Role of Sialylglycoconjugate(s) in the Initial Phase of Metastasis of Liver-metastatic RAW117 Lymphoma Cells

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To elucidate the early events of blood-borne metastasis under actual blood flow, real-time trafficking of RAW117 large cell lymphoma cells, namely parental RAW117-P and liver-metastatic RAW117-H10 cells, was investigated using positron emission tomography (PET). Both types of cells accumulated in the liver immediately after injection via the portal vein, and were eliminated from the liver time-dependently. The elimination rate of RAW117-H10 cells, however, was slower than that of RAW117-P cells, suggesting that RAW117-H10 cells interact more strongly with hepatic sinusoidal endothelium than the parental cells. This result correlated with the metastatic potential of these cells: RAW117-H10 cells metastasized in the liver to a greater extent than RAW117-P cells after injection via this route. To investigate the role of sialylglycoconjugates in the interaction of RAW117-H10 cells with the hepatic endothelium after injection via the portal vein, the trafficking of RAW117-H10 cells was examined after the cells had been treated with sialidase. The elimination rate of RAW117-H10 cells from liver was observed to be greatly accelerated by sialidase treatment. To elucidate what kind of sialylglycoconjugates is related to this phenomenon, we analyzed the distribution of sialyl Lewis A and sialyl Lewis X antigens of both sublines of RAW117 by using flow cytometry. RAW117-H10 cells were found to express a much higher level of sialyl Lewis A than RAW117-P cells, whereas the amount of sialyl Lewis X did not differ significantly. These findings suggest that some sialylglycoconjugates, perhaps sialyl Lewis A in particular, play an important role in the initial interaction of RAW117-H10 cells with the hepatic endothelium, leading to metastasis.

Key words: Tumor cell trafficking — Positron emission tomography (PET) — Sialylglycoconjugate — Metastasis — Sialyl Lewis A

Metastasis occurs through a complex cascade of events including dissociation from the primary site, intravasation, adhesion to the vascular endothelium of the target organ followed by extravasation, and growth at the colonization site.[1–4] Through a number of *in vitro* analyses, the role of target tissue adhesion and invasion properties of metastatic tumor cells and that of the growth capability of the cells in the organ microenvironment have become clear.[5, 6] Also, many investigators have demonstrated that adhesion of metastatic tumor cells to the endothelium of the target organ might be mediated by several classes of adhesion molecules, including selectins, integrins, and their ligands.[7–11] Among these adhesion molecules, it is proposed that selectins and their ligands play an important role in the primary adhesion of tumor cells. Sialyl Lewis A (sialyl-Le^a) and sialyl Lewis X (sialyl-Le^x) antigens, which have been shown to be specific ligands for endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin),[12] are known to be involved in the metastatic process.[13] In fact, the metastatic organ of B16F10 melanoma, of which the cells express a plethora of sialyl-Le^a owing to stable transfection with cDNA for $\alpha(1,3/1,4)$ fucosyltransferase, was redirected in E-selectin transgenic mice.[14, 15] Furthermore, the expression level of sialyl-Le^a on certain tumor cells, such as human colon cancer cell line and gastric cancer cells, is associated with the acquisition of potential of the cells for metastasis to the liver.[16–18] Thus, certain glycoconjugates containing sialyl-Le^a antigens on metastatic tumor cells are likely to regulate the adhesion of metastatic tumor cells to the liver endothelium in an early step of metastasis.

The presumption of tumor cell trafficking during metastasis has not been fully confirmed, since there was no non-invasive technique available for the determination of real-time tumor cell trafficking *in vivo*. We previously developed a non-invasive method to investigate the real-

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time tumor cell trafficking under conditions of blood flow by using positron emission tomography (PET), and demonstrated that, following injection via a tail vein, highly lung-metastatic sublines of rat adenocarcinoma cells accumulated in lung tissue more extensively than did poorly metastatic ones.[19] Therefore, the early accumulation of metastatic tumor cells is well correlated with the metastatic character. In addition, we observed that liposomal sialyl-Le[x] suppressed the accumulation of B16BL6 melanoma cells in the lung, as well as the establishment of lung metastasis, after injection via a tail vein.[20] These findings suggest the importance of early interactions, especially interactions mediated by selectins, between tumor cells and the target organ in the establishment of metastasis.

In this study, we investigated, using PET analysis, the trafficking of liver-metastatic RAW117 cells after portal-vein injection and evaluated the correlation between liver accumulation of metastatic cells and metastatic potential. Injection via the portal vein is a favorable route to elucidate specific interaction between liver-metastatic tumor cells and the hepatic endothelium in organ-specific metastasis, since the liver is the first organ encountered after injection via this route. Therefore, this injection route has been applied in liver-metastasis models, e.g., human colon cancer models,[17, 18] and is also applicable for videomicroscopic analysis, which is an invasive but useful method to investigate the adhesion and invasion of metastatic tumor cells *in vivo*.[21–23] In addition, to clarify the involvement of sialylglycoconjugates in the early events of tumor metastasis, we compared the cell trafficking of sialidase-treated RAW117-H10 cells with that of nontreated cells after portal-vein injection in the present study, and further analyzed the distribution of sialyl-Le[a] and sialyl-Le[x] antigens on the surface of the tumor cells.

