, N. and Akagi, T. Expression of lymphocyte adhesion molecule (CD44) in malignant lymphomas: relevance to primary site, histological subtype and clinical stage. *Acta Med. Okayama,* **47,** 215–222 (1993).
27) Seiter, S., Arch, R., Reber, S., Komitowski, D., Hofmann, M., Ponta, H., Herrlich, P., Matzku, S. and Zöller, M. Prevention of tumor metastasis formation by anti-variant CD44. *J. Exp. Med.,* **177,** 443–455 (1993).
28) Jalkanen, S., Barfatze, R. F., de los Toyos, J. and Butcher, E. C. Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85–95-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. *J. Cell Biol.,* **105,** 983–990 (1987).
29) Argraves, W. S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M. D., and Ruoslahti, E. Amino acid sequence of the human fibronectin receptor. *J. Cell Biol.,* **105,** 1183–1190 (1987).
30) Kita, Y., Takashi, T., Iigo, Y., Tamatani, T., Miyasaka, M. and Horiuchi, T. Sequence and expression of rat ICAM-1. *Biochim. Biophys. Acta,* **1131,** 108–110 (1992).
31) Stoolman, L. M., Yednock, T. A. and Rosen, S. D. Homing receptors on human and rodent lymphocytes — evidence for a conserved carbohydrate-binding specificity. *Blood,* **70,** 1842–1850 (1987).
32) Wu, N. W., Jalkanen, S., Streeter, P. R. and Butcher, E. C. Evolutionary conservation of tissue-specific lymphocyte-endothelial cell recognition mechanisms involved in lymphocyte homing. *J. Cell Biol.,* **107,** 1845–1851 (1988).
33) Picker, L. J., Michie, S. A., Rott, L. S. and Butcher, E. C. A unique phenotype of skin-associated lymphocytes in humans. Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am. J. Pathol.,* **136,** 1053–1068 (1990).