Butanol-Extractable and Detergent-Solubilized Cell Surface Components From Murine Large Cell Lymphoma Cells Associated With Adhesion to Organ Microvessel Endothelial Cells

Robert J. Tressler and Garth L. Nicolson

Department of Tumor Biology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Abstract Metastatic variant cell lines of the murine RAW117 large cell lymphoma were used to study the cell surface components involved in syngeneic tumor cell/microvessel endothelial cell interactions. Poorly liver-metastatic parental RAW117-P cell line adhered to murine hepatic sinusoidal endothelial cell monolayers at significantly lower rates than the liver-selected, highly liver-metastatic RAW117-H10 line and both cell lines were poorly adherent to lung microvessel and bovine aorta endothelial cells. Viable, 2% 1-butanol–treated RAW117-H10 tumor cells formed fewer liver tumor nodules in experimental metastasis assays than untreated H10 cells and were significantly less adherent to murine hepatic sinusoidal endothelial cell monolayers. When 2% 1-butanol extracts of metabolically labeled or CHAPS detergent lysates of cell surface-labeled tumor cells were analyzed for their ability to bind to fixed microvessel endothelial cell monolayers, quantitative differences were found in the extractable tumor cell surface components that bound to the different organ-derived microvessel endothelial cells. Cell surface components (1-butanol extractable), of $M_r \sim 85,000–90,000$ and $\sim 37,000–40,000$ bound to hepatic sinusoidal endothelial cell monolayers to a greater extent than to murine lung microvessel endothelial or bovine aortic endothelial cell monolayers, whereas tumor cell surface components of $M_r \sim 45,000$, $\sim 33,000$, and $\sim 25,000$ bound similarly to endothelial cells regardless of origin. The results suggest but do not prove that tumor cell/endothelial cell adhesion involves multiple tumor cell surface components, some of which commonly bind to various endothelial cells and others for which binding may be dictated by the tissue origin and type of endothelial cell. Particular RAW117 butanol-extractable cell membrane components were associated with tumor cell–endothelial cell adhesion, and these components could be responsible, in part, for the preferential adhesion of RAW117 cells to liver sinusoidal endothelial cells and metastasis to liver.

Key words: cell adhesion, metastasis, tumor cells, receptors

The process of blood-borne metastasis formation at distant sites for many tumors cannot be explained solely by mechanical or anatomical

considerations, such as the random arrest of malignant cells in the microcirculation [1–4]. Once malignant cells enter the blood vascular system, their successful colonization of distant organs requires implantation in a microvessel, usually by tumor cell adhesion to microvascular endothelial cells. Arrested metastatic cells subsequently penetrate the vascular basement membrane, invade surrounding tissue, and establish a favorable microenvironment for survival and growth [3,4]. When malignant cells metastasize to particular organs, specific tumor cell and host properties are thought to be involved [3,4]. One of the critical events in this process, the adhesion of tumor cells to organ microvessel endothelial cells, is related to the organ preference of metastasis formation in several tumor systems [2–6].

To study the organ preference of metastasis we have used an Abelson virus-induced murine

large cell lymphoma parental line (RAW117-P) and have selected variant lines in vivo for enhanced liver (RAW117-H10) [7] or lung plus liver (RAW117-L17) [8] colonization properties. Compared with poorly metastatic parental cells, highly metastatic RAW117 lines (such as RAW117-H10) show loss of lectin-binding sites [9,10], RNA tumor virus-encoded cell surface gp70 and internal components p30 and p15 [11], increased partitioning in aqueous 2-phase solutions [12], increased expression of cell surface gp150 [13], sialoglycoproteins [14], lymphoid antigens, and loss of particular glycolipids [15]. None of these properties, however, has been related to the organ preference of colonization of RAW117 cells or to endothelial cell adhesion.

The cell surface is important in many of the steps involved in metastasis formation [1–4]. To demonstrate the involvement of cell surface components in metastasis, they have been selectively removed by extraction of highly metastatic tumor cells with 1-butanol [15,16]. LeGrue [16] reported that 1-butanol extraction decreased the experimental metastatic properties of murine B16 melanoma and MCA-F fibrosarcoma cells, and Joshi et al. [14] found that experimental metastases of 1-butanol–treated murine RAW117 cells were reduced without affecting cell viability or tumorigenicity. Here we report on the effects of 1-butanol extraction on the adhesion of RAW117 cells to syngeneic organ-derived microvascular endothelial cells and the development of an endothelial cell affinity assay to identify butanol-extractable tumor cell surface components associated with adhesion to particular organ-derived endothelial cells.

MATERIALS AND METHODS
Tumor Cells

RAW117-P and RAW117-H10 cell lines were grown as suspension cultures in plastic Petri dishes (Falcon Plastics, Lincoln Park, PA) in SDMEM (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated FBS (Hyclone, Logan, UT) and 2.2 mM D-glucose without antibiotics. Cells were tested routinely for *Mycoplasma* contamination by Hoescht staining and Gen Probe℗ analysis (Gen Probe, Inc., San Diego, CA) and were found to be negative.

Endothelial Cells

Bovine aorta (BAE), murine lung microvessel endothelial (LE), and murine hepatic sinusoidal endothelial (HSE) cells were isolated and characterized as described previously [17,18]. Briefly, microvessel trees from various murine organs were isolated, collagenase digested, and cultured. Colonies of cells having endothelium-like morphologies were then isolated and characterized biochemically, histologically, enzymatically, and electron microscopically. For example, the endothelial cells were characterized by their cell morphologies and presence of intercellular junctions, lack of platelet binding, presence of Von Willebrand factor antigen and angiotensin converting enzyme, binding of acetylated low-density lipoprotein, and synthesis of a subendothelial matrix containing laminin and type IV collagen [17,18]. Endothelial cells were cultured in 1:1 (v/v) DMEM/F12 (GIBCO) containing 2% horse serum (Hyclone), 5% FBS (Hazelton, Lenexa, KS), non-essential amino acids (GIBCO), 1 mM sodium pyruvate (GIBCO), 0.1 mM L-glutamine (GIBCO), and 0.1 mg/ml endothelial mitogen (Biomedical Technologies, Inc., Stoughton, MA) in gelatin-coated 12-well plastic tissue culture dishes (Costar, Cambridge, MA) at 37°C in a 5% CO_2–95% air atmosphere.

