Sialoadhesin on Macrophages: Its Identification as a Lymphocyte Adhesion Molecule

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Summary

In this study we present evidence that the mouse and rat sialoadhesin (originally named sheep erythrocyte receptor) on macrophages can function as a lymphocyte adhesion molecule. Lymphocytes were shown to bind to the splenic marginal zone, and lymph node subcapsular sinus and medulla in a frozen section assay. Selective depletion experiments showed that binding was mediated by macrophages. Adhesion was blocked by preincubation of the sections with monoclonal antibodies against mouse or rat sialoadhesin. Binding was temperature dependent, divalent cation independent, and involved sialic acid residues on the lymphocyte, as it could be inhibited by prior neuraminidase treatment or addition of the ganglioside GD1a. Binding to sialoadhesin was confirmed using the purified receptor and was observed among T cells, T blasts, B cells, and B blasts. Isolated macrophages or dendritic cells showed little binding. Sialoadhesin provides the first example of a macrophage-restricted lymphocyte adhesion molecule.

Cell-cell interactions provide an important means of communication within the immune system. They are instrumental in the regulation of proliferation, differentiation, and migration of lymphocytes. In lymphoid organs, lymphocytes encounter specialized microenvironments in which local interactions with other lymphocytes, nonlymphoid cells, or the extracellular matrix regulate their functions. These interactions involve cell surface adhesion receptors, many of which have now been identified (1).

Sialoadhesin (sheep erythrocyte receptor; SER)[1] has recently been characterized as a mouse macrophage-restricted cell surface receptor that binds glycoproteins and glycolipids containing the terminal oligosaccharide Neu5Acα2,3Galβ-1.3GalNAc (2). Sialoadhesin was originally identified as a SER on resident bone marrow macrophages (RBMM) (3, 4). This subpopulation naturally forms clusters with developing hemopoietic cells (5).

By immunocytochemistry, the expression of sialoadhesin was shown to be restricted to macrophages localized in particular compartments of lymphoid tissues. These included macrophages of the splenic marginal zone and the lymph node subcapsular sinus and medulla (6). In the rat, the ED3 antigen is expressed on a similar subpopulation of macrophages (7). Subsequent studies showed that the ED3 mAb blocks sialic acid–dependent erythrocyte binding in frozen section overlay assays (8). In both rodent species, the binding characteristics and the tissue distribution of sialoadhesin distinguish it from other erythrocyte binding receptors, including CD2 on T cells and CD22 on B cells (9, 10). Experiments in vitro have shown that expression of the receptor can be induced by lymphokines (11), a factor from homologous serum (12), and corticosteroids (13). In contrast, IFN-γ has a potent down-regulating effect (3, 11).

The physiological functions of sialoadhesin are unknown. A direct role in the scavenging or in the uptake of particulate antigens seems unlikely because binding of cellular ligands to sialoadhesin does not trigger phagocytosis (3, 8). Another possibility is that sialoadhesin is involved in cell-cell interactions. Indirect evidence supporting this idea was provided by immunoelectron microscopy of bone marrow clusters where sialoadhesin on resident macrophages was shown to be selectively localized at contact regions with myeloid precursor cells (5). Here we present evidence that sialoadhesin can function as an adhesion receptor for lymphocytes.

Materials and Methods

Animals. Male BALB/c mice and Wistar rats were obtained from Bomholtgard (Ry, Denmark) and Harlan/Olac/CPB (Zeist,

[1] *Abbreviations used in this paper:* RBMM, resident bone marrow macrophages; SER, sheep erythrocyte receptor.

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The Netherlands), respectively. All animals were maintained under conventional laboratory conditions and were allowed free access to food and water.

Antibodies. The following rat anti-mouse mAbs were used as culture supernatants: SER-4 and 3D6 (recognizing different epitopes of sialoadhesin) (6, Crocker et al., manuscript in preparation), MOMA-1 (specific for metallophilic macrophages [14]), ERTR9 (binding to marginal zone macrophages [15]); 3OH12 (recognizing Thy-1.2 [16]); 187-1 (recognizing κ Ig chains [16]); MECA-367 (recognizing the mucosal adressin [17]); MEL-14 (recognizing the peripheral homing receptor L-selectin [18]); and LPAM-1 (recognizing the mucosal homing receptor [19]). Mouse anti-rat mAbs were used to ascites. They included: ED3 and ED16 (both recognizing sialoadhesin [7, 8]), ED9 (recognizing a surface molecule on macrophages, including the sialoadhesin-positive populations, and granulocytes [20]), OX19 (binding to CD5 [21]; Serotec, Oxford, UK), and HIS14 (pan-B cells [22]). Polyclonal antisialoadhesin was produced by immunization of a rabbit with purified mouse sialoadhesin and was shown to specifically recognize mouse sialoadhesin by immunoprecipitation. Crossreactivity with the rat molecule was shown by immunocytochemistry of tissue sections. The antiserum was affinity purified by absorption to purified sialoadhesin that had been transferred to nitrocellulose after SDS-PAGE.

