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LECTIN-MEDIATED AGGLUTINATION OF MURINE LYMPHOMA CELLS

CELL SURFACE DEFORMABILITY AND REVERSIBILITY OF AGGLUTINATION BY SACCHARIDES

GARTH L. NICOLSON and GEORGE POSTE

Department of Developmental and Cell Biology, University of California, Irvine, CA 92717 and Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, NY 14263 (U.S.A.)

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Summary

Agglutination of S49 mouse lymphoma cells by *Ricinus communis* I agglutinin can be reversed by the competing haptenic saccharide, lactose, soon after agglutination, but after further incubation in the absence of lectin the agglutination reaction could not be reversed by lactose and the cells remained as multicell aggregates. The irreversibility of S49 cell agglutination was time, temperature and lectin concentration dependent and its onset correlated with ultrastructurally observed deformation of adjacent cell surfaces and an increase in the proportion of adjacent cell surface areas in close apposition within multicell aggregates. Pretreatment of S49 cells with cytochalasin B or cytochalasin B plus vinblastine enhanced *R. communis* I agglutinin-mediated agglutination, while vinblastine alone and fluoride plus azide had essentially no effect. When drug-treated cells were agglutinated and then incubated in lectin-free drug-containing media for various times prior to lactose addition, the drug effects were more pronounced. Cytochalasin B alone or with vinblastine inhibited lactose reversal of S49 cell agglutination compared to the drug-free controls, while fluoride plus azide enhanced hapten reversibility. Electron microscopic analysis revealed that the onset of agglutination irreversibility correlated with cell surface deformation in the drug-treated cells. Cell aggregates that were more readily reversible by lactose (fluoride plus azide) were unchanged or less deformed, while S49 aggregates treated with cytochalasin B plus vinblastine

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

were more deformed compared to controls without drugs. These experiments suggest a role for cell surface deformability as an important secondary effect during lectin-mediated cell agglutination of S49 lymphoma cells.

Introduction

Many different types of tumor cells are highly agglutinable by plant lectins which bind to cell surface oligosaccharides [1,2]. Although several general proposals have been published to explain lectin-mediated cell agglutination at the molecular level (reviews: Refs. 2—4), none of these adequately deal with the diverse surface properties displayed by widely different cell types. One aspect of cell agglutination by lectins that is receiving increasing attention in generalized models for the agglutination process is the possible role of secondary, non-lectin-mediated effects occurring after initial lectin-mediated cell aggregation [3]. These secondary effects are seen, for example, when lectin agglutination is not readily reversed by addition of the appropriate sugar hapten. During initial lectin-mediated agglutination, or shortly thereafter, addition of sufficient competing sugar hapten generally produces complete dispersal of the cell aggregates to single cells [5,6]. However, in many systems agglutination is not readily reversible by specific sugar haptens [7—10] suggesting that forces other than those contributed by the carbohydrate-binding specificities of the lectin-bridging molecules are involved in holding cells together within multicell aggregates. There are several possible secondary forces that could contribute to irreversible agglutination: cell surface deformability, cell-cell adhesion, surface charge, hydrophobic interactions of surface molecules on adjacent cells (reviewed in Refs. 2—4,10,11) and undoubtedly others as well. Cell surface deformability has been proposed as an essential secondary force in determining stability of agglutination in certain studies [12—14], while in other systems the formation of cell-to-cell adhesions seems to be important [9,10,15—17].

In the present study we have examined secondary effects occurring during lectin-mediated cell agglutination of a murine S49 lymphoma cell line that grows in suspension as isolated cells and which does not establish detectable cell-to-cell adhesions. The effects of cell adhesion on lectin-mediated agglutination are thus minimized in this system, enabling other agglutination parameters to be examined. We found previously that S49 cell agglutination by *Ricinus communis* I agglutinin was completely reversible by lactose addition, but only if the competing sugar hapten was added shortly after the initial formation of multicell aggregates [8]. In this paper we show that the loss of agglutination reversibility correlates with gross cell surface deformability and the formation of areas of close apposition between adjacent agglutinated cells.

Methods

Cells. The murine lymphoma S49 · 1TB · 2 (S49) [18] was obtained from Dr. R. Hyman of The Salk Institute (San Diego, CA) and grown as a suspension culture in Dulbecco-modified Eagle's medium supplemented with 10% heated horse serum [19]. Cell viability was determined by dye exclusion using trypan blue or by plating efficiency.

Lectins and labeling of lymphoma cells. R. communis I agglutinin was affinity purified as described previously [20,21] and labeled with ^{125}I by established procedures [21,22]. ^{125}I-labeled *R. communis* I preparations varied from 0.5—$1 \cdot 10^6$ cpm/μg protein before dilution with unlabeled lectin. Cell labeling techniques are presented elsewhere [21,22].