Metastasis Assays

RAW117 cells were washed three times in serum-free SDMEM and injected intraveneously (5,000 viable cells) in 0.2 ml serum-free SDMEM into groups of 5–6-week-old BALB/c mice. Experiments were continued either until the mice died or until 12–14 days after injection at which time the remaining mice were sacrificed and necropsied. Visible tumor colonies were counted in all major organs [7–10].

CHAPS Detergent and 1-Butanol Extracts

RAW117 tumor cells were metabolically labeled with 10 μCi/ml [^{35}S]methionine (ICN, Irvine, CA; specific activity 400 Ci/mmol) for 5–8 h in methionine-free medium (GIBCO) plus 5% FBS (HyClone) at 37°C. The cells were washed by centrifugation (500g), and resuspension 2 to 3 times at a concentration of 5×10^7 cells/ml DPBS at room temperature. They were then solubilized for 15 min on ice in a solution containing 2% CHAPS (Sigma, St. Louis, MO), 0.05 mM $CaCl_2$, 10 mM Tris-HCl (pH 7.2), 10% sucrose, 5% glycerol, 1 mM $MgCl_2$, 1 μM phenylmethylsulfonyl fluoride (Sigma), and 1 μM leupeptin (Sigma). After solubilization, the detergent lysates were centrifuged at 11,000g for 5 min, and the supernatants collected and used immedi-

ately or stored frozen at −80°C. The method of LeGrue [16] was used for 1-butanol extraction of RAW117 tumor cells. [35S]methionine-labeled RAW117 cells were collected by centrifugation at 500g and resuspension in DPBS. After repeating this procedure twice, the cells were suspended at a concentration of 1.5 × 10^7 cells/ml in 2% 1-butanol in DPBS and incubated for 5 min at room temperature in this solution. In some experiments the cells were pelleted by centrifugation, as described above, resuspended in adhesion medium, and immediately used in the adhesion assays. The 1-butanol extracts were diluted with 3 vol of DPBS and concentrated to 2.5–5 × 10^7 cell equivalents/ml with Amicon YM-10 membranes to remove excess 1-butanol, and the extracts were then equilibrated with DPBS containing 1% BSA, 1% CHAPS (Sigma), 1 μM phenylmethylsulfonyl fluoride (Sigma), and 1 μM leupeptin (Sigma) and stored in aliquots at −70°C until used in the binding assays.

Gel Electrophoresis

Cellular extracts and endothelial cell–adherent tumor cell components were mixed with 0.5 vol of 6% SDS, 3% 2-mercaptoethanol, 1.5 mM EDTA, 30% glycerin, and 187.5 mM Tris-phosphate buffer, pH 6.8 (3X sample buffer). After centrifugation for 5 min at 10,000g, the supernatants were collected and heated at 100°C for 10 min. SDS-PAGE was performed in 1 mm thick 5–15% linear gradient polyacrylamide gels as described previously [12,13]. The gels were treated with EnHance (New England Nuclear, Boston, MA) and dried, and radioactive proteins were identified by fluorography with X-AR5 film (Kodak, Rochester, NY). The developed films were scanned with a densitometer (Beckman model DU-8) to quantitate relative band intensities.

Adhesion Assays

Adhesion assays were performed, in general, as described previously [19]. Confluent monolayers of endothelial cells cultured in 24-well tissue culture dishes (Costar) were washed once with 1 ml of serum-free medium and incubated for 3 h in 1 ml of serum-free medium. The medium was then removed, and 1 ml of fresh serum-free medium was added. RAW117 cells (2 × 10^6) in 30 ml of complete medium were labeled overnight with 0.5 μCi/ml of [35S]methionine, washed in serum-free medium 3 times, and added to wells containing confluent endothelial cell mono-

layers (approximately 2 × 10^5 cells/well) in HEPES-buffered DMEM plus 1% BSA (adhesion medium). In some experiments the tumor cells were extracted with 1-butanol as described above prior to the adhesion assays; otherwise, the endothelial cell monolayers were fixed with 0.0125% glutaraldehyde in DPBS for 30–45 min with rocking at room temperature, excess glutaraldehyde was removed, and the fixed monolayers were washed 3 times with DPBS, incubated with 1% BSA in DPBS for 1 h, and then stored at −70°C or used as described above in the adhesion assays. The tumor cells were incubated on the endothelial monolayers at 37°C with or without fluid shear [19]. At various times the nonadherent tumor cells were removed by gentle aspiration, followed by addition of serum-free medium. This was repeated four times, and the remaining adherent tumor cells were solubilized with 150 μl 1 M NaOH, neutralized with 150 μl 1 M acetic acid, and mixed with ScintiVerse II scintillation cocktail (Fisher Scientific, Pittsburgh, PA), and radioactivity was quantitated with a Beckman Model LS 7500 scintillation counter.

Inhibition of RAW117 cell adhesion by tumor cell surface components was carried out as follows. CHAPS detergent lysates (100 μl) or 1-butanol extracts (50 μl) of RAW117 cells were incubated with fixed endothelial cell monolayers overnight at 4°C under the same conditions as described for the quantitative binding assays. After being rinsed with 0.05% Tween 20 in DPBS, the endothelial cell monolayers were rinsed 3 times with DPBS alone, incubated for 1 h at room temperature with 1 ml of adhesion medium, and used in adhesion assays with radiolabeled RAW117 cells. Control samples contained equivalent amounts of CHAPS solubilization buffer preincubated on endothelial monolayers. The number of adherent tumor cells was calculated using the equation

No. bound cells/well =
$$\text{(Total CPM bound/well)}/\text{(CPM/cell)}.$$

The assays were performed in triplicate, and the standard deviation was calculated for each experiment. ANOVA statistical analysis was performed on various time points in the assays.

