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Reversible binding of sialidase-treated rat lymphocytes by homologous peritoneal macrophages*

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ABSTRACT

After sialidase treatment, lymphocytes disappear from the blood stream, but reappear after a few hours. The behavior of sialidase-treated rat lymphocytes was investigated by *in vitro* binding studies with homologous peritoneal macrophages. A lymphocyte mixture from thymus and spleen was treated with sialidase and cultured up to 55 h, and at various times, the binding of the lymphocytes to glass-adherent macrophages was studied by light and electron microscopy; vital lymphocytes were only bound but not phagocytosed, and the interaction with macrophages was inhibited by D-galactose. During culture of lymphocytes, either separately or with macrophages, the binding was more and more reduced, and a second sialidase treatment of cultured lymphocytes led again to increased binding which could be inhibited by D-galactose. This change did not occur in the presence of *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid, an inhibitor of sialidases, showing the sialic acid specificity of this phenomenon. Thus, the reversibility of lymphocyte binding could be explained by resynthesis of cell surface sialic acids.

INTRODUCTION

Lymphocytes are cells of the immune system that circulate from blood to lymph, "home" into defined areas of lymphoid organs, and recirculate into the blood stream. *In vivo* studies by Woodruff and Gesner[1,2] demonstrated that sialidase-treated and retransfused rat T-lymphocytes do not migrate into lymph nodes, but are trapped by liver and spleen; however, after several hours they reappear in the circulation. The same behavior was observed with antigen-specific and sialidase-treated mouse T-lymphocytes[3]. In contrast, sialidase treatment of rat erythrocytes leads to an irreversible sequestration from the blood stream as a consequence of phagocytosis by liver and spleen macrophages[4].

In vitro studies have shown that sialidase-treated lymphocytes are bound by homologous hepatocytes and Kupffer cells as well as by peritoneal macrophages[5,6]. These interactions were found to be mediated by D-galactose-specific receptor molecules isolated from the surface of the macrophages[6–8], and it was concluded that this receptor is involved in the unusual "homing" of sialidase-treated lymphocytes[1–3]. The release of the trapped lymphocytes into circulation was only assumed[1–3], but never proved, to be due to the resynthesis of sialid acids. Consequently, we used peritoneal macrophages as they are easily available and express well the D-galactose lectin.

* Dedicated to Professors Nathan Sharon and Toshiaki Osawa.

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EXPERIMENTAL

Materials. — Medium RPMI 1640 and L-glutamine were obtained from Biochrom (D-1000 Berlin); fetal calf serum, Hepes [4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid], and *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid from Boehringer (D-6800 Mannheim); the sodium salt of EDTA, 2-mercaptoethanol, Trypan Blue, glutardialdehyde for electron microscopy, and pararosaniline from Merck (D-6100 Darmstadt); penicillin, streptomycin, sodium diatrizoate, D-galactose, $NaHCO_3$, and α-naphthyl acetate from Sigma Chemical Co. (D-8024 Deisenhofen). Sialidase of *Vibrio cholerae* (specific activity 20 U/mg protein) was obtained from Behringwerke (D-3550 Marburg) and density gradient medium Ficoll 100 from Pharmacia (D-7800 Freiburg). Male Wistar rats weighing 180–220 g were obtained from the animal house of the University of Kiel.

Isolation of lymphocytes. — Lymphocytes were isolated and handled under sterile conditions at 4° by use of the procedure described by Jibril *et al.*[6], which was slightly modified. The cells used for the experiments consisted of B- and T-lymphocytes and were a mixture obtained from thymus and spleen. Thymus was cut into 2–3-mm fragments in a small volume of isolation medium containing RPMI 1640 (10.39 g), $NaHCO_3$ (2 g), Hepes (5 g), and water per 1000 mL, pH 7.4; it was sterilized by filtration, and 1% each of penicillin and streptomycin were added before use. Spleen, put into a small volume of isolation medium, was pressed out with two blunt tweezers. The disintegrated tissues were filtered under stirring through a nylon gauze (100 μm) with ~ 500 mL of isolation medium. The cells in the filtrate were washed once with NaCl–phosphate buffer (2.7mM KCl, 8.1mM Na_2HPO_4, and 1.5mM KH_2PO_4, pH 7.2, sterilized in the autoclave) by centrifugation at 250*g* for 10 min. The pellet was resuspended in NaCl–phosphate buffer (30 mL) containing 0.26mM EDTA and layered on the top of 20 mL of a density-gradient medium (8.3% Ficoll 400 and 13.11% sodium diatrizoate in water; *d* 1090 g/L) according to Jibril *et al.*[6] After centrifugation at 800*g* for 25 min at 23°, the cells at the interphase were collected and washed twice with cold isolation medium as described above. Monocytes–macrophages were removed by incubation of the cell suspension (5×10^6 cells/mL of medium) in glass vessels at 37° for 1 h in humidified air containing 5% CO_2, allowing the cells to adhere to the glass surface. The lymphocytes were separated from the monocytes–macrophages by careful stirring of the glass vessels in ice for 5 min. The lymphocytes were then collected from the supernatant by centrifugation and characterized in air-dried smears which were stained according to Löffler[9] (see below). Vital lymphocytes were those which did not take up Trypan Blue.