Cell Populations. All cells were cultured in RPMI 1640 (Flow Laboratories, Irvine, Scotland) supplemented with 10% FCS (HyClone Laboratories, Logan, UT), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. TK-1, a mouse T lymphoblastic tumor cell line, and 38C13, a mouse B lymphoblastic tumor cell line, were a gift of Dr. E. C. Butcher (Stanford University, Stanford, CA) (23). Cell suspensions of spleens and lymph nodes were prepared from mice and rats by cutting the tissue into small fragments and pressing them through a nylon screen. Red cells were lysed using ammonium chloride. Fractionation of the populations into T cells (mouse, rat) and B cells (rat) was performed using magnetic beads in a negative selection procedure. B cells, preincubated with 187-1 mAb (mouse cells) or without first-stage antibody (rat cells), were removed from the suspensions by absorption to magnetic beads coated with sheep anti-rat IgG (Dynal, Oslo, Norway). Mouse T cells were removed from the suspensions by incubation with mAb 3OH12, followed by complement-mediated lysis using guinea pig serum. The purity of each of these populations exceeded 90% as estimated in cytospin preparations stained with anti-Thy-1 and anti-K (mouse), or OX19 and HIS14 (rat). Mouse and rat T blasts were obtained by culturing T cells for 3 d in medium containing 0.3 μg/ml Con A (Flow Laboratories) and 0.05 mM β-mercaptoethanol (β-ME). Mouse B cells were stimulated for 3 d using 50 μg/ml LPS (*Escherichia coli*, L-3254; Sigma Chemical Co., St. Louis, MO) and β-ME. Mouse and rat peritoneal macrophages were obtained by peritoneal lavage from which B lymphocytes were removed by negative selection. Dendritic cells were isolated from lymph node suspensions as described (24).

Adhesion Assay. Cryostat sections (8 μm) were picked up on three-well (Θ 14 mm) slides (Nutacon, Schiphol-Oost, the Netherlands), air dried for 30 min and frozen (−20°C) until further use. The cells under investigation were washed extensively and resuspended in 5 mM Hepes-buffered RPMI 1640, containing 5% FCS, at a concentration of 10^7/ml. Cryostat sections were incubated, under horizontal rotation (85 rpm; radius, 0.4 cm), with the cells (100 μl/well) for 30 min at 37°C. The slides were inverted to remove unbound cells from the sections and immediately fixed in 1% glutaraldehyde. Binding was examined by light microscopy.

Blocking studies with various reagents were performed as following. Cells or sections were incubated with saturating concentrations of antibodies for 30 min at room temperature, washed once, and used in the binding assay. For enzymatic removal of sialic acid residues, cells (10^6) were pretreated with 0.004 U/ml neuraminidase (from *Vibrio cholerae*; Behringwerke, Marburg, FRG) in PBS for 30 min at 37°C and washed extensively. To study the effect of divalent cations, the binding assay was performed in presence of 2 mM EDTA (Merck, Darmstadt, FRG) in PBS, after both cells and sections had been preincubated with the same buffer. The gangliosides GM1 and GD1a (from bovine brain; Sigma Chemical Co.) were added (final concentration 100 μM) to the sections before the cells, and the assay was performed in their continued presence.

To quantitate and compare the binding affinities of the different cell populations, TK-1 cells were labeled with TRITC as described (25), mixed (1:1) with the cells under study, and after binding the ratio of TRITC-positive and -negative cells was determined by counting under a fluorescence microscope.

Macrophage Elimination In Vivo. Macrophages were removed from spleens of mice and rats by intravenous injection with 200 μl and 2 ml, respectively, of dichloromethylene diphosphonate (Cl_2MDP)-containing liposomes. Cl_2MDP liposomes were prepared as described previously (26). Control animals were treated with PBS-containing liposomes. The spleens of these animals were removed for adherence assays after 3 d. Mouse popliteal lymph nodes were depleted of macrophages by injection with 75 μl of Cl_2MDP liposomes subcutaneously in the footpad. Lymph nodes were used 5 d later. The absence of macrophages was confirmed by staining with acid phosphatase and macrophage-specific mAbs (27).

Immunocytochemistry. Immunoperoxidase staining of tissue sections and cytospin preparations was performed as described (28), using rabbit anti-mouse IgG peroxidase (P 260; Dakopatts, Glostrup, Denmark) or rabbit anti-rat IgG peroxidase (P 162; Dakopatts) as the second step and 3,3′-diaminobenzidine-tetrahydrochloride (Sigma Chemical Co.) as the substrate. Double labeling of macrophages and binding lymphocytes was done as following. TRITC-labeled lymphocytes were allowed to bind to tissue sections and they were fixed using 2% paraformaldehyde in PBS for 30 min. Macrophages were visualized using SER-4- and FITC-conjugated rabbit anti-rat IgG (F 238; Dakopatts).

Binding of Purified Sialoadhesin. Sialoadhesin was purified and iodinated with ^{125}I by the Iodogen method, as described previously (2), to a specific activity of 7.5×10^6 cpm/μg. Cell populations were washed in PBS, fixed for 10 min at room temperature with 0.5% glutaraldehyde in PBS, and washed extensively with PBS containing 1% BSA. Control experiments had shown that this treatment did not affect their sialoadhesin-binding capacity. Cells, resuspended at different concentrations in 96-well round-bottomed bacterial plastic plates (Greiner, Alphen a/d Ryn, the Netherlands), were incubated for 1 h at room temperature with ~30 ng of ^{125}I-sialoadhesin in 50 μl PBS containing 1% BSA. After four washes in PBS/0.1% BSA, cell-bound radioactivity was determined in a gamma counter (Berthold). Nonspecific binding was determined after a 1-h preincubation of ^{125}I-sialoadhesin with 50 μg/ml $F(ab')_2$ fragments of the antisialoadhesin mAb 3D6.