Cell Affinity Binding Assays

Confluent BAE, HSE, or LE cell monolayers in 12-well tissue culture dishes were incubated for 2–3 days after reaching confluency with

DMEM/F12 plus 5% FBS, rinsed once with se-rum-free medium, and incubated for 3 h in serum-free medium (1 ml/well) at 37°C. The wells were rinsed 2 times with DPBS and fixed with 0.0125% glutaradehyde (Ted Pella, Tustin, CA) in DPBS for 45 min at room temperature with rocking, then rinsed 2 times with DPBS and incubated with 30 mM ethanolamine in PBS for 1 h with rocking at room temperature. The fixed endothelial cell monolayers were then stored frozen at −70°C or used immediately. Frozen endothelial cell monolayers were thawed and incubated with 1 ml of 1% BSA in DPBS for 1 h at room temperature immediately prior to use. The medium was aspirated, 0.5 ml of DPBS plus 0.05% Tween 20 was added to each well, and [^{35}S]methionine-labeled CHAPS detergent lysates or 1-butanol extracts of RAW117 cells (25 or 50 μl) were added to each well. After an incubation at room temperature for 2–4 h with rocking, the wells were rinsed 4 times with DPBS/Tween 20. The radiolabeled, adherent tumor cell components were eluted with 0.5% SDS in H$_2$O and the TCA-precipitable radioactiv-ity determined. Endothelial cell–bound compo-nents were then analyzed by SDS-PAGE, and fluorography and densitometric scanning were performed.

For the preclearance assays, 1-butanol ex-tracts of RAW117 cells were incubated on fixed endothelial monolayers (usually BAE cell mono-layers) for 4 h at room temperature. The nonad-herent fractions were collected and reincubated on various endothelial monolayers, and the ad-herent components were quantitated by the TCA-precipitable radioactivity. ANOVA statisti-cal analysis was performed to determine signifi-cance.

RESULTS

When 5 × 10^3 RAW117-P cells were injected i.v. into BALB/c mice, few liver (median = 0, range = 0–12) or lung (median = 0, range = 0) tumor nodules were visible 12–14 days later. Injection of the same number of RAW117-H10 cells resulted in liver tumors too numerous to count (median = > 200, range = > 200) but few lung nodules (median = 1, range = 1–8) [19].

When the endothelial cell adhesive properties of RAW117 cells were examined, the highly liver-metastatic H10 variant adhered at a signifi-cantly higher rate to HSE cell monolayers than the poorly metastatic P cell line (P < 0.001) (Fig. 1). RAW117 cell lines were significantly
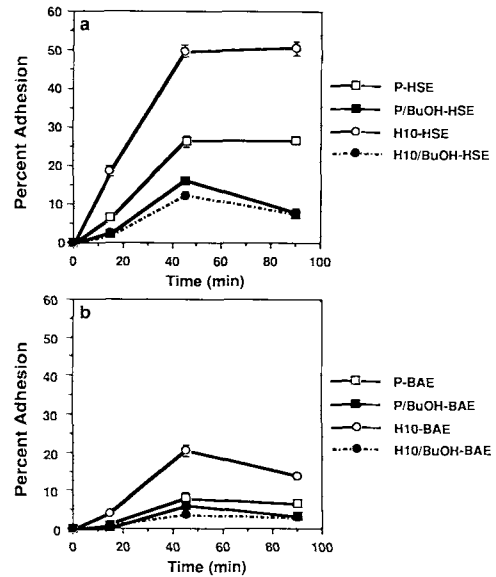

Fig. 1. Effect of 2% 1-butanol extraction of RAW117 cells on their rate of adhesion to murine hepatic sinusoidal endothelial (HSE) or bovine aorta endothelial (BAE) cell monolayers with-out shear. Values are expressed as cells bound (percent of total cells ±SD) to a 24-well tissue culture plate (2 × 10^5 cells added per well; quadruplicate samples). a: Adhesion of RAW117-P or -H10 cells with (P/BuOH-HSE, H10/BuOH-HSE) or without (P-HSE or H10-HSE) prior 2% 1-butanol treatment to HSE cell monolayers. b: Adhesion of RAW117-P or -H10 cells with (P/BuOH-BAE; H10/BuOH-BAE) or without (P-BAE; H10-BAE) prior 2% 1-butanol treatment to BAE cell monolayers.

more adherent to HSE than to LE monolayers and BAE cell monolayers (P < 0.0001) (Fig. 1). Thus the quantitative difference in tumor cell adhesion to the endothelial cell monolayers par-alleled their organ colonization properties [19]. Similar patterns of adhesion were obtained when viable RAW117 cells were allowed to adhere to fixed endothelial cell monolayers (Fig. 2). Al-though the rates and extents of adhesion of RAW117 cells to the fixed endothelial monolay-ers were lower than to viable endothelial cell monolayers, possibly due to the lack of mem-brane receptor mobility on the cell surfaces of the fixed endothelial monolayers or the inactiva-tion of receptors by fixation, significant differ-ences between the rates of H10 and P adhesion to HSE cell monolayers were preserved (P < 0.0001), as well as the preference of both of these cell lines to adhere to HSE cell monolayers at higher rates than to BAE cell monolayers (P < 0.0001) (Fig. 2).