Sialidase treatment of lymphocytes. — Lymphocytes (10^8) were incubated according to Jibril *et al.*[6] with *Vibrio cholerae* sialidase (EC 3.1.1.18) (50 mU) in phosphate buffer (1 mL), pH 7.4, for 1 h at 37° in a humidified atmosphere containing 5% CO_2, and centrifuged at 250*g* for 10 min at 4°, followed by washing (3 times) in phosphate buffer (50 mL each). This sialidase treatment was carried out at the beginning of the experiment with freshly isolated lymphocytes, and in some cases for a second time, after the

sialidase-treated lymphocytes had been incubated for various times. In a parallel experiment, to test the specificity of the sialidase, a mM solution of the sialidase inhibitor *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid (50 μL) was added to a lymphocyte suspension in NaCl–phosphate buffer (450 μL; 10^8 lymphocytes/mL) which included 25 mU of sialidase. In the control, the inhibitor was omitted and NaCl–phosphate buffer (50 μL) was added instead. Incubation and washing were performed as described above.

Culture of lymphocytes. — When used for culture, sialidase-treated and control lymphocytes (10^6 cells/mL) were suspended in a culture medium (pH 7.4) of the following composition: Medium RPMI 1640 (10.39 g), $NaHCO_3$ (2 g), Hepes (5 g), 2-mercaptoethanol (1:300, diluted with NaCl–phosphate buffer; 2.5 mL), 200mM L-glutamine (10 mL), and inactivated fetal calf serum (100 mL) were diluted with water to 1000 mL, and sterilized by filtration; penicillin and streptomycin were added to a 1% concentration to the medium before use. The cells were incubated in culture flasks (200 mL; NUNC, D-6200 Wiesbaden) for a maximum time of 55 h at 37° in humidified air containing 5% CO_2. After each 6–8 h period, lymphocytes were collected and centrifuged off (10 min, 250*g*, room temperature), followed by resuspension in fresh culture medium (10^6 cells/mL), and the culture was continued as just described.

Isolation and culture of macrophages. — Rat peritoneal macrophages were prepared under sterile conditions as described earlier[10]. Cells were transferred into silicon chambers (5×10^5 cells/chamber) of flexiperm (Heraeus, D-6450 Hanau), mounted on slides, and incubated in isolation medium (200 μL) at 37° for 1 h in humidified air containing 5% CO_2. Nonadherent cells were removed by washing twice with cold isolation medium. These glass-adherent macrophages were cultured up to 55 h in the silicon chamber system with culture medium (0.5 mL/chamber) under the same conditions as lymphocytes.

Binding assays with lymphocytes and macrophages. — The number of sialidase-treated and control lymphocytes bound by homologous macrophages was determined with cells either freshly isolated or incubated for various times. Freshly prepared or cultured sialidase-treated or control lymphocytes (10^6) in isolation medium (100 μL) were added to adherent, freshly isolated or cultured macrophages (see above) in isolation medium (200 μL) in each chamber. The D-galactose specificity of the interaction was ascertained by the addition of 30mM D-galactose to the medium. In the case of cultured lymphocytes, the cells were harvested from the culture medium and washed twice with NaCl–phosphate buffer by centrifugation at 250*g* for 10 min at room temperature. When cultured macrophages were used, they were washed twice with NaCl–phosphate buffer before addition of the lymphocytes. After 1 h of incubation (37°, humidified air, 5% CO_2), the unbound lymphocytes were removed by two washes with NaCl–phosphate buffer (1 mL/chamber). The remaining cells were fixed by the addition of 0.25% glutardialdehyde in NaCl–phosphate buffer (100 μL) to each chamber and incubation for 2 h at room temperature, and then the flexiperm was removed and the slide washed with 50% aqueous ethanol. The cells were stained for acid esterase with α-naphthyl acetate as substrate and counterstained with hematoxyline (Medite, D-3167 Burgdorf) according to Löffler[9]. Binding of dark-brown stained lymphocytes to