Cell agglutination. Lectin-mediated cell agglutination was assessed by the procedures of Oppenheimer and Odencrantz [23]. S49 cells (final concentration, approx. $4 \cdot 10^6$ cells/ml) were incubated with various concentrations of *R. communis* I agglutinin in 16-mm wells of 54 Linbro trays at final volumes of 0.4 ml in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)-buffered medium. The cell suspension was gyrated for various times at approx. 1.5 Hz on a rotary table with a radium of gyration of approx. 11 cm [24]. At specified times the samples were removed from the wells, placed in vials on ice and 10 ml of filtered 0.2% formalin/Isoton (Coulter Electronics) was added to each sample. Agglutination was determined with an electronic particle counter (Celloscope, Particle Data) using an assay which detects disappearance of single cells to form cell aggregates. For the sugar hapten reversibility experiments, S49 cells were agglutinated with *R. communis* I agglutinin, washed with phosphate-buffered saline to remove excess lectin, and incubated in the same wells with gyration for various times at different temperatures in Hepes-buffered medium before addition of lactose (50 mM final concentration unless stated otherwise). The cell samples were then incubated an additional 10 min with gyration and samples were removed for assay.

Drug treatments were performed as follows. Cells (usually $4—8 \cdot 10^6$/ml) were incubated with cytochalasin B (10 μg/ml final concentration; Sigma Chemical Co., St. Louis, MO) containing 0.1% dimethylsulfoxide vinblastine sulfate (1 μM final concentration; Sigma Chemical) or sodium fluoride (5 mM final concentration) plus sodium azide (2 mM final concentration) for 15 min at 37°C. Some incubations contained cytochalasin B (10 μg/ml) plus vinblastine sulfate (1 mM). After preincubation with drugs, S49 cells were then agglutinated by *R. communis* I agglutinin in the presence of the drug(s). All solutions used in further manipulations contained the drug(s) at the same concentrations until final diluation with formalin/Isoton.

Electron microscopy. Ferritin-*R. communis* I agglutinin conjugates were synthesized and purified by previous procedures [25] or by the following method [26]. Affinity-purified lectin was added to purified ferritin (Immuno-Diagnostics, Solana Beach, CA) in a ratio of 1 : 5 (w/w) in 0.2 M sodium chloride/0.005 M phosphate buffer, pH 7.2, containing 0.2 M D-galactose. The reaction mixture was split into two equal portions and 10 μl of 1% glutaraldehyde in distilled water was added to one of the samples. 10 min later and at each successive 10 min interval 10 μl of 1% glutaraldehyde was added to each reaction mixture. When the sample containing the additional 10 μl of glutaraldehyde displayed a slight turbidity under a strong beam of visible light, the reactions in both samples were terminated by addition of 0.25 vol. of 1 M glycine in the phosphate buffer. The samples were centrifuged at 27 000 $\times g$ for 15 min at 4°C and the small pellets discarded. The supernatants were pooled and centrifuged at 160 000 $\times g$ for 120 min onto a 0.5 ml cushion of Sepharose 4B beads (Pharmacia, Piscataway, NJ). After removal of the supernatants containing

unconjugated lectin, the pellet of ferritin and ferritin-lectin was gently resuspended in phosphate-buffered saline and purified by affinity chromatography [26]. Ferritin-lectin conjugate activities were monitored by agglutination of rabbit erythrocytes [25].

Results

Reversibility of lectin-mediated agglutination

S49 lymphoma cells were chosen for the present experiments because they grow in a suspension as single cells and show virtually no tendency to form homotypic aggregates. When S49 cells are agglutinated by *R. communis* I lectin, agglutination occurs as a dose- and temperature-dependent process (Fig. 1). After treatment with lectin for 10 min the multicell aggregates formed can be completely reversed by the immediate addition of lactose (50—100 mM final concentration) to form a single cell suspension (Fig. 1). S49 agglutination by concanavalin A is also completely reversible by α-methyl-D-mannoside (50—100 mM final concentration) after similar short incubation with lectin (data not shown). However, if *R. communis* I-agglutinated S49 cells are washed once to remove excess lectin and then incubated for an additional 60 min before lactose is added, agglutination can no longer be reversed (Fig. 1). The failure of lactose to reverse lectin agglutination occurs in a temperature-dependent manner and is not due to loss in cell viability. Incubation of cells for 60 min at 4°C after initial agglutination by low concentrations of *R. communis* I agglutinin (0.1—1 μg/ml) results in nearly complete dispersal of the cell aggregates by lactose, but after washing and incubation for 60 min at 37°C only 50% of the agglutinated cells can be dispersed by lactose (Fig. 1).

The time course of the onset of the irreversible agglutinated state was

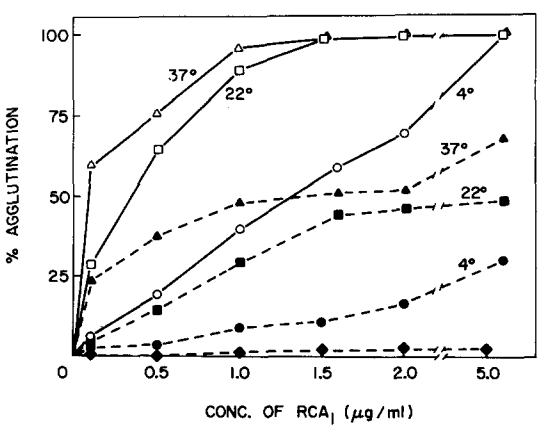
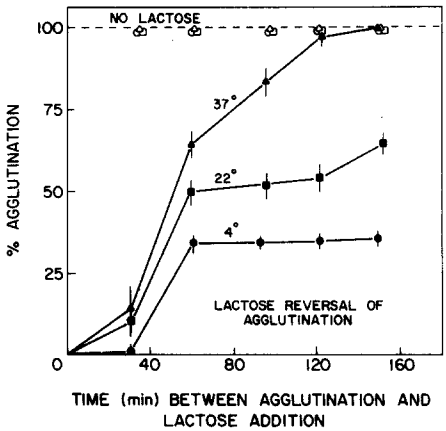


Fig. 1. Lectin concentration dependence of S49 cell agglutination and dispersal of multicell aggregates by lactose. Cells (4—8 · 10^6/ml) were incubated for 10 min with various concentrations of *R. communis* I agglutinin at 37°C (△), 22°C (□) or 4°C (○). Similar samples were treated at various concentrations of *R. communis* I agglutinin for 10 min, washed and incubated for 60 min at 37°C (▲), 22°C (■) or 4°C (●) prior to addition of 50 mM lactose for 10 min at the same temperature. Control experiments received lectin for 10 min at 37°C, 22°C or 4°C prior to immediate addition of 50 mM lactose for 10 min (◆). Data have been averaged from three separate experiments.



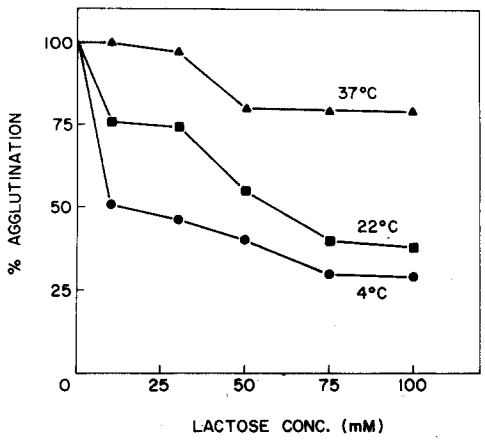


Fig. 2. Temperature dependency of lactose dispersal of *R. communis* I-mediated S49 cell aggregates. Cells (4—8 · 10^6/ml) were agglutinated for 10 min at 22°C with 5 μg/ml lectin, washed and then incubated for various times at 37°C (▲), 22°C (■) or 4°C (●) prior to 50 mM lactose addition. Open symbols indicate samples in which lactose addition was omitted. Standard deviations are indicated by bars.

Fig. 3. Dependence on lactose concentration for dispersal of *R. communis* I-agglutinated S49 cells. Cells were agglutinated for 10 min at 22°C with 5 μg/ml lectin and washed. After 90 min post-agglutination, various concentrations of lactose were added to duplicate samples and agglutination assessed after 10 min at 22°C.

assessed by agglutinating S49 cells for 10 min at 22°C with 5 μg/ml *R. communis* I agglutinin and then incubating the washed, agglutinated cells for various times prior to lactose addition. Incubation at 4, 22 or 37°C after agglutination does not cause cell dispersal unless lactose is added (Fig. 2), and there is no effect on cell viability when assessed by dye exclusion. Agglutination at 22°C, followed by washing and incubation at 4°C results in almost complete lactose dispersal of the cell aggregates 0—30 min after *R. communis* I agglutination. However, lectin-agglutinated S49 cells show only about 70% lactose reversibility (i.e. 30% of the cells remain agglutinated) after 60—120 min incubation at 4°C (Fig. 2). At incubation temperatures of 22 or 37°C, fewer cell aggregates could be dispersed by lactose addition. After a 120 min incubation at 37°C, pre-agglutinated S49 cells are not affected by hapten addition (Fig. 2). In Fig. 3 the dependency on lactose concentration for dispersal of cell aggregates after a 90 min post-agglutination incubation indicates that 50—70 mM lactose is optimal.

Ultrastructural examination of S49 cells agglutinated by ferritin-*R. communis* I agglutinin suggests that deformation of the lymphoma cell surface occurs during the lectin-free incubation after ferritin-lectin agglutination. S49 cells agglutinated for 