The 1-butanol extraction of P and H10 cells resulted in decreased rates of adhesion of the extracted cells to HSE and BAE cell monolayers
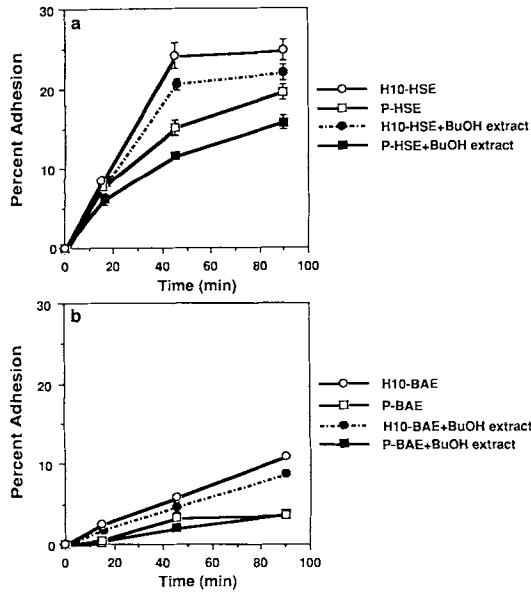
Fig. 2. Effect of 2% 1-butanol extracts of RAW117-H10 cells on the rate of adhesion of RAW117 cells to fixed endothelial cell monolayers. Values are expressed as cells bound (percent of total cells ±SD) to a 24-well tissue culture plate (2 × 10⁵ cells added per well; quadruplicate samples). **a:** RAW117-P or -H10 cell adhesion to HSE cell monolayers with (H10-HSE + BuOH extract; P-HSE + BuOH extract) or without (P-HSE or H10-HSE) preincubation of the monolayers with H10-1-butanol–extracted components (50 μl/well). **b:** RAW117-P or -H10 cell adhesion to BAE cell monolayers with (P-BAE + BuOH extract; H10-BAE + BuOH extract) or without (P-BAE or H10-BAE) preincubation of the monolayers with H10-1-butanol–extracted components (50 μl/well).
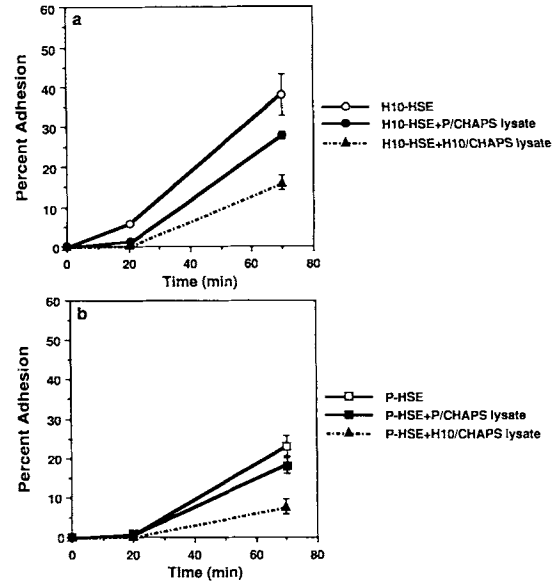
Fig. 3. Effect of CHAPS detergent lysates of RAW117 cells on the rate of adhesion of RAW117 cells to fixed endothelial cell monolayers. Values are expressed as cells bound (percent of total cells ±SD) to a 24-well tissue culture plate (2 × 10⁵ cells added per well; quadruplicate samples). **a:** RAW117-H10 cell adhesion to HSE cell monolayers with (H10-HSE + H10/CHAPS lysate; H10-HSE + P/CHAPS lysate) or without preincubation of the monolayers with H10 or P/CHAPS detergent lysates (50 μl/well). **b:** RAW117-P cell adhesion to HSE cell monolayers with (P-HSE + H10/CHAPS lysate; P-HSE + P/CHAPS lysate) or without preincubation of the monolayers with H10 or P/CHAPS detergent lysates (50 μl/well).

and abolished the differences in adhesion between H10 and P cells to HSE cell monolayers (Fig. 1). Thus the effect of 1-butanol extraction on adhesion of RAW117 cells was not equivalent for the two cell lines. After incubation for 40–45 min with the endothelial cell monolayers, 74% fewer H10 cells ($P < 0.0001$) and 49% fewer P cells ($P < 0.0001$) were adherent to HSE cell monolayers after 1-butanol extraction (Fig. 1a). In a similar incubation, adhesion of 1-butanol–extracted H10 and P cells to BAE cell monolayers was decreased by 82% ($P < 0.0001$) and 25% ($P < 0.0001$), respectively, after 1-butanol extraction (Fig. 1b).

The ability of RAW117-P and -H10 dialyzed CHAPS lysates to inhibit the binding of viable RAW117 cells to fixed endothelial cell monolayers was assessed in a 70 min adhesion assay (Fig. 3). The adhesion of H10 cells to HSE cell monolayers was inhibited 21 ± 2% by P CHAPS cell lysates, whereas H10 CHAPS cell lysates inhibited H10 adhesion to HSE cell monolayers

by 56 ± 3% (Fig. 3a). Preincubation of glutaraldehyde-fixed HSE cell monolayers with P cell CHAPS lysates did not significantly inhibit the adhesion of viable P cells to HSE cell monolayers, whereas the H10 cell lysates inhibited P cell adhesion to fixed HSE cell monolayers by 58 ± 2% ($P < 0.002$) (Fig. 3b). CHAPS cell lysates from other cell types (murine hepatocytes, human erythrocytes, murine lymphocytes) had no significant effect on RAW117 cell adhesion to fixed HSE cell monolayers (data not shown).

We also examined the effects of 2% 1-butanol extracts of RAW117 cells on RAW117–endothelial cell adhesion (Fig. 2). Preincubation of HSE cell monolayers with H10 1-butanol extracts inhibited P cell adhesion to HSE cell monolayers by 25 ± 1% at 45 min ($P < 0.001$), whereas H10 cell adhesion to HSE monolayers in the presence of H10 1-butanol extracts was decreased by 15 ± 1% ($P < 0.01$) (Fig. 2a). Differences in RAW117 cell adhesion to BAE cell monolayers in the presence or absence of RAW117 1-butanol extracts were not significant (Fig. 2b).