Western Blotting. Mouse and rat mesenteric lymph nodes from a single animal were homogenized in 1 ml 10 mM Tris-HCl, pH 8.0, containing 150 mM NaCl and the following protease inhibitors: 2 mM PMSF, 5 mM EDTA, 5 mM iodoacetamide, 0.1 mg/ml soybean trypsin inhibitor, 1 μg/ml pepstatin, 2.5 μg/ml aprotinin, and 0.5 μg/ml leupeptin. Octylglucoside was added to a final concentration of 1% and incubated for 30 min at 4°C. Nuclei and other insoluble materials were removed by centrifugation at 10,000 *g*, for 15 min at 4°C, followed by ultracentrifugation at 100,000

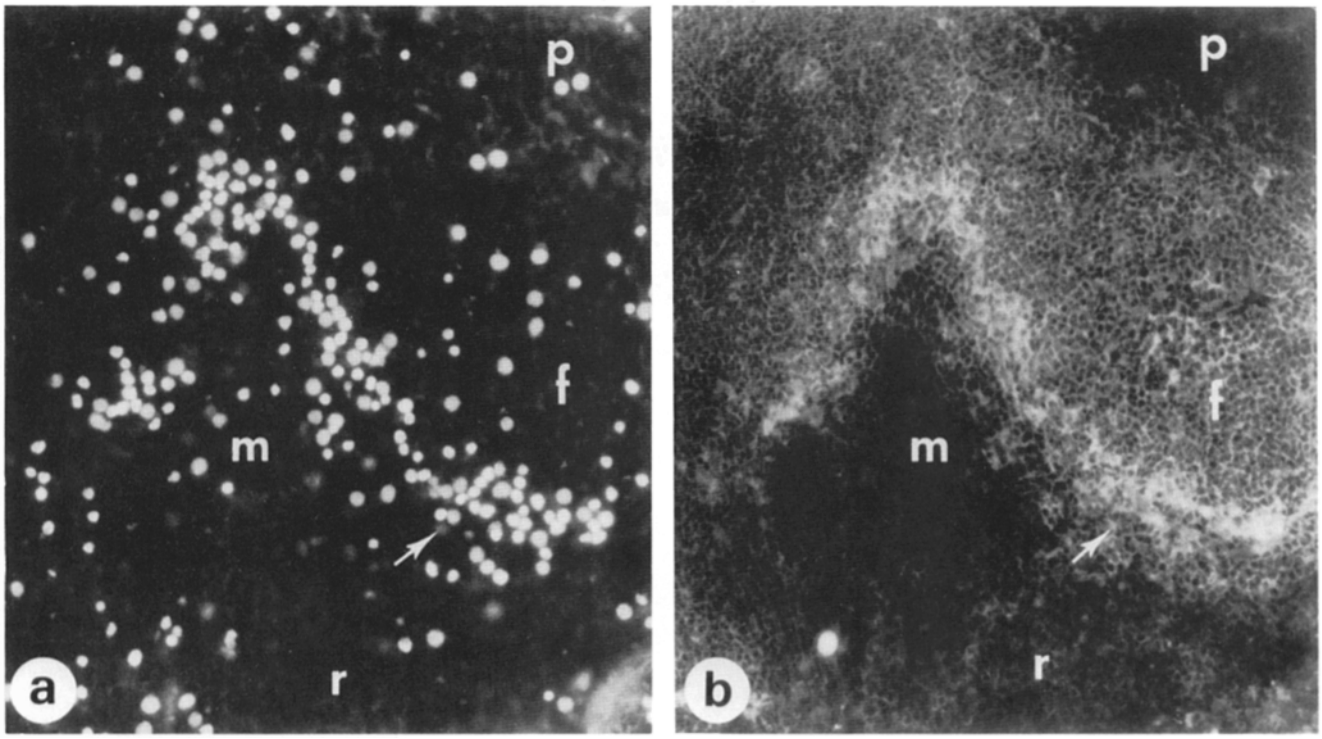

Figure 1. Binding of TK-1 lymphoma cells to the marginal zone of the mouse spleen coincides with the expression of sialoadhesin. TK-1 cells were labeled with TRITC and allowed to bind to cryostat sections of mouse spleen (*a*). Adhering cells were fixed in 2% paraformaldehyde and sections stained with SER-4 mAb and rabbit anti-rat/FITC (*b*). Binding is predominantly confined to the rim of metallophilic macrophages (*arrow*), which selectively express sialoadhesin. *f*, follicle; *m*, marginal zone; *p*, periarteriolar lymphocyte sheath; *r*, red pulp.

g in a 100.3 rotor (Beckman Instruments, Inc., Palo Alto, CA) for 10 min at 4°C. The lysates were subjected to SDS-PAGE (6.5% polyacrylamide), transferred to nitrocellulose, and probed with affinity-purified rabbit antisialoadhesin followed by ^{125}I-horse F(ab')$_2$ anti-rabbit IgG.