MATERIALS AND METHODS

Cell culture and characterization of metastatic potential Both parental line (RAW117-P) and a highly liver-metastatic variant subline (RAW117-H10) of RAW117 mouse large cell lymphoma cells were gifts from Dr. G. L. Nicolson (Institute for Molecular Medicine, Irvine, CA). These cells were cultured in high-glucose Dulbecco's modified Eagle's medium (hG-DMEM) supplemented with 5% fetal calf serum (JRH Biosciences, Lenexa, KS) under a humidified atmosphere of 5% CO_2 in air.

The metastatic potential of these cells was determined as follows. Female BALB/c mice (Japan SLC Inc., Hamamatsu) were cared for according to the animal facility guidelines of the University of Shizuoka. Seven-week-old mice were anesthetized with sodium pentobarbital (0.05 mg/g mouse body weight). For the injection of lym-

phoma cells into the hepatic portal system, an incision was made along the midline of the abdomen to expose the large vein located in the mesentery. Cultured RAW117 cells were washed three times with hG-DMEM to remove the serum, and injected (1×10^6 cells/0.2 ml) into the mice via the portal vein. The animals (6 per group) were killed at day 5 after injection, and the removed organs, i.e., liver, spleen, and lung, were weighed to determine the extent of metastasis, since the colonies of RAW117 cells were difficult to count. Metastasis establishment was also confirmed by histochemical analysis.

PET analysis of tumor cell trafficking RAW117 cells were labeled with [2-^{18}F]2-fluoro-2-deoxy-D-glucose ([2-^{18}F]FDG) by a modification of the method described previously.[20] In brief, RAW117 cells were washed with DMEM and incubated in the presence of 1 GBq of [2-^{18}F]FDG for 15 min at 37°C. After removal of the free [2-^{18}F]FDG by centrifugation, a single-cell suspension (1×10^7 cells/ml) was obtained. Viability of the labeled cells was determined by the trypan blue dye exclusion method and was greater than 90% throughout the experiment. In some experiments, sialidase treatment of the cells was performed before labeling with [2-^{18}F]FDG by the same procedure described below (in the section on "Cell adhesion assay").

The cells labeled with [2-^{18}F]FDG (1×10^6 cells in 0.2 ml of medium) were injected into syngeneic 8-week-old female BALB/c mice via the portal vein under anesthesia with sodium pentobarbital after a 30-min transmission scan with a [68]Ge/^{68}Ga ring source (18.5 MBq). The emission scan of PET was performed with an animal PET camera (SHR-2000; Hamamatsu Photonics, K.K., Shizuoka) as previously described.[20] The initial 60 frames were taken at a rate of 1 every 1 min, and the next 12 frames were taken at a rate of 1 every 2.5 min, so that 72 frames were taken during the PET scan. PET images of 14 slices were simultaneously obtained, where the 9th slice was set at the xiphisternum. The radioactivity in the form of coincidence γ photons was measured and converted to Bq/cm^3 of tissue volume by a calibration after correction for decay and attenuation. The time-activity curve was obtained from the mean pixel radioactivity in the region of interest of the composed PET images, in which the injected dose was calibrated as 740 kBq. The elimination rate of cells from the liver was calculated from the slope of 5–20 min ^{18}F-accumulation changes on the time activity curves. Each experiment was repeated three times, and similar results were obtained in repeated experiments; although each figure presents a typical result obtained from a single animal.

Cell adhesion assay Hepatic sinusoidal microvessel endothelial cells (HSE) were seeded on a 24-well culture dish (2×10^5 cells/well) precoated with gelatin and incubated overnight in a CO_2 incubator. RAW117 cells were

incubated with 0.1 mM 3'-O-acetyl-2'-, 7'-bis(carboxyl-ethyl)-4- or 5-carboxylfluorescein, diacetoxymethyl ester (BCECF-AM, Dojindo, Laboratories, Kumamoto) for 30 min as described previously.[24] After having been washed with phosphate-buffered saline (pH 7.4, PBS), fluorescence-labeled cells were suspended in hG-DMEM containing 0.1% bovine serum albumin (BSA). A fluorescence-labeled cell suspension (250 μl, 2×10⁵ cells/ml) was applied to each well of a 24-well culture dish pre-seeded with HSE. After incubation for selected times under a non-static condition (100 rpm), non-adherent cells were removed by washing three times; and adherent cells were lysed with 1% Triton X-100. The percentage of adherent cells was determined fluorometrically by use of a fluorescence microplate reader (Corona MTP-100F). In some experiments, RAW117 cells were treated with sialidase at a final concentration of 100 mU/ml for 3 h at 37°C prior to BCECF-AM labeling, as described previously.[19]