TABLE I. Binding of Surface-Radiolabeled RAW117 Cell Detergent Lysates or 1-Butanol Extracts to Fixed Endothelial Cell Monolayers*

Preparation/cell type	CPM radioactivity bound ± SD (% cell equivalents bound ± SD) to fixed endothelial cell monolayers	
	HSE	BAE
[^{125}I]Surface-labeled CHAPS detergent lysate		
RAW117-H10	19,622 ± 3728 (100 ± 19)	12,754 ± 1275 (65 ± 10)
RAW117-P	13,175 ± 1581 (67 ± 12)	7,246 ± 435 (36 ± 6)
[^{35}S]Methionine-labeled 2% 1-butanol extract		
RAW117-H10	9,960 ± 398 (100 ± 4)	6,574 ± 329 (66 ± 5)

*[^{125}I]Surface-labeled CHAPS detergent lysates (50 μl) or [^{35}S]methionine-labeled 2% 1-butanol extracts (50 μl) (protein yield approximately 320 μg/1.5 × 10^7 cells; final concentration 1,070 μg/ml at 1,380 cpm/μg) were incubated on glutaraldehyde-fixed endothelial cell monolayers for 2–4 h, washed, and bound TCA-precipitable radioactivity determined. The values represent CPM bound ± SD for triplicate samples and represent approximately 7.2 μg protein bound to HSE and 4.8 μg protein bound to BAE cell monolayers. HSE > BAE, $P < 0.005$; H10 > P, $P < 0.005$.

We next measured the amounts of CHAPS solubilized or 1-butanol extracted cell membrane components from H10 or P cells that bound to fixed HSE or BAE cell monolayers. Significantly more TCA-precipitable radioactivity from CHAPS lysates or 1-butanol extracts from cell-surface labeled P or H10 cells bound to HSE than to BAE cell monolayers ($P < 0.005$), suggesting that endothelial cells of different tissue origins have differing abilities to bind RAW117 tumor cell surface components and that some components bind to various types of endothelial cells regardless of their origin (Table I). In addition, significantly more TCA-precipitable radioactivity from CHAPS lysates of H10 cells bound to HSE or BAE cell monolayers than did P/CHAPS lysates ($P < 0.005$) (Table 1). Preincubation of HSE cell monolayers with unlabeled H10 1-butanol extracts resulted in a 50% decrease (from 10,105 to 5,088 cpm) in the binding of radioactivity from cell-surface labeled H10 CHAPS lysates to the fixed HSE cell monolayers. The specificity of binding of the cell-surface labeled H10 CHAPS lysates to the fixed HSE cell monolayers was assessed in experiments where murine hepatocytes or human erythrocytes were substituted for HSE cell monolayers. In such experiments, low levels of radioactivity were bound to the murine hepatocytes and human erythrocytes, and the addition of unlabeled H10 1-butanol extracts failed to inhibit binding (data not shown).

The 1-butanol–extractable components that bound to the various endothelial monolayers were analyzed by SDS-PAGE and autoradiography (Figs. 4, 5). Since in these experiments the

cell equivalents were adjusted for maximum binding of radioactivity, differences in the binding of radiolabeled P or H10 to HSE cell monolayers (as in Table I) were not seen (Figs. 4, 5). Some of the endothelial cell–bound components from RAW117 cells were commonly bound to all of the endothelial cell lines tested, such as the major P and H10 cell surface components of $M_r \sim 45,000$, $\sim 33,000$, and $\sim 25,000$ that were adherent to all of the endothelial cell monolayers (Fig. 4a, lanes 1–6; Fig. 4b, scans 1–6; Fig. 5, lanes 1–6). Other components bound in differing amounts to the various endothelial cell monolayers. For example, 1-butanol–extractable components of $M_r \sim 85,000–90,000$ and $\sim 37,000–40,000$ from P or H10 cells bound preferentially to HSE cell monolayers rather than to LE or BAE cell monolayers (Fig. 4a, lanes 3, 4; Fig. 4b, scans 3, 4; Fig. 5, lanes 1, 2).