Results

Previous investigations in mice and rats have shown that erythrocytes can bind to cryostat sections of spleen and lymph nodes in a sialoadhesin-dependent manner (8). To test whether lymphocytes were able to bind to the marginal zone of the

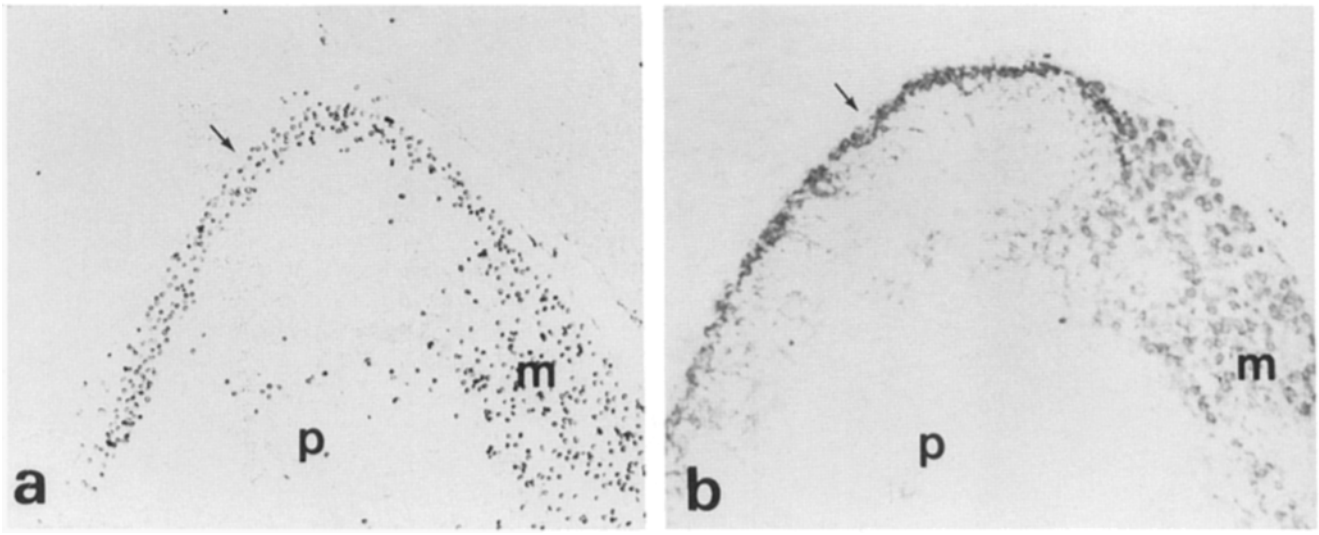

Figure 2. Adhesion of TK-1 cells to a mouse lymph node section (*a*). Binding occurs in the subcapsular sinus (*arrow*) and medullary region (*m*). For comparison, a lymph node section was stained with SER-4 mAb by the indirect immunoperoxidase method (*b*). Binding of cells occurs in areas that contain sialoadhesin-positive macrophages.

mouse spleen, we initially used the T lymphoblastic cell line TK-1. Under the binding conditions used, TK-1 cells strongly adhered to the marginal zone, and in particular to the inner part of this region, which contains the marginal metallophilic macrophages (Fig. 1 *a*). In peripheral lymph nodes, binding was observed in the subcapsular sinus and medullary region (Fig. 2 *a*). TK-1 cells also interacted with follicles and with high endothelial venules (HEV) in Peyer's patches. Occasionally, a small number of cells bound to the splenic red pulp.

These binding patterns were also found with freshly isolated T cells, Con A–stimulated T blasts, B cells, and LPS-stimulated B blasts. The B lymphoblastic cell line 38C13 bound to the same locations, but did not adhere to Peyer's patch HEV. In contrast to lymphocytes, no binding to the marginal zone was observed when peritoneal macrophages or isolated dendritic cells were used. A similar pattern of binding was found when rat tissue sections were used in the binding assay. Adhesion to rat tissue was also observed when the murine TK-1 cells were used, suggesting that the binding mechanism is similar in mice and rats.

Binding to the Marginal Zone, Subcapsular Sinus, and Medulla Is Mediated by Macrophages. The involvement of macrophages in the binding of lymphocytes was investigated using lymphoid tissues from mice and rats that had been selectively depleted of macrophages by injection with Cl_2MDP-encapsulated liposomes (Fig. 3). Elimination of macrophages resulted in the complete loss of binding to the marginal zone of spleen and lymph node subcapsular sinus and medulla. This was observed with TK-1 cells, T cells, and T blasts from rats and mice.

Involvement of Sialoadhesin in Macrophages-Lymphocyte Binding. The lymphocyte binding pattern correlated very well with the expression of sialoadhesin on macrophages in mouse and rat lymphoid tissues (Figs. 1 and 2). To test whether sialoadhesin was responsible for the interaction with lymphocytes, the effects of several conditions that are known to interfere with the receptor activity were tested in the binding assay (Table 1). Preincubation of mouse tissue sections with mAb SER-4 completely blocked binding of TK-1 cells to the macrophages in the splenic marginal zone (Fig. 4) as well as those in the lymph node subcapsular sinus and medulla. The same was observed when another antisialoadhesin mAb, 3D6, or its $F(ab')_2$ fragments were used. The mAbs MOMA-1 or ERTR-9, which recognize marginal zone macrophages or marginal metallophilic macrophages, respectively, had no effect on lymphocyte binding. In the rat anti-sialoadhesin mAb

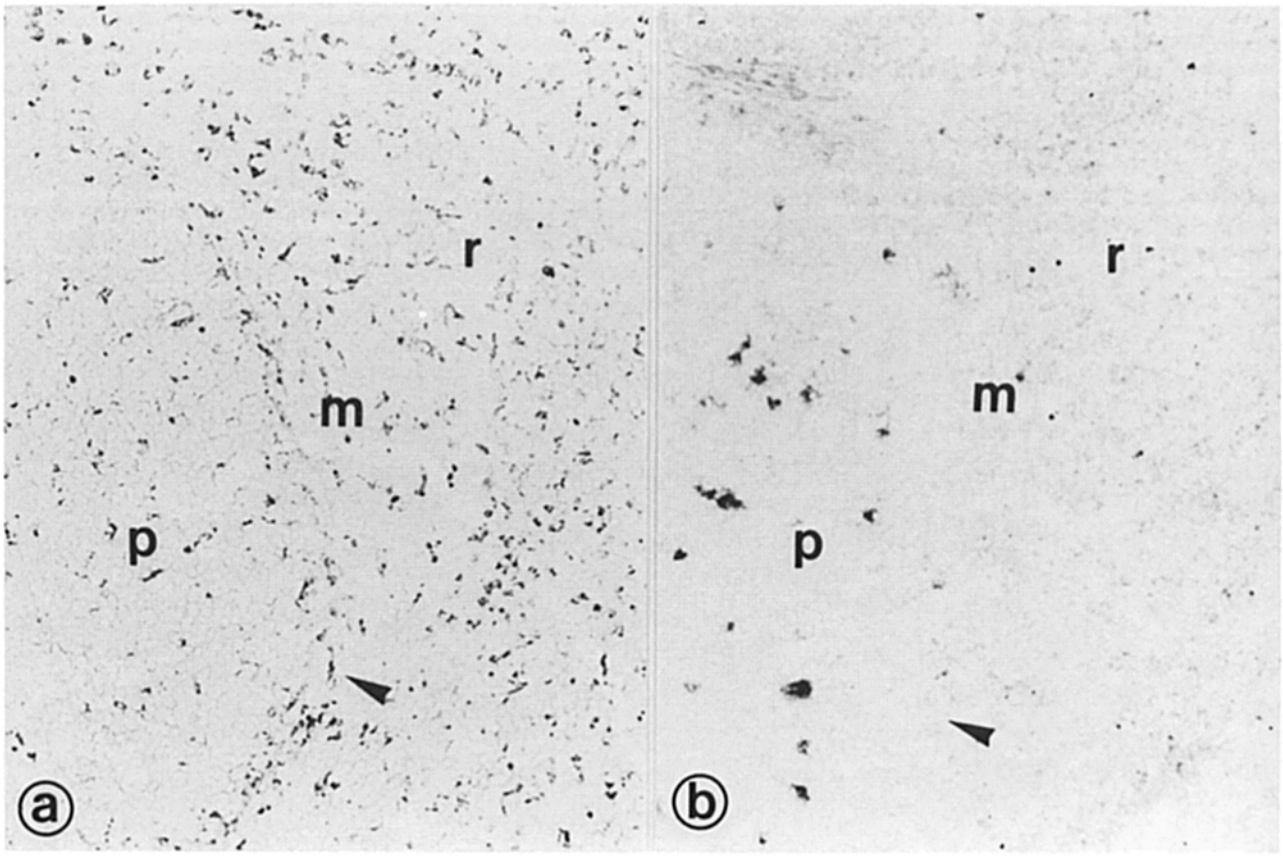

Figure 3. Macrophage elimination from mouse spleen using Cl_2MDP-encapsulated liposomes. Mice were injected with 200 μl of liposomes containing PBS (*a*) or Cl_2MDP (*b*). Spleens were removed after 3 d, and the presence of macrophages was analyzed using acid-phosphatase staining. Cl_2MDP-liposome treatment causes complete removal of methallophils, marginal zone macrophages, and red pulp macrophages. Elimination of white pulp macrophages, which show a swollen appearance, is incomplete. Arrow indicates the border between white pulp and marginal zone. *m*, marginal zone; *p*, periarteriolar lymphocyte sheath; *r*, red pulp.

Table 1. *Effect of Different Conditions on the Interaction of TK-1 Cells with the Splenic Marginal Zone and Lymph Node Subcapsular Sinus and Medulla from Mouse and Rat*

	Mouse			Rat		
	MZ	SCS	Med	MZ	SCS	Med
Control*	++‡	++	++	++	++	++
Temperature: 4°C	−	−	−	−	−	−
20°C	+	+	++	+	+	+
37°C	++	++	++	++	++	++
EDTA§	++	++	++	++	++	++
Neuraminidase‖	−	−	−	−	−	−
Gangliosides: GM1¶	++	++	++	++	++	++
GD1a	−	−	−	−	−	−
mAb:**SER-4	−	−	−	ND	ND	ND
3D6	−	−	−	ND	ND	ND
3D6 $F(ab')_2$	−	−	−	ND	ND	ND
MOMA-1	++	++	++	ND	ND	ND
ED3	ND	ND	ND	−	−	−
ED16	ND	ND	ND	−	−	−
ED9	ND	ND	ND	++	++	++

* Performed at 37°C in medium containing 5% FCS.
‡ ++, many cells binding; +, small number of cells binding: −, no cells binding.
§ In the presence of 2 mM EDTA.
‖ Pretreatment of TK-1 cells with 4 mU/ml neuraminidase *(Vibrio cholerae)* for 30' at 37°C.
¶ In the presence of 100 μM ganglioside.
** Pretreatment of sections for 30 min at room temperature.

ED3 and ED16 were inhibitory, whereas the control mAb ED9 had no effect.

The characteristics of lymphocyte binding to spleen and lymph node sections (Table 1) were consistent with those previously described for sialoadhesin (4, 8). These included the lack of requirement for divalent cations and dependency of sialic acid on the lymphocyte surface for binding. Potent inhibition was observed when the disialylated ganglioside GD1a (100 μM) was added during binding. The effect was specific, since it did not occur when similar concentrations of the structurally related monosialylated ganglioside GM1 were used. The only difference was the temperature dependence, since virtually no binding of lymphocytes occurred at 4°C, in contrast to sheep erythrocytes (4, 8). Although Table 1 only shows the data for TK-1 cells, the results of blocking experiments with antibodies and gangliosides are representative for other lymphocyte populations as well (not shown), including: mouse thymocytes, T cells, T blasts, B cells, and B blasts, for binding to mouse tissues; and rat T cells and T blasts for binding to rat tissues.

The binding of lymphocytes to the HEV and follicles appeared to be mediated by different mechanisms, since binding to these sites was unaffected by antisialoadhesin antibodies. The involvement of other adhesin molecules was studied in antibody blocking experiments. Preincubation of lymphocytes with anti-LFA-1, MEL-14 (recognizing the peripheral homing receptor L-selectin), or anti-LPAM-1 (recognizing the mucosal homing receptor) did not affect their binding to the sialoadhesin-positive locations. At the same time MEL-14 blocked adhesion to popliteal lymph node HEV, while anti-LPAM-1 strongly inhibited binding to Peyer's patch (PP) HEV. The latter mAb also prevented adhesion of lymphocytes to PP follicles.

Characterization of Mouse and Rat Sialoadhesin. To compare the nature of the mouse and rat sialoadhesin, lysates of mesenteric lymph nodes from both species were subjected to SDS-PAGE followed by Western blotting (Fig. 5). In both mouse and rat, an affinity-purified rabbit polyclonal antisialoadhesin antibody detected a major single band migrating at 175 kD (nonreduced) or 185 kD (reduced). These data are consistent with previous observations for mouse sialoadhesin using mAb SER-4 (2, 6).

Binding of Different Leukocyte Populations to Sialoadhesin-positive Macrophages. To compare the binding capacity of different cell populations, a method was employed in which TK-1 cells were used as an internal standard. TK-1 cells were labeled with TRITC, mixed 1:1 with cell populations of interest, and the ratio of unlabeled cells vs. labeled cells binding in marginal zone or subcapsular sinus was determined by counting.

As can be seen in Fig. 6, TRITC labeling did not alter the binding by TK-1 cells, since mixing of labeled and unlabeled TK-1 cells resulted in values close to 1. Compared with the other populations of lymphocytes, TK-1 cells showed superior binding. However, relatively small differences were observed among thymocytes, T cells, Con A–stimulated T blasts, B cells, and LPS-stimulated B blasts. Peritoneal macrophages or dendritic cells showed very little or no binding.

Binding of Purified Sialoadhesin to Different Leukocyte Populations. It was important to obtain direct proof that sialoadhesin alone could mediate the observed specificity in the adhesion of lymphocyte populations. For this purpose, binding of purified radiolabeled receptor to the cell populations was determined. Binding was specific since >95% could be inhibited in the presence of 3D6 $F(ab')_2$ fragments. The specificity was also demonstrated by the strict dependency of binding on sialic acid, since neuraminidase pretreatment of cells reduced binding by >90% in every population examined (data not shown). For all cells investigated, higher binding was observed when the number of cells in the assay was increased fivefold. However, striking differences between the cell populations were found when the number of cells required to bind a given amount of sialoadhesin were compared (Fig. 7). As reference, human erythrocytes that bind high amounts of sialoadhesin were used. In agreement with the results from the frozen section assay, highest binding of sialoadhesin was observed using TK-1 cells. Within the T cell lineage, highest binding was observed with Con A–stimulated T cells, followed by freshly isolated T cells, whereas thymocytes showed relatively low binding. In contrast, binding of B cells and

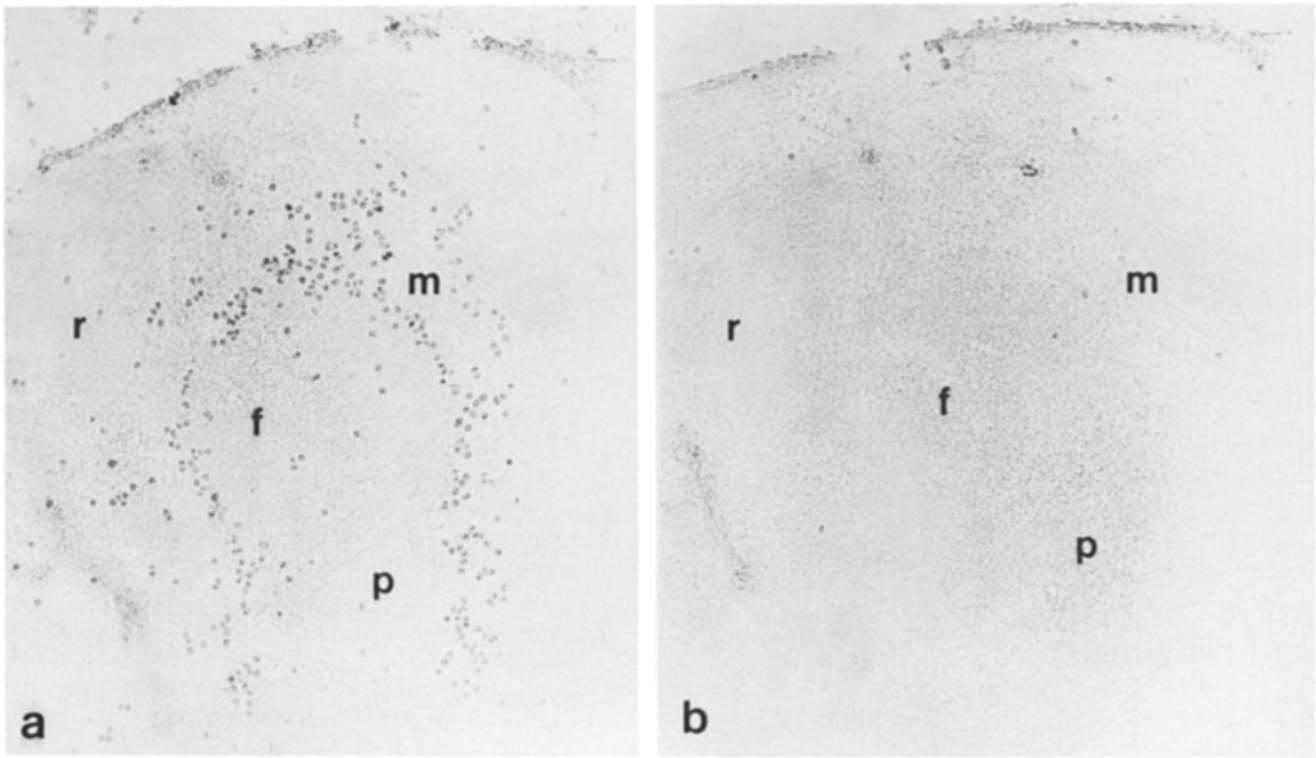

Figure 4. Adhesion of TK-1 cells to the marginal zone is blocked by preincubation with SER-4 mAb. Adjacent mouse spleen sections were incubated with control mAb MOMA-1 (*a*) or with SER-4 (*b*), and were subsequently tested for binding of TK-1 cells. Normal binding is observed in control sections, whereas binding to the marginal zone is absent after preincubation with SER-4.

B blasts was almost identical. Peritoneal macrophages displayed relatively little binding, and only minimal binding was observed for mouse erythrocytes that exhibited up to ~100-fold less binding than human erythrocytes.

Discussion

In this study we describe the characterization of the macrophage receptor, sialoadhesin, as an adhesion molecule for lymphocytes. The initial evidence for this was obtained using a frozen section adhesion assay in which it was demonstrated:

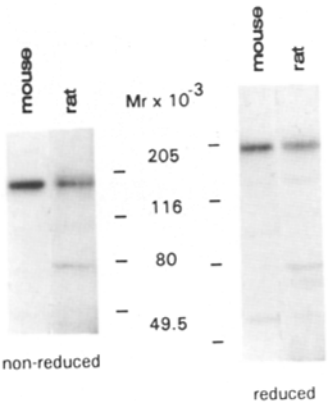

Figure 5. Molecular characterization of mouse and rat sialoadhesin. Octylglucoside lysates of lymph nodes from both species were subjected to SDS-PAGE followed by Western blotting. Blots were probed with an affinity-purified rabbit antisialoadhesin antiserum and ^{125}I-horse F(ab')$_2$ anti-rabbit IgG. Mouse and rat sialoadhesin have a similar size, migrating at 175 kD (nonreduced) or 185 kD (reduced).

(*a*) that lymphocytes bind to the sialoadhesin-positive macrophage populations in spleen and lymph nodes; and (*b*) that this interaction could be blocked specifically by antisialoadhesin mAbs, GD1a, and by neuraminidase treatment of lymphocytes. Direct evidence that sialoadhesin can function as a lymphocyte adhesion molecule was obtained in cell binding assays with purified receptor. The binding mechanism is very similar to the one previously described for the binding of sheep erythrocytes to mouse and rat macrophages (4, 8).

The conditions used in our assay also allowed adhesion of lymphocytes to other sites, namely the follicles and HEV. However, antibody blocking experiments clearly demonstrated that these interactions involve other receptor systems, and this is consistent with previous findings (29–31). Our experiments also suggested that adhesion molecules expressed by lymphocytes such as LFA-1, VLA-4, and L-selectin do not function as ligands for sialoadhesin.

The finding that mouse cells adhere to the putative rat sialoadhesin molecule and vice versa demonstrates that the binding mechanism is conserved between these species. In the mouse, sialoadhesin has been characterized as a 175-kD (nonreduced) or 185-kD (reduced) lectin-like surface molecule (2, 6). We have recently characterized the rat equivalent of sialoadhesin in an erythrocyte binding assay (8), and the present results demonstrate that the rat sialoadhesin is similar in size, providing strong additional evidence for the existence of a sialoadhesin homologue in the rat.

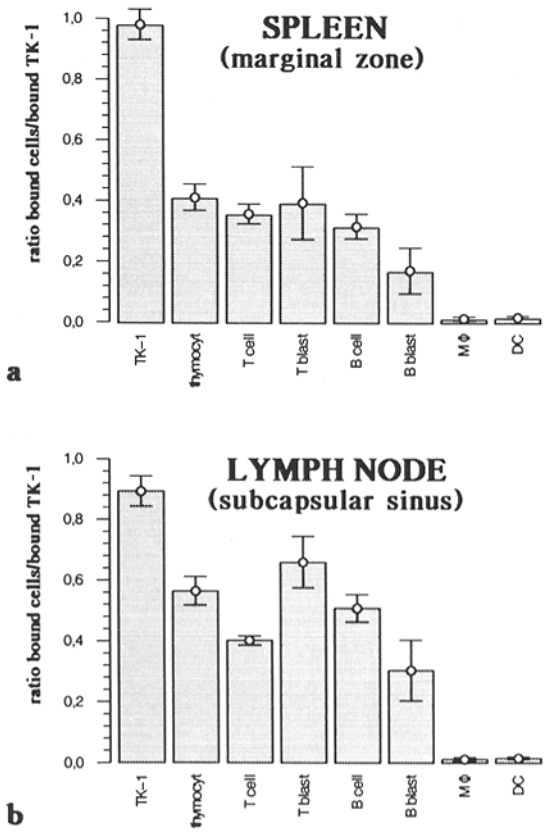

Figure 6. Binding of different mouse cell types to sialoadhesin-positive macrophages in spleen and lymph node sections. Each cell population was mixed 1:1 with TRITC-labeled TK-1 cells. After adhesion to cryostat sections, the ratio of unlabeled vs. labeled cells was determined by counting at least 500 cells. The results are expressed as the mean ± SD of three experiments.

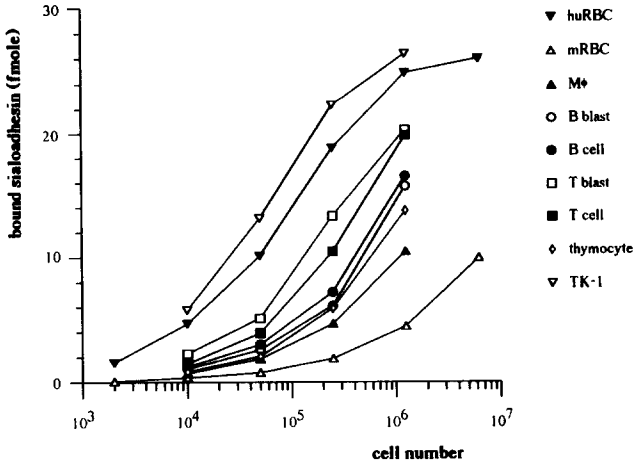

Figure 7. Binding of purified ^{125}I-sialoadhesin to different mouse cell populations. Different numbers of glutaraldehyde-fixed cells were incubated with 160 fmol of ^{125}I-sialoadhesin, washed, and cell-bound sialoadhesin was determined by counting. Binding in the presence of 3D6 F(ab')$_2$ fragments was measured at every point, subtracted from the values shown, and never exceeded 5% of the binding in its absence. Data shown are from a single representative experiment of four.

It has recently been shown that sialoadhesin preferentially recognizes the oligosaccharide sequence Neu5Acα2,3Galβ1, 3GalNAc in either sialoglycoproteins or gangliosides (2). However, the nature of the cell surface molecules that act as ligands for sialoadhesin have not been identified. Both erythrocytes and lymphocytes are able to bind to sialoadhesin, but they differ with respect to their temperature dependence; erythrocytes bind at 4°C (4, 8), whereas lymphocytes do not (this study) Whether this is due to the nature of the ligands on the two cell types or other differences is not known at present.

In this study we have demonstrated with two essentially different methods that sialoadhesin binds both B and T lymphocytes. Lymphocytes bind to sialoadhesin much better then peritoneal macrophages and dendritic cells. High binding of sialoadhesin to neutrophils has been shown in an independent study (Crocker et al., manuscript in preparation). An interesting finding was the high levels of binding of TK-1 lymphoma cells and some other lymphoma cell lines (our unpublished observation). Whether the presence of high level of sialoadhesin ligands is related to the tumorigenic nature of these cells remains to be established. Interestingly, we observed increasing activity for sialoadhesin in the order: thymocytes < T cells < activated T cells in the assay using the purified receptor, indicating that adhesion may vary with maturation stage in this lineage. However, these differences were less clear in the frozen section assay. One possible explanation would be that in this assay a minor population of cells with higher affinity are selected by sialoadhesin. For example, recent studies demonstrated selective binding of certain memory T cells through surface glycoconjugates by E-selectin (32, 33). However, it cannot be excluded that besides density and affinity of the ligands for sialoadhesin, other factors like membrane motility, cytoskeletal interactions, cell size/shape, or other adhesive mechanism could influence binding. The contribution of these factors could depend on experimental conditions of the assay used.

Although we have shown in this study that sialoadhesin can function as a lymphocyte adhesion molecule, the significance of this adhesive interaction between lymphocytes and macrophages is not clear. Under normal conditions, the expression of sialoadhesin is restricted to distinct subpopulations of macrophages in lymphoid tissues. The presence of sialoadhesin on medullary macrophages and marginal metallophils suggests that the receptor may be involved in macrophage adhesive functions. Furthermore, since macrophages expressing sialoadhesin are Ia negative, it seems unlikely that the function of sialoadhesin is directly related to antigen presentation (3, 34). The lymph node medulla and the outer part of the splenic periarteriolar lymphocyte sheath are considered to be important microenvironments for the maturation of antibody-forming cells, and intimate associations between B cells and local macrophages have been observed in situ (34). Sialoadhesin could contribute to those interactions and thereby play a role in B cell activation. In addition, because the splenic marginal zone functions as the main port of entry for recirculating lymphocytes, it is plausible that

sialoadhesin plays a role in the recruitment of lymphocytes into the white pulp. In this case sialoadhesin could be considered as a splenic equivalent of the so-called "adressins" expressed by high endothelial cells in, e.g., lymph nodes, which mediate the entry of lymphocytes. Possibly, this recruitment could be restricted to certain lymphocyte subpopulations. In conclusion, we have identified sialoadhesin as a lymphocyte adhesion molecule. Its highly restricted distribution makes sialoadhesin the first example of a macrophage-specific lymphocyte adhesion molecule.

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