Immunofluorescence staining and flow-cytometric analysis of sialylglycoconjugates on the cell surface RAW117 cells (2×10⁶ cells) were washed twice with washing buffer (PBS with 0.1% BSA) and resuspended in 1 ml of the washing buffer. The cells (5×10⁵ cells) were then fixed in 1% (v/v) formaldehyde solution in PBS for 30 min at room temperature and washed twice with the washing buffer. The washed cells were treated with a 1:50 diluted mouse anti-sialyl-Le^a monoclonal antibody (IgG$_1$,

Wako Pure Chemical Industries, Ltd., Osaka), a 1:500 diluted mouse anti-sialyl-Le^x monoclonal antibody (IgM, Wako Pure Chemical Industries, Ltd.), or a 1:50 diluted isotype-matched control mouse antibody (Wako Pure Chemical Industries, Ltd.) for 30 min on ice. These cells were washed twice in the washing buffer, and subsequently stained with a 1:200 diluted FITC-conjugated AffiniPure goat anti-mouse IgG+IgM (H+L, Jackson ImmunoResearch Lab., Inc., West Grove, PA) for 30 min on ice. The stained cells were washed twice in the washing buffer, and then subjected to flow-cytometric analysis.

Table I. Experimental Metastasis Produced by RAW117 Cells Injected via the Portal Vein

Cells	Tissue weight (g±SD)		
	Lung	Liver	Spleen
Without cells	0.15±0.01	1.06±0.00	0.13±0.03
RAW117-P	0.20±0.04	0.94±0.05	0.11±0.02
RAW117-H10	0.25±0.05	1.47±0.09**	0.16±0.03*

Significant difference from RAW117-P: ** $P<0.001$, * $P<0.05$. An incision was made in female BALB/c mice to expose the portal vein located in the mesentery, and RAW117 cells (1×10⁶ cells/0.2 ml) were injected via this vein. The mice ($n=6$) were killed at day 5 after injection, and the removed organs, i.e., liver, spleen and lung, were weighed to determine the extent of metastasis.
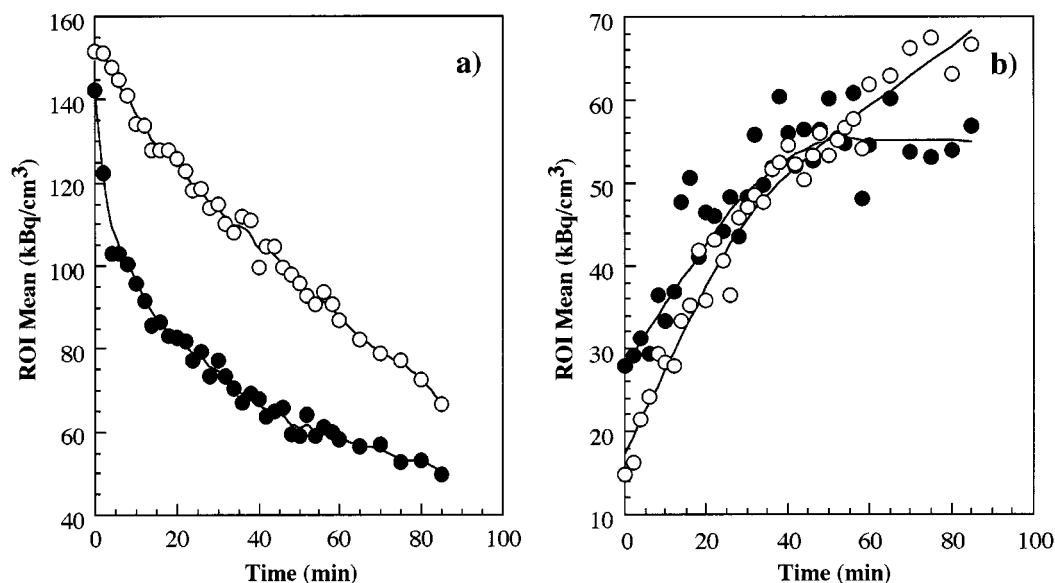

Fig. 1. Time-activity curves of ¹⁸F accumulation in the liver and lung after the injection of [2-¹⁸F]FDG-labeled RAW117 cells via the portal vein. Eight-week-old BALB/c mice were injected with [2-¹⁸F]FDG-labeled RAW117 cells (1×10⁶ cells/0.2 ml) via the portal vein. The PET emission scan was performed for 90 min. Time-activity curves of ¹⁸F in the liver (a) and lung (b) after the injection of [2-¹⁸F]FDG-labeled RAW117-P cells (●) or RAW117-H10 cells (○) were obtained as described in "Materials and Methods."

Table II.   Time-dependent Change in Distribution of [2-^{18}F]FDG-labeled RAW117 Cells after Portal-vein Injection

Cells	Release from liver (%/min)	Half-life in liver (min)	Liver-lung ratio at 60 min post-injection
RAW117-P	1.75	21.5	0.83
RAW117-H10	0.97	52.0	1.34
Sialidase-treated			
RAW117-H10	2.74	17.8	0.87
Change in distribution of RAW117 cells after tail-vein injection (reproduced from Ref. 26)			
RAW117-P	2.78	21.6	0.35
RAW117-H10	2.05	30.6	0.33

The release rate of RAW117 cells from the liver was calculated from the slope of 5 to 20 min accumulation changes in the time-activity curves of ^{18}F accumulation in the liver after the injection of [2-^{18}F]FDG-labeled RAW117-H10 and RAW117-P cells via the portal vein, as was that after the injection of sialidase-treated RAW117-H10 cells. The half-life of the cells in liver and the liver-lung ratio at 60 min post-injection were also calculated from the time-activity curves. The release rate of RAW117 cells from lung, the half-life of the cells in lung, and the liver-lung ratio at 60 min after tail-vein injection are reproduced from Ref. 26 for comparison.

Flow cytometry was performed with an Epics XL System II flow cytometer (Coulter Corp., Hialeah, FL), as described previously.[25]

RESULTS

Metastatic potential of RAW117-H10 and RAW117-P cells after injection via the portal vein   To investigate the relation between liver-specific metastasis and early interactions of liver-metastatic cells with target endothelium, we first examined the metastatic potential of RAW117 cells after portal-vein injection. When RAW117-H10 and RAW117-P cells were injected via the portal vein of BALB/c mice, the liver weight of mice injected with RAW117-H10 cells rose significantly, whereas the lung and spleen weights did so only to a small extent (Table I). On the other hand, injection of RAW117-P cells did not cause any weight gain in liver, even though the liver is the first organ encountered after portal-vein injection of the cells.

Trafficking of RAW117 cells after injection via the portal vein   To confirm the correlation between specific interactions of tumor cells with target endothelium and metastatic potential, we employed PET analysis to examine the real-time trafficking of the RAW117 cells after injection via the portal vein. The PET images of 14 slices indicated that most of the cells accumulated in the liver, that some of the cells also accumulated in the lung, and that specific accumulation in heart, kidney, spleen and other tissues examined could not be observed (data not shown). Fig. 1 shows the time-activity curve of the accumulation of these cells in the liver and lung. The accumulation of RAW117-H10 cells in the liver was more intense than that of RAW117-P during the first 90 min after the portal injection, whereas the accumulation of RAW117-
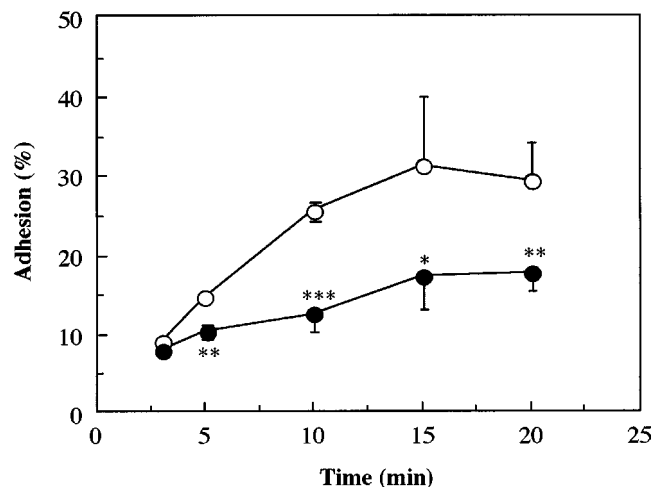

Fig. 2. Effect of sialidase treatment on the adhesion of RAW117-H10 cells to HSE cells. RAW117-H10 cells were treated with sialidase at a final concentration of 100 mU/ml for 3 h at 37°C. Sialidase-treated (●) and -untreated RAW117-H10 cells (○) were labeled with 0.1 mM BCECF-AM, and fluorescence-labeled cells (250 μl, 2×10^5 cells/ml) were incubated for the indicated times at 37°C with HSE cells preseeded on a 24-well culture dish (2×10^5 cells/well) on a shaker (100 rpm). Adherent cells were determined as described in "Materials and Methods." Each point and bar represent the mean and SD (n=4). Significant differences (∗ $P<0.05$, ∗∗ $P<0.01$, ∗∗∗ $P<0.001$) from the adhesion of untreated RAW117-H10 cells are shown.

H10 cells in lung was comparable with that of RAW117-P cells during the first 60 min after the injection. The accumulated cells in liver were released time-dependently from the organ, although the release of RAW117-H10 cells was slower than that of the RAW117-P cells. The
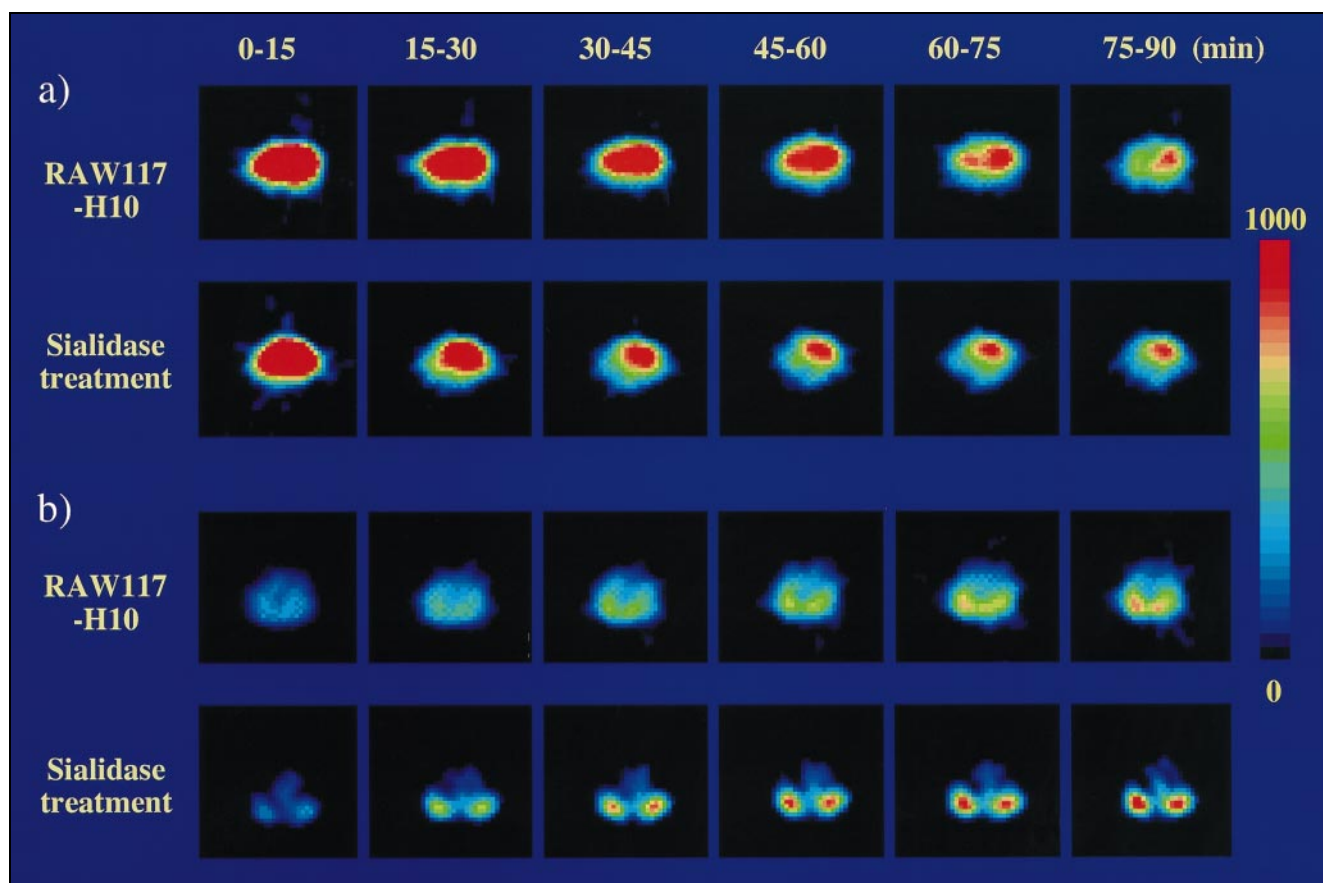
Fig. 3. PET images of the accumulation of [2-^{18}F]FDG-labeled RAW117-H10 cells pretreated with sialidase. RAW117-H10 cells were treated with sialidase at a final concentration of 100 mU/ml for 3 h at 37°C. Sialidase-treated and untreated cells were labeled with [2-^{18}F]FDG, and were injected (1×10^6 cells/0.2 ml) into BALB/c mice via the portal vein. The emission scan of PET was started immediately after injection of the cells. Accumulations for 15 min in the liver (a) and lung (b) are shown. Gradation is comparable in all images. Similar results were obtained in two separate experiments, although the images shown represent a result obtained from a single animal.

release of these cells from the liver after portal-vein injection and that from the lung after tail-vein injection for reference (data from Ref. 26) are summarized in Table II. The values of the elimination rate of RAW117-H10 and RAW117-P cells from the liver after portal-vein injection were 0.97%/min and 1.75%/min, and the biological half-lives of these cell lines in the liver were 52.0 min and 21.5 min, respectively. These results suggested that RAW117-H10 cells interact with hepatic endothelium more strongly than do the parental cells, at least shortly after injection.

Effect of sialidase treatment on the adhesion of RAW117-H10 cells to HSEs *in vitro* and on the trafficking of the cells *in vivo* To confirm the effect of sialylglycoconjugates on the metastatic property of RAW117 cells, we examined the adhesion properties of the cells to

HSE after treatment with sialidase under a non-static condition where the interaction mediated via E-selectin would be strengthened. Non-treated RAW117-H10 cells adhered to HSEs, and sialidase treatment reduced the adhesiveness (Fig. 2). This result is attributable to the removal of sialic acids from sialylglycoconjugates on the cell surface membrane, instead of artifactual damage to the cell membrane, since the treatment of the cells with the sialidase did not affect cell viability or cell growth.

On the basis of the above results, we further examined the trafficking of sialidase-treated RAW117-H10 cells after injection via the portal vein. As shown in Figs. 3 and 4, the accumulation of sialidase-treated RAW117-H10 cells in the liver was much lower than that of the non-treated cells, and a remarkable decrease in accumulation of the former was observed up to the first 40 min after
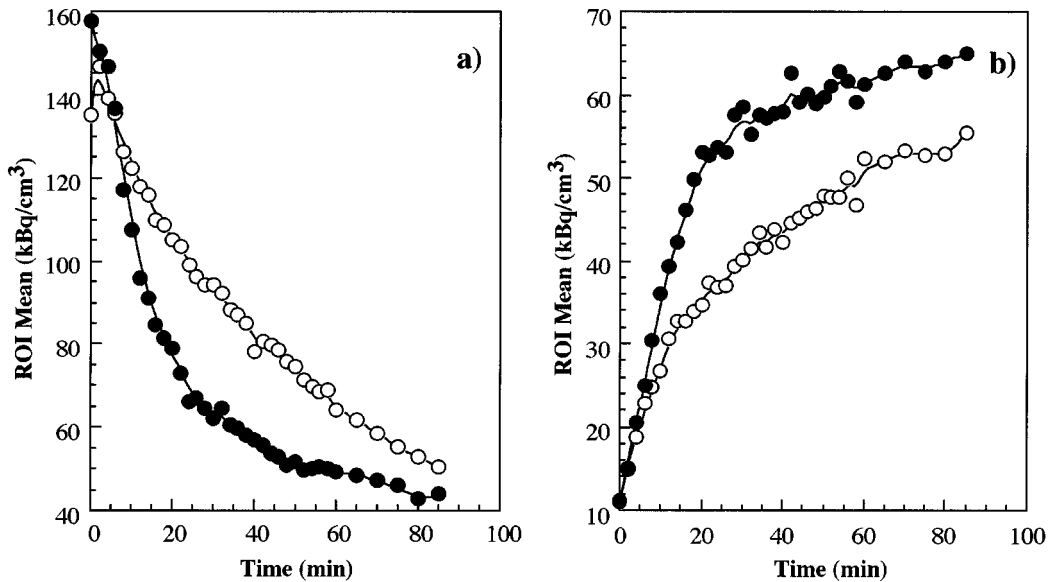
Fig. 4. Time-activity curves of ^{18}F accumulation in the liver and lung after the injection of [2-^{18}F]FDG-labeled RAW117 cells pre-treated with sialidase. PET scan of sialidase-treated RAW117-H10 cells (●) and -untreated RAW117-H10 cells (○) was performed as described in the legend to Fig. 3, and time-activity curves of ^{18}F in the liver (a) and lung (b) after the portal-vein injection of the cells were obtained.

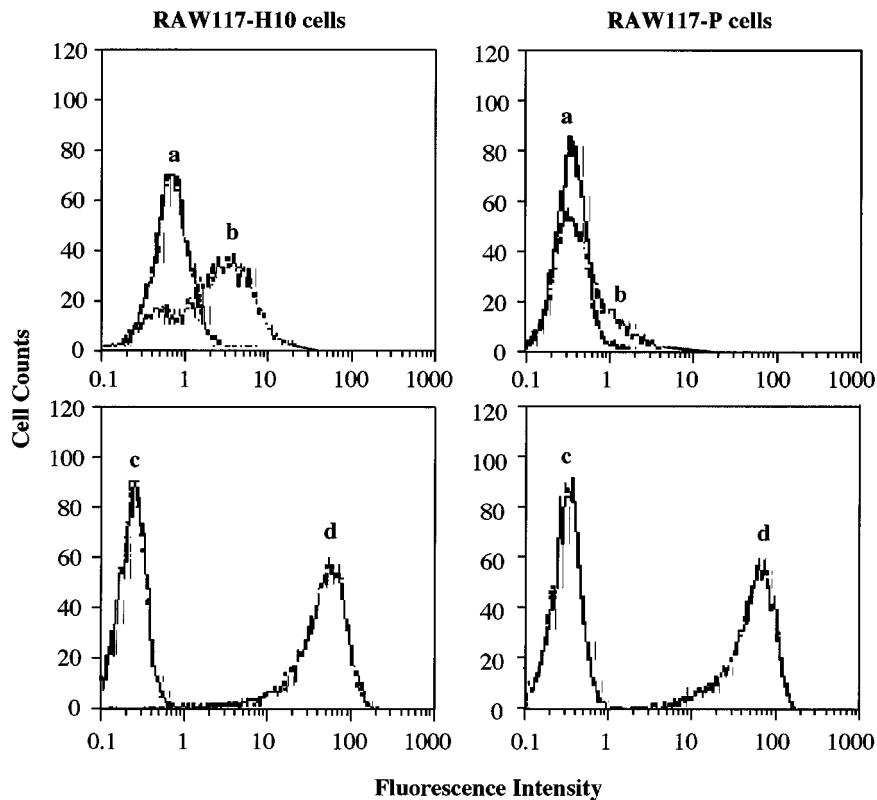Fig. 5. Distribution of sialyl-Le^a and sialyl-Le^x antigens on RAW117 cells. RAW117-H10 (left panels) and RAW117-P (right panels) cells (2×10^6 cells) were fixed with formaldehyde, and treated with control mouse antibody (a, c), mouse anti-sialyl-Le^a monoclonal antibody (b), or mouse anti-sialyl-Le^x monoclonal antibody (d). These cells were stained with FITC-conjugated goat anti-mouse antibody, and then applied to a flow cytometer as described in "Materials and Methods."

portal-vein injection. The elimination rate of RAW117-H10 cells from the liver was also increased, as compared with that of the non-treated cells (Table II). On the other hand, accumulation of sialidase-treated RAW117-H10 cells in the lung was rather higher than that of non-treated cells, suggesting that decreased interaction of the cells with the hepatic endothelium by sialidase treatment resulted in an increase in the number of cells reaching the lung. These results indicate that interaction between RAW117-H10 cells and the hepatic endothelium in the initial stage might be canceled by the sialidase treatment of the cells.

Distribution of sialyl-Le^a and sialyl-Le^x antigens on RAW117 cells The results of the present study and our previous data[19] indicate the importance of sialylglycoconjugates on the tumor cell surface in the initial arrest of metastatic tumor cells at the target organ. Furthermore, liposomal sialyl-Le^x could reduce the initial arrest of lung metastatic tumor cells and metastatic establishment.[24] Thus, E-selectin ligands containing sialyl-Le^a and/or sialyl-Le^x moieties are most probably the sialylglycoconjugates responsible for the liver accumulation of RAW117-H10 cells observed here. However, the distribution of sialyl-Le^a and sialyl-Le^x antigens on RAW117 cells has not been investigated so far. Therefore, using flow cytometry, we analyzed the distribution of sialyl-Le^a and sialyl-Le^x antigens on the surface membranes of RAW117-P and RAW117-H10 cells. Both types of cells expressed sialyl-Le^x antigens to a similar extent. In contrast, sialyl-Le^a antigen was predominantly expressed on the surface of the highly liver-metastatic RAW117-H10 cells, as compared with the expression on the RAW117-P cells (Fig. 5).

DISCUSSION

In the early stage of blood-borne metastasis, metastatic tumor cells intravasating from the primary site may adhere to and extravasate through the vascular endothelium of the target organ,[4–6] and this adhesion process is thought to be a prerequisite for metastasis establishment. The interaction of metastatic cells with the endothelium may be initiated via the interaction between selectin molecules and their ligands, such as sialyl-Le^a and sialyl-Le^x.[13] Recently, we demonstrated that the metastatic potential of various tumor cells injected via a tail vein is well correlated with the early accumulation of the cells in the target organ, suggesting that the target organ might be initially engaged by specific interactions between adhesion molecules on tumor cells and those of the target endothelium.[26]

The data in Table I and the data from Ref. 26 indicate that RAW117-H10 cells have metastatic potential for the liver after injection via either the portal vein or the tail vein, suggesting that the RAW117-H10 cell line is a useful model system for liver-specific blood-borne metastasis, since the cells metastasize in the liver independently of the first organ passed after i.v. injection. Experimental metastasis established by portal-vein injection, however, is thought to reflect the actual metastasis of liver metastatic tumors. Furthermore, this injection route is favorable to elucidate the specific interaction between liver-metastatic tumor cells and the hepatic endothelium, because the first organ reached via this injection route is the liver. Thus, in the present study, we investigated the trafficking of RAW117-H10 and parental RAW117-P cells after injection via the portal vein, in comparison with that after injection via a tail vein. As shown in Fig. 2, both RAW117-P and RAW117-H10 cells accumulated in the liver immediately after injection via the portal vein. This phenomenon is well correlated with the lung accumulation of these cells after injection via a tail vein[26]; liver and lung are the first organ of passage after injection via the portal and tail vein, respectively. Although the accumulated cells in the lung after the tail-vein injection moved off similarly for both types of RAW117 cells,[26] the elimination rate of RAW117-H10 cells from the liver was slower than that of RAW117-P cells after the portal-vein injection (Table II). These results indicate that RAW117-H10 cells interact strongly with the hepatic endothelium *in vivo* compared with RAW117-P cells; both types of cells interact similarly and weakly with the lung endothelium. The results of PET analysis also suggest that the initial interaction of RAW117 cells with the hepatic sinusoidal endothelium regulates the liver metastasis of the cells, since RAW117-H10 cells were highly metastatic to the liver (Ref. 26 and Table I).

RAW117-H10 cells injected via the portal vein would encounter the microvascular system in the liver, where the adhesion of the cells to the microvascular endothelium would occur as a prerequisite for extravasation. It has been reported that several high-molecular-weight glycoproteins having distinct carbohydrate chains are differentially expressed in different classes of tumor tissues and that the direction of change in expression is rather consistent with malignancy or metastatic topology.[12, 27] Among these differences, one of the most remarkable phenotypes found in the advanced and metastatic tumors is increased expression of sialyl-Le^x and/or sialyl-Le^a antigens.[28–32] In the case of RAW117 lymphoma cell lines, it has been reported that the expression of a glycosphingolipid, GD1α, on RAW117-H10 cells is twice as great as that on RAW117-P cells.[33] Integrin αVβ3, which may interact with extracellular matrix components at later stages, after selectin-mediated interactions,[20] is also reported to be expressed on RAW117-H10 cells, but not on parental cells.[34] However, other phenotypic changes, including expression of sialyl-Le^x and/or sialyl-Le^a antigens of RAW117-H10 cells, remain unclear. To examine such

phenotypic changes in RAW117 cells, we determined the distribution of sialyl-Le^x and sialyl-Le^a antigens on the cells by flow cytometry, and observed significant expression of sialyl-Le^a on the surface of highly liver-metastatic RAW117-H10 cells, whereas little was detected on RAW117-P cells. On the other hand, sialyl-Le^x antigens were expressed on the surface of both types of cells in the same amount.

If sialylglycoconjugates, such as sialyl-Le^a and GD1α, play an important role in the early adhesion of RAW117-H10 cells to the hepatic endothelium, removal of sialyl residues from the surface of RAW117-H10 cells would be expected to reduce the early accumulation of the cells in the liver. In fact, our previous study showed that highly lung-metastatic MTLn3 cells injected via a tail vein accumulated less in the lung after sialidase treatment compared with the non-treated cells.[19] Thus, the trafficking of RAW117-H10 cells after sialidase treatment was examined by PET. As shown in Fig. 2, enzymatic removal of sialic acid residues from RAW117-H10 cells reduced the adhesion capability of RAW117-H10 cells to HSE *in vitro*, indicating the requirement of terminal sialic acid of sialylglycoconjugates for the interaction of the cells with HSE. Furthermore, sialidase-treated RAW117-H10 cells were eliminated quite rapidly from the liver compared with the non-treated cells (Table II). Thus, sialylglycoconjugates may play an important role in adhesion of RAW117-H10 cells to the hepatic endothelium. Sialidase treatment may also accelerate the degradation of the cells by the host immune system, since Kupffer cells and pit cells, the latter being large granular lymphocytes with natural killer activity, are reported to defend against hepatic metastasis.[35, 36] However, this may not be the reason for the rapid elimination of the cells from liver, since siali-

dase-treated RAW117-H10 cells gradually accumulated in the lung (Fig. 2). On the basis of these findings, we concluded that the rapid elimination of sialidase-treated RAW117-H10 cells from liver might be due to a reduced interaction between sialylglycoconjugates on the cell surface and hepatic endothelial cells, rather than degradation of the cells by the immune system.

Adhesion of certain human carcinoma cells to endothelial cells is reported to be mediated in part by E- and P-selectins.[37–40] We previously observed that sialyl-Le^x-conjugated liposomes suppressed both the lung accumulation of B16BL6 melanoma cells and the lung metastasis after tail-vein injection.[20] Furthermore, the importance of sialylglycoconjugates, including sialyl-Le^a determinants, in liver metastasis has been reported.[13, 31, 32] Our present data indicate that certain sialylglycoconjugates on the surface of liver-metastatic RAW117 cells may play an important role in the adhesion of the cells to the hepatic endothelium, and thus in metastasis establishment. We propose that certain sialylglycoconjugates decide the topology of metastasis and that sialyl-Le^a antigen is a candidate for the determinant of hepatic metastasis, at least in the case of RAW117 metastatic tumor cells.

ACKNOWLEDGMENTS

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