DISCUSSION

The organ preference of metastasis appears to be dictated, in part, by cell adhesion molecules whose expression appears to be tissue dependent in particular organs [1–6, 19–23]. Adhesion molecules involved in cell "homing" are also important in normal biological processes, including neural development, tissue-specific lymphocyte trafficking, and embryogenesis [24–28]. Various related families of cell adhesion molecules, such as integrins [29], cadherins [30], neural cell adhesion molecules [31], and intercellular adhesion molecule-1 [32], can be expressed by different cell types and function in the positioning or homing of cells to various tissues or organs. Nakache et al. [28] identified an $M_r \sim$ 58,000–66,000 tissue-specific "vascular addres-
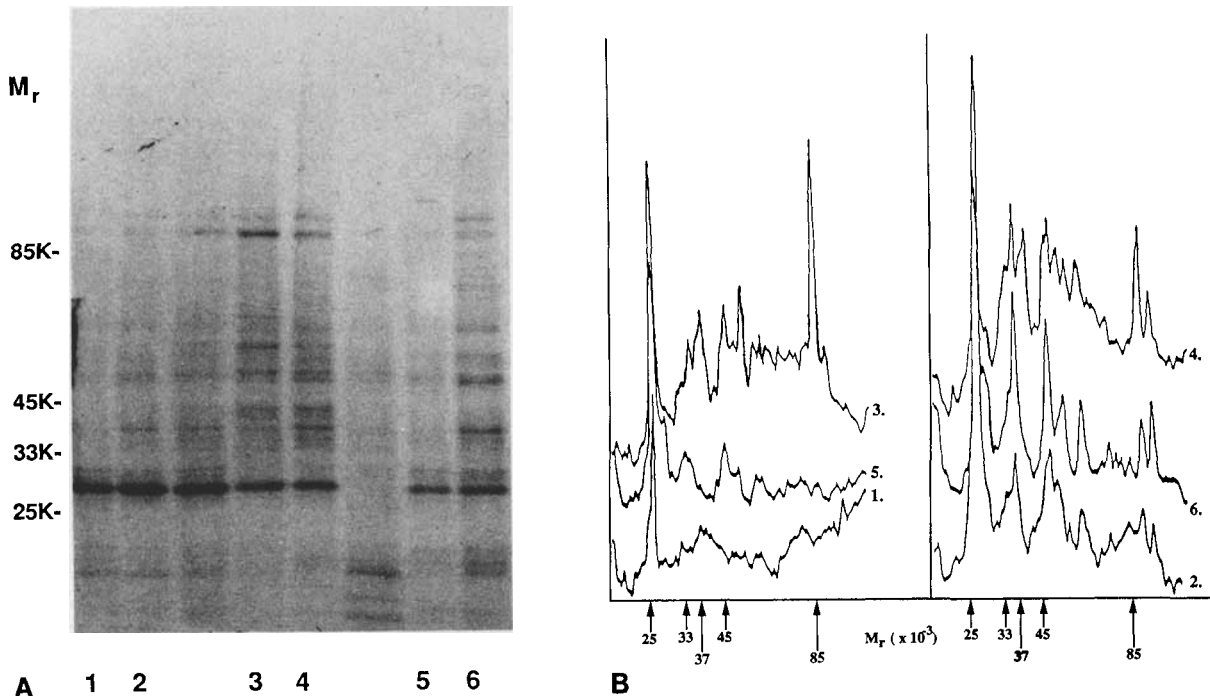
Fig. 4. SDS-PAGE analysis of [³⁵S]methionine-labeled RAW117 1-butanol–extracted components that bound to fixed bovine aorta (BAE), lung microvessel (LE), or hepatic sinusoidal (HSE) endothelial cell monolayers. a: Fluorograph of [³⁵S]methionine-labeled RAW117 1-butanol–extracted components that bound to various endothelial cell monolayers. Lane 1, RAW117-P 1-butanol–extracted components bound to BAE cell monolayers; lane 2, RAW117-H10 1-butanol–extracted components bound to BAE cell monolayers; lane 3, RAW117-H10 1-butanol–extracted components bound to HSE cell monolayers; lane 4, RAW117-P 1-butanol–extracted components bound to HSE cell monolayers; lane 5, RAW117-P 1-butanol–extracted components bound to LE cell monolayers; lane 6, RAW117-H10 1-bu-

tanol–extracted components bound to LE cell monolayers. b: Densitometric scans of fluorographs of labeled RAW117 1-butanol–extracted components that bound to various endothelial cell monolayers. Scan 1, RAW117-P 1-butanol–extracted components bound to BAE cell monolayers; scan 2, RAW117-H10 1-butanol–extracted components bound to BAE cell monolayers; scan 3, RAW117-P 1-butanol–extracted components bound to HSE cell monolayers; scan 4, RAW117-H10 1-butanol–extracted components bound to HSE cell monolayers; scan 5, RAW117-P 1-butanol–extracted components bound to LE cell monolayers; scan 6, RAW117-H10 1-butanol–extracted components bound to LE cell monolayers.

sin" adhesion molecule on high endothelial venules, and this molecule appeared to be involved in lymphocyte homing to mucosal tissues. The lymphocyte receptors for vascular addressins have been identified as $M_r \sim 90,000$ glycoproteins [33,34].

While the ability of tumor cells to adhere to extracellular components is important in the process of invasion and secondary tumor formation at distant organ sites [35–38], the initial arrest and adhesion of tumor cells occurs to the endothelial cells lining the microvasculature of the target organ. It is only after damage and/or retraction of the endothelial cells that the tumor cells are able to bind to the subendothelial matrix or basement membrane. Thus tumor cell–endothelial cell adhesion is probably the first step in the process of organ preference of metastasis, whereas the adhesion of tumor cells to the subendothelial basement membrane is sub-

sequently important in the invasion of tumor cells into the target organ after their initial arrest [39]. In the RAW117 system the rates of adhesion of these large-cell lymphoma cells to microvessel endothelial cells were always higher than to subendothelial extracellular matrix, and in contrast to organ-derived microvessel endothelial cells differences were not found in the rates of adhesion of RAW117-P or -H10 cells to subendothelial extracellular matrix derived from various organ sources [19].

Various investigators have shown that treatment of tumor cells with 1-butanol can alter their growth and dissemination properties [14,16,40–46]. Most of these studies suggested that 1-butanol–extracted components functioned by affecting the host's immune response to a tumor. Using the murine RAW117 large-cell lymphoma tumor model Joshi et al. [14] showed that injection with 1-butanol–extracted H10 cells
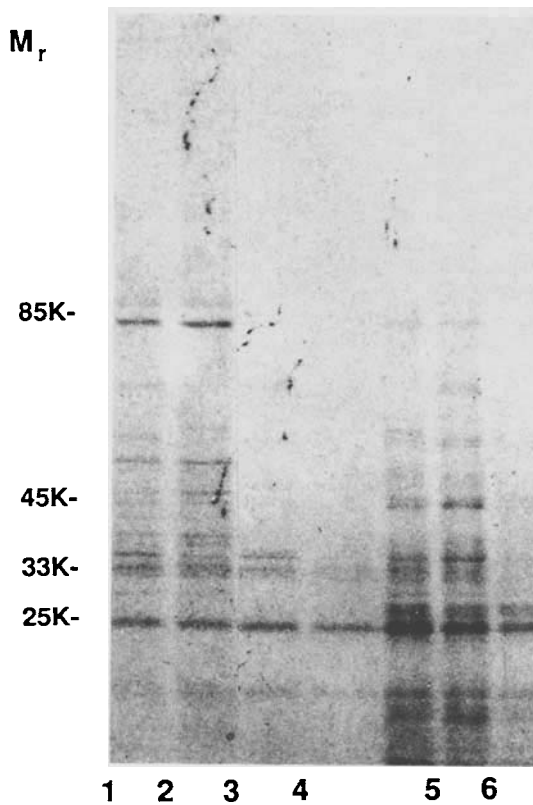
Fig. 5. Fluorograph of [^{35}S]methionine-labeled RAW117 1-butanol–extracted components that bound to various endothelial cell monolayers (SDS-PAGE analysis). Lane 1, RAW117-H10 1-butanol–extracted components bound to HSE cell monolayers; lane 2, RAW117-P 1-butanol–extracted components bound to HSE cell monolayers; lane 3, RAW117-H10 1-butanol–extracted components bound to BAE cell monolayers; lane 4, RAW117-P 1-butanol–extracted components bound to BAE cell monolayers; lane 5, RAW117-H10 1-butanol–extracted components bound to LE cell monolayers; lane 6, RAW117-P 1-butanol–extracted components bound to LE cell monolayers.

resulted in fewer liver tumor colonies than with untreated H10 cells. This result could have been due to the reduced endothelial cell adhesive properties of 1-butanol–treated cells. We have characterized the in vitro endothelial cell adhesion properties of metastatic variants of RAW117 cells and found that their adhesion properties parallel their metastatic potential [19], suggesting that the ability of RAW117 cells to preferentially adhere to HSE cells is necessary for their ability to colonize the liver with high efficiency. Additional evidence supporting this premise is that when we sequentially selected RAW117-P cells for increased adhesion to HSE monolayers, the selected tumor cell variants also showed increased metastatic potential to liver (unpublished data). In contrast, RAW117-P cells that were sequentially selected for increased resis-

tance to interferon-γ were significantly less adherent to HSE monolayers and also less metastatic than unselected RAW117-P cells (H. Zhang, R.J. Tressler, R. LaBiche and G.L. Nicolson, manuscript in preparation).

We have analyzed the properties of 1-butanol–treated RAW117 cells and the cell surface components extracted with 1-butanol that are associated with endothelial cell adhesion. We found that the rate of adhesion of 1-butanol–extracted highly metastatic H10 cells to HSE cell monolayers was decreased to a level similar to that of 1-butanol–extracted P cells, paralleling the in vivo effect of 1-butanol extraction on the liver colonization properties of these cells [14]. Ours is the first attempt at elucidating the molecular basis of the altered metastatic phenotype of 1-butanol–extracted cells and demonstrating that the 1-butanol–extractable cell surface components are associated with tumor cell adhesion to endothelial cells. The ability of the 1-butanol–extracted components to inhibit the adhesion of RAW117 cells to various fixed endothelial cell monolayers, although significant, was not as great as that of the detergent lysates of the same tumor cells. This difference could be due to various factors. The 1-butanol extraction is not nearly as good a membrane protein solubilizer as CHAPS detergent, or the extraction of adhesion-associated components with 1-butanol could have a deleterious effect on their affinity or avidity to the endothelial cell monolayers. Alternatively, the 1-butanol extraction of RAW117 cells could remove peripheral membrane components essential to the activity or display of nonextractable integral membrane adhesion components, or such treatment could stimulate turnover of integral membrane components. When the 1-butanol–extracted H10 cells were allowed to recover for 3 days in culture, they remained viable, grew, and regained their high liver colonization potential [14], indicating that 1-butanol extraction of H10 cells does not modify cell viability or growth properties, nor does it have a permanent effect on the metastatic phenotype of these cells.

When the 1-butanol–extracted components of RAW117 cells that bound to different organ-derived microvessel endothelial cell monolayers were analyzed by SDS-PAGE, multiple cell surface components were identified. The 1-butanol–extracted RAW117 cell surface components of $M_r \sim 45,000$, $\sim 33,000$, and $\sim 25,000$ bound to all of the endothelial cell monolayers tested,

whereas components of $M_r \sim 85,000-90,000$ and $\sim 37,000-40,000$ showed preferential adhesion to HSE cell monolayers established from the target organ for metastasis formation for RAW117 cells. Kraal et al. [47] have generated a monoclonal antibody, CT4, that is able to inhibit guinea pig lymphocyte/high endothelial venule adhesion, and this antibody immunoprecipitates a $M_r \sim 36,000$ component from the surface of guinea pig lymphocytes. It is not known at this time whether the $M_r \sim 37,000-40,000$ 1-butanol–extractable component from murine RAW117 cells that binds preferentially to HSE cell monolayers is related to this protein from guinea pig lymphocytes. In addition, other molecules, such as glycolipids, could be involved in the 1-butanol extract inhibition of RAW117/endothelial cell adhesion.

While many studies have characterized integral membrane components in the process of cell adhesion, the results of our study are the first to implicate 1-butanol–extractable peripheral membrane components in the process of tumor cell–endothelial cell adhesion. Future experiments will focus on the possible role of the individual 1-butanol–extracted components, if any, in endothelial cell adhesion. Dissecting the role of individual tumor cell–endothelial cell adhesion components in the organ preference of metastasis could lead to the development of specific agents that might prevent organ implantation of blood-borne malignant cells in the microcirculation. Our results suggest but do not prove that RAW117 cell/endothelial cell adhesion is mediated by multiple molecular interactions, some components common to various endothelial cells and others more restricted in their organ expression. If this proves to be correct, then it might be possible to selectively interfere with adhesion by blocking combinations of common and unique tumor cell/endothelial cell adhesion molecules [48].

ACKNOWLEDGMENTS

REFERENCES

1. Nicolson GL: Biochim Biophys Acta 495:113–176, 1982.
2. Kiernan MW, Longenecker BM: Cancer Metastasis Rev 2:165–182, 1983.
3. Nicolson GL: Cancer Metastasis Rev 7:143–188, 1988.
4. Nicolson GL: Biochim Biophys Acta 948:175–224, 1988.
5. Nicolson GL: J Histochem Cytochem 30:214–220, 1982.
6. Alby L, Auerbach R: Proc Natl Acad Sci USA 81:5739–5743, 1984.
7. Brunson KW, Nicolson GL: J Natl Cancer Inst 61:1499–1503, 1978.
8. Nicolson GL, Mascali JJ, McGuire EJ: Oncodevelop Biol Med 4:149–159, 1982.
9. Reading CL, Brunson KW, Torrianni M, Nicolson GL: Proc Natl Acad Sci USA 77:5943–5947, 1980.
10. Nicolson GL, Reading CL, Brunson KW: In Crispen RG (ed): "Tumor Progression." Amsterdam: Elsevier/North-Holland, 1980, pp. 31–48.
11. Joshi SS, Sharp JG, Gharpure HM, Brunson KW: Clin Exp Metastasis 5:89–101, 1987.
12. Miner KM, Walter H, Nicolson GL: Biochemistry 20:6244–6250, 1981.
13. Irimura T, Tressler RJ, Nicolson GL: Exp Cell Res 165:403–416, 1986.
14. Joshi SS, Tilden PA, Jackson JD, Sharp JG, Brunson KW: Cancer Res 47:3551–3557, 1987.
15. Appella E, Law LW, Henriksen O: Cancer Res 36:3539–3544, 1976.
16. LeGrue SJ: Cancer Metastasis Rev 4:209–219, 1985.
17. Belloni PN, Nicolson GL: J Cell Physiol 136:398–410, 1988.
18. Belloni PN, Carney DH, Nicolson GL: Microvasc Res (in press).
19. Nicolson GL, Belloni PN, Tressler RJ, Dulski K, Inoue T, Cavanaugh PG: Invasion Metastasis 9:102–116, 1989.
20. Korach S, Poupon MF, DuVillard JA, Becker M: Cancer Res 46:3624–3629, 1986.
21. Bargatze RF, Wu NW, Weissman IL, Butcher EC: J Exp Med 166:1125–1131, 1987.
22. Roos E, Tulp A, Middelkoop OP, van de Pavert IV: J Natl Cancer Inst 72:1173–1180, 1984.
23. Auerbach R, Lu WC, Pardon E, Gumkowski F, Kaminski G, Kaminski M: Cancer Res 47:1492–1496, 1987.
24. Gallatin WM, Weissman IL, Butcher EC: Nature 304:30–34, 1983.
25. Masuyama J, Minato N, Kano S: J Clin Invest 77:1596–1605, 1986.
26. Yednock TA, Butcher EC, Stoolman LM, Rosen SD: J Cell Biol 104:725–731, 1987.
27. Hamann A, Jablonski-Westrich D, Scholz K-U, Duijvestijin A, Butcher EC, Thiele H-G: J Immunol 140:737–743, 988.
28. Nakache M, Berg EL, Streeter PR, Butcher EC: Nature 337:179–181, 1989.
29. Rouslahti E, Hayman EG, Pierschabacher MD: Arteriosclerosis 5:581–594, 1985.
30. Hatta KS, Takagi S, Fujisawa H, Takeichi M: Dev Biol 120:215–227, 1987.
31. Crossin KL, Chuong C-M, Edelman GM: Proc Natl Acad Sci USA 82:6942–6946, 1985.
32. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: J Immunol 137:245–254, 1986.
33. Berg EL, Goldstein LA, Jutila MA, Nakache M, Picker LJ, Streeter PR, Wu NW, Zhou D, Butcher EC: Immunol Rev 108:5–18, 1989.
34. Picker LJ, de los Toyos J, Telen MJ, Haynes BF, Butcher EC: J Immunol 142:2046–2051, 1989.
35. Gehlsen KR, Argraves WS, Pierschabacher MD, Ruoslahti E: J Cell Biol 106:925–930, 1988.
36. Savagner P, Imhof BA, Yamada KM, Thiery JP: J Cell Biol 103:2715–2727, 1986.

37. Welch DR, Lobl TJ, Seftor EA, Wack PJ, Aeed PA, Yohem KH, Seftor REB, Hendrix MJC: Int J Cancer 43:449–457, 1989.
38. Roos E, Roosien FF: J Cell Biol 105:553–559, 1987.
39. Tryggvason K, Hoyhtya M, Salo T: Biochim Biophys Acta 907:191–217, 1987.
40. LeGrue SJ, Pellis NR, Riley LB, Kahan BD: Cancer Res 45:3164–3172, 1985.
41. Nomi S, Pellis NR, Kahan BD: J Natl Cancer Inst 73:943–950, 1984.
42. Motyckya K, Bostik J, Bures L, Slavik K, Jirasek A: Neoplasma 33:177–185, 1986.

43. Sato N, Kikuchi K: J Natl Cancer Inst 74:883–888, 1985.
44. Coggin JH Jr, Gillis LD, Payne WJ Jr: J Natl Cancer Inst 72:853–862, 1984.
45. Labateya N, Thomson DM, Durko M, Shenouda G, Robb L, Scanzano R: Cancer Res 47:1058–1064, 1987.
46. Hao W, Lundquist DL, McDonald TL, Joshi SS: Proc Am Assoc Cancer Res 30:1274a, 1989.
47. Kraal G, Twisk A, Tan B, Scheper R: Eur J Immunol 16:1515–1519, 1986.
48. Nicolson GL: Curr Opin Oncol 3:75–92, 1991.