red-brown macrophages was observed with a photomicroscope from Zeiss (D-7082 Oberkochen) at 750-fold magnification. The number of lymphocytes bound or phagocytosed by 100 macrophages was counted in a representative area.

Coculture experiments. — When lymphocytes were cultured together with macrophages, sialidase-treated or control lymphocytes (10^6 in 100 μL of culture medium) were added to all chambers of the flexiperm slides (see above) containing freshly isolated and glass-adherent macrophages (5×10^5 in 200 μL of culture medium each). Each chamber was filled with culture medium (300 μL) which was then incubated up to 55 h. To test the D-galactose specificity of this interaction, 30mM D-galactose was added to the medium. After 6–8-h intervals of culture time, one slide was taken from the culture, the cells were washed twice with NaCl–phosphate buffer (1 mL) to remove unbound lymphocytes, fixed, and stained in the same way as described above. The medium of the remaining incubation mixture was changed by washing with isolation medium and adding fresh culture medium (600 μL for each). Further culturing was performed as described earlier.

Scanning-electron microscopy. — For electron microscopy, the binding assays were carried out on glass coverslips placed on the bottoms of a multi vial (NUNC, D-6200 Wiesbaden). After the interaction of macrophages with lymphocytes, the cells were fixed with 2.5% glutardialdehyde in NaCl–phosphate buffer for 2 h and washed three times with NaCl–phosphate buffer, followed by dehydration with aqueous ethanol at increasing concentrations (30, 50, 70, and 100% for 10 min each). The cells were treated with 2:1, 1:1, and 1:2 ethanol–Frigen, followed by two times pure Frigen for 30 min each, and finally pure Frigen for 24 h. The next step was "critical point"-drying (Critical point dryer, SPC-900/Ex), and finally the cells were coated three times with gold for 1.5 min each in a Mini Coater (Commonwealth Scientific). The coverslips were examined with a Cambridge Stereoscan S4-10 scanning microscope.

Transmission-electron microscopy. — The binding assays and glutardialdehyde fixation were carried out in micro vials as described for scanning-electron microscopy. The cells were subsequently fixed in 2% OsO_4 in water for 1 h, followed by three washes with NaCl–phosphate buffer and dehydration with aqueous ethanol at increasing concentrations of 30, 50, 70, and 100% for 10 min each. Thereafter, the preparations were treated with 1:1 ethanol–Araldite containing 3% accelerator (Ciba–Geigy, D-7867 Wehr) for 30 min, then embedded in pure Araldite with 2% accelerator, followed by polymerization at 37° for 4 days and at 65° during the last 4 h. The Araldite blocks were removed from the vials and cut into ultrathin sections, which were then stained with uranyl acetate and lead citrate[11]. The sections were examined with a Siemens Elmiscope 101 electron microscope.

RESULTS

By use of methods described previously for the isolation of lymphocytes, about 8×10^8 cells were obtained per rat. Lymphocytes composed 80–90% of these cells, 60% from thymus and 40% from spleen, more than 90% being viable. The other cells were macrophages–monocytes, granulocytes, and erythrocytes.

Fig. 1. Light microscopy of a rosette of freshly isolated, sialidase-treated rat lymphocytes bound by a peritoneal macrophage. After staining as described in the Experimental section, the macrophages appear as red-brown cells showing a distribution of esterase activity in the whole cytoplasm, whereas lymphocytes are stained dark brown.

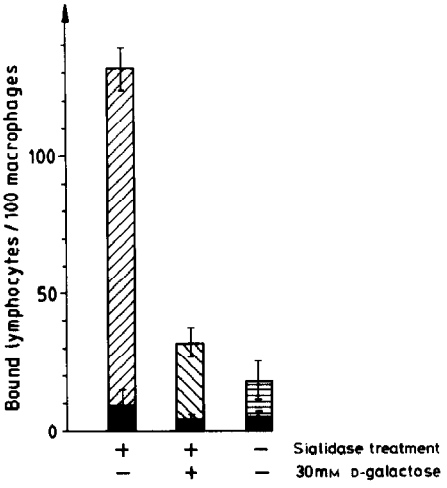

Fig. 2. Binding of freshly isolated rat lymphocytes to homologous peritoneal macrophages to phagocytosis: ▨, Number of bound lymphocytes after sialidase treatment; ▧, number of bound sialidase-treated lymphocytes in the presence of 30mM D-galactose ("non-D-galactose-specific binding"); ▤, number of bound control lymphocytes ("unspecific binding"); and ■, number of phagocytosed lymphocytes. The values are means of five experiments each.

By light microscopy, macrophages appeared as intensively stained red-brown cells with a homogenous distribution of esterase activity in the whole cytoplasm, whereas lymphocytes showed no esterase activity and were counterstained dark brown. Cells were considered to be phagocytosed when surrounded by macrophage cytoplasm. Sialidase-treated lymphocytes formed rosettes with macrophages (Fig. 1), whereas untreated lymphocytes adhered to macrophages in much smaller amounts. Fig. 2 showed that, under the experimental conditions used in these studies, about 130 sialidase-treated lymphocytes and about 20 untreated lymphocytes were bound by 100 peritoneal macrophages. The attachment of sialidase-treated lymphocytes was much reduced (to about 30 lymphocytes bound by 100 macrophages) in the presence of 30mM D-galactose (Fig. 2), thus proving the D-galactose specificity of this binding, in accordance with earlier observations made by Jibril *et al.*[6] The number of sialidase-treated, control, and noninteracting-specifically with D-galactose lymphocytes phagocytosed by macrophages was maximally 10 cells per 100 macrophages (Fig. 2).

Scanning-electron microscopy showed that intact sialidase-treated lymphocytes were simply bound without deformation of their cell shape. The binding to the macrophages occurred either by extended (Fig. 3a) or more point-like (Fig. 3b) contact zones. These two types of contact zones were verified by transmission-electron microscopy, as first observed by Jibril *et al.*[6] The morphological features of the lymphocytes changed neither after sialidase treatment nor during culture. Remarkably, only lymphocytes showing signs of destruction, such as a damaged membrane or loss of cytoplasm, were phagocytosed.

Reversibility of binding of sialidase-treated lymphocytes by macrophages. — Two types of experiments were carried out in order to study the interaction of sialidase-treated lymphocytes with macrophages during longer incubation times. Firstly, sialidase-treated and control lymphocytes were kept in culture and mixed, after various times, with macrophages either freshly isolated or cultured in parallel. Secondly,

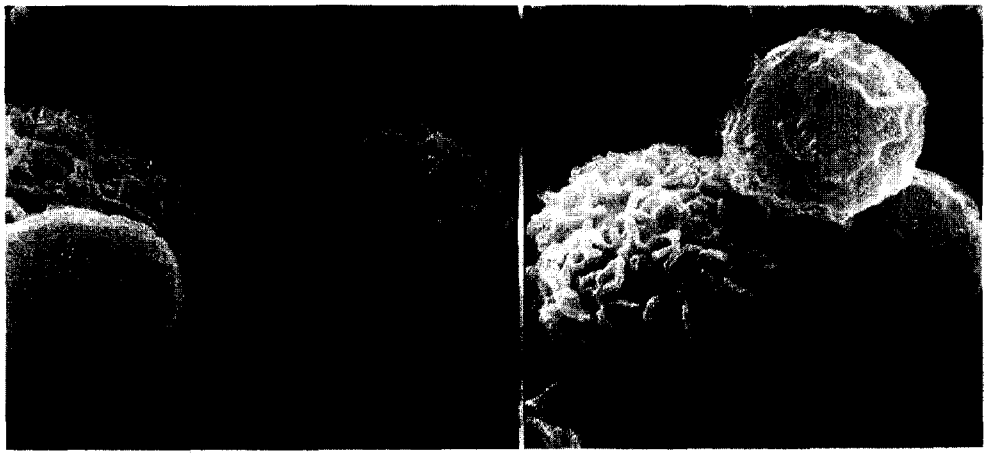

Fig. 3. Scanning-electron microscopy of cultured sialidase-treated lymphocytes (L) bound by a macrophage (M): (a) The binding occurred either by an extended contact zone or (b) in a more point-like manner by microvilli. In both cases lymphocytes were bound without deformation of their cell shape.

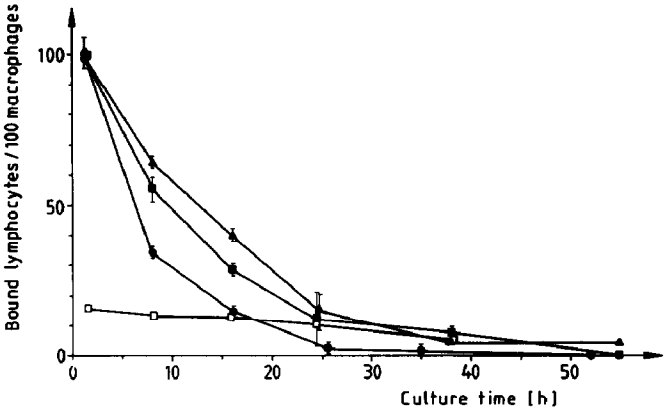

Fig. 4. Decrease of the D-galactose-specific binding of cultured sialidase-treated lymphocytes to homologous rat peritoneal macrophages *vs.* duration of incubation: The macrophages were either freshly prepared for each test (■) or cultured in parallel (▲) before the addition of cultured lymphocytes, or they were cultured together with sialidase-treated lymphocytes (●). In all experiments, the number of bound control lymphocytes remained nearly constant, corresponding to the binding of cultured control lymphocytes to macrophages freshly prepared for each test (□). The values are means of three experiments each.

lymphocytes were cultured together with macrophages and the number of bound cells was quantitatively determined at each time point. Fig. 4 showed that the ability of cultured sialidase-treated lymphocytes to adhere to macrophages decreases during incubation, leading to a 50% reduction of the D-galactose-specific binding by freshly isolated macrophages after 10 h and by separately cultured macrophages after 13 h of incubation. When cultured together, 50% of the sialidase-treated lymphocytes were found to be dissociated from the macrophages already after 7 h of culture. After 26 h, the D-galactose-specific binding of lymphocytes had disappeared in this and, in the other types of culture, after 55 h. In all tests, the number of bound control lymphocytes remained about the same as that of freshly isolated control lymphocytes (Fig. 4).

The experiments suggested that the lower extent of binding correlates with a decrease in D-galactose-specific interactions during incubation, because the residual binding capacities could be reduced almost to the level of control-lymphocyte binding with free galactose at any time and in all types of experiments. The degree of phagocytosis of sialidase-treated, control, and noninteracting-specifically with D-galactose lymphocytes was similar (on average 13 cells/100 macrophages) in all experiments and did not increase significantly in the course of culture. The decrease of binding was not due to a loss of receptors on the macrophage membrane, because the D-galactose-specific interaction of freshly isolated and sialidase-treated lymphocytes with separately cultured macrophages did not change during culture of the macrophages (Fig. 5).

Effect of sialidase on cultured sialidase-treated lymphocytes. — A second sialidase treatment of once sialidase-treated lymphocytes after various times of culture (8, 24.5, and 38 h) restored most of the original extent of binding, especially after the shorter incubation times indicated (100, 77, and 58%, respectively; for example, see Fig. 6). Sialidase treatment of control lymphocytes after this time of incubation also resulted in

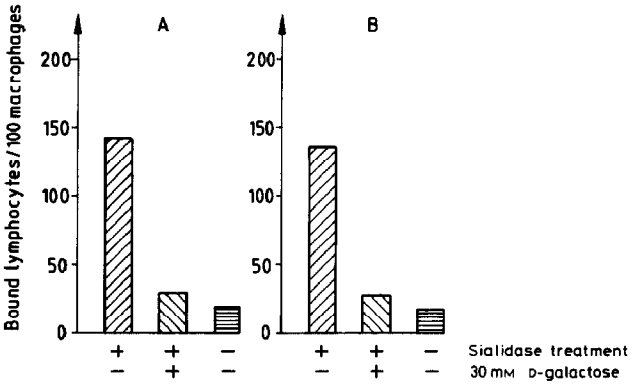

Fig. 5. Comparison of the capacity of freshly isolated (A) and cultured (24 h) macrophages (B) to bind freshly prepared lymphocytes: ▨, Number of bound lymphocytes after sialidase treatment; ▧, number of bound sialidase-treated lymphocytes in the presence of 30mM D-galactose; and ▤, number of bound control lymphocytes. The values are means from three incubations each.

a marked increase of binding to macrophages (96, 77, and 58%, respectively). When this enzyme treatment was performed in the presence of a 1mM concentration of the sialidase inhibitor, *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid, no increase of binding of sialidase-treated or of control lymphocytes occurred.

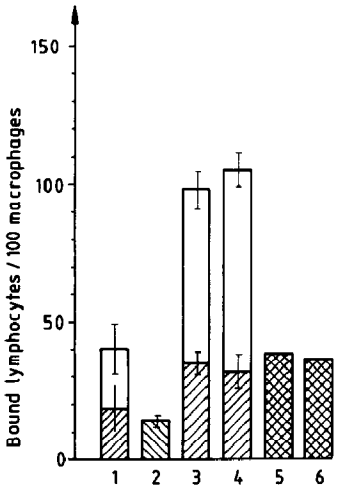

Fig. 6. Influence of a second sialidase treatment on the binding of sialidase-treated and control lymphocytes that had already been cultured for 24.5 h. Binding of: (1) Sialidase-treated lymphocytes, (2) control lymphocytes, (3) sialidase-treated lymphocytes after a second sialidase treatment, (4) control lymphocytes after sialidase treatment, (5) sialidase-treated lymphocytes after a second sialidase treatment in the presence of the inhibitor, *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid, and (6) control lymphocytes after sialidase treatment in the presence of *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid. The part of D-galactose specificity of the interaction in (1), (3), and (4) in the presence of D-galactose is symbolized by (□); the part of non-D-galactose-specific binding is symbolized by (▨). The values are means of three experiments (1–4) each, and one experiment each (5 and 6), respectively. Each experiment consisted of three incubation mixtures. The experimental details are described in the Experimental section.

DISCUSSION

The enzymic cleavage of sialic acids from lymphocytes leads to their sequestration from circulation and to selective trapping by liver and spleen, because the demasked cells are considered to be recognized as "foreign" or "non-self"[1-3,12]. After 24–48 h, these lymphocytes reappear in the blood stream. The present *in vitro* studies have shown the validity of the assumption of Woodruff and Gesner[1,2], and Kaufmann *et al.*[3] that this phenomenon is due to the restoration of cell surface sialic acids which decreases the binding of the lymphocytes to macrophages. As described by Jibril *et al.*[6] and also observed in the present study, about 3.3 nmol of sialic acids can be released by 50 mU of sialidase from 10^8 lymphocytes, corresponding to 30% of the total amount of surface sialic acids. Thus, D-galactose and *N*-acetyl-D-galactosamine units that serve as recognition determinants for the D-galactose receptor on peritoneal macrophages are exposed on sialidase-treated lymphocytes, as earlier *in vitro* studies with peritoneal macrophages[6] and Kupffer cells[5,12] had shown, and as the present assays with D-galactose as inhibitor of this interaction have ascertained. Since intact lymphocytes are only bound and not phagocytosed, in contrast to damaged cells, the question arose about the mechanism hindering their phagocytosis. When compared to erythrocytes, which are phagocytosed after binding[4,10,13], this difference may be due to membrane flexibility. A reason for this rigidity may be that the ligands are able to move in the membrane, as is known from different nucleated cells[14]. This clustering of the membrane molecules under the influence of macrophage receptors could prevent the deformation and phagocytosis of lymphocytes.

Lymphocytes are nucleated cells that are able to metabolize their membrane components, including sialic acids[15]. Thus, another reason for the absence of phagocytosis of lymphocytes may be the capacity of these cells to rapidly restore their membrane sialic acids. Indeed, the present results clearly demonstrate the reversibility of D-galactose-specific lymphocyte attachment to macrophages. Since the results are similar, whether lymphocytes are cultured separately or together with macrophages, the capacity of loosing attachment to macrophages must be a property of the lymphocytes. Strong evidence for the ability of lymphocytes to restore their enzymically-removed membrane sialic acids was obtained from lymphocyte binding after a second sialidase treatment. The renewed high amount of bound lymphocytes as the consequence of such a treatment was assumed to be due to the exposure of D-galactose ligands, which had been masked by sialic acid units newly added during incubation. The involvement of D-galactosyl groups also after this second sialidase treatment was confirmed by inhibition of the interaction with D-galactose. The action of sialidase could be inhibited with *N*-acetyl-2,3-didehydro-2-deoxyneuraminic acid, thus giving strong evidence that the decrease of lymphocyte binding was sialic acid-specific and not due to the loss of receptors on the macrophage membrane. Since sialidase treatments of cultured control lymphocytes after various incubation times also led to high-binding rates, the culture conditions applied do not seem to infuence significantly the sialic acid-specific membrane properties of lymphocytes. However, the binding rate of cultured, sialidase-

treated and control lymphocytes decreased when a sialidase treatment was performed after longer incubation times, as compared to freshly isolated cells. This indicated that a part of the lymphocytes had changed their membrane properties during culture, *e.g.*, by shedding or endocytosis of ligands. The observation that, after about 24 h, almost no D-galactose-specific binding of sialidase-treated lymphocytes and almost complete dissociation of these cells from macrophages in coculture experiments occurred is in accordance with the *in vivo* effects that sialidase-treated and retransfused lymphocytes reappear in the normal circulation after about the same time[1-3].

Nothing is known, so far, about the mechanism of resynthesis of sialic acids in rat lymphocyte membranes. Sialidase demasks D-galactosyl groups on the surface of lymphocytes and leads to their binding *via* D-galactose ligands to specific receptors on peritoneal macrophages. These ligands are probably mainly located on sialoglycoproteins according to the analysis of sialoglycoconjugates of mouse T-lymphomas[16], which has shown that sialic acid residues are derived primarily from glycoproteins (93%) with only a small proportion from glycolipids (7%). A major membrane sialoglycoprotein of various white blood cells, leukosialin, was described by Fukuda and Carlsson[17], and it has been shown that the removal of sialic acid from the membrane sialoglycoconjugates induces the *de novo* synthesis of sialoglycoproteins in these T-lymphoma cells[16]. On the other hand, resialylation of membrane asialoglycoproteins may occur *via* an endocytotic pathway by the activity of sialyltransferases that are found preponderantly in the Golgi compartment[18-20], as shown for the specific antigenic determinants of T-lymphocytes[21] and for the transferrin receptors of human erythroleukemia cells[22]. Furthermore, inhibition with pyrimidine antagonists of sialic acid regeneration in leukemic cells showed that the reappearance of cell surface sialic acids correlated with sialyltransferase activity[23]. Thus, it may be assumed that the observed restoration of sialic acids on the rat lymphocyte surface uses a similar mechanism. A third possibility would be the mechanism of plasma membrane resialylation by membrane-bound exosialyltransferase activity that has been described for Hodgkin's cells[24] and thymocytes[25].

The weakening of attachment of D-galactose ligands in lymphocytes to macrophages may be explained by endocytosis, by the lymphocytes themselves, of the ligands reversibly attached to the receptor molecules; the ligands are then replaced by newly synthesized or regenerated sialoglycoconjugates which do not bind to macrophage receptors. Alternately, the whole receptor-ligand complexes may get internalized by the macrophages, which are known to endocytose such complexes with soluble ligands where the receptors recycle and the ligands are lysosomally digested[26-28]. The study of the inhibition by sialic acid of lymphocyte binding to macrophages[29] indicated that the restored sialic acid units may influence the macrophage receptor allosterically, so that the attachment of the remaining D-galactose-specifically bound ligands to the macrophage receptors are more and more weakened. The present results with lymphocytes suggested a model to explain how reversible sialylation mediated by sialidase and sialyltransferase activities may regulate cellular interactions (see Fig. 7). This model of reversibility of interaction between lymphocytes and macrophages may also be applied to similar interactions in other cell systems.

Fig. 7. Model showing the reversible interaction between cells mediated by the action of sialidase and sialyltransferase (model modified after Fig. 13 from Ref. 13): ■, D-galactose, ⬭, sialic acid, ⌴, D-galactose-recognizing receptor, and ⬚, recognized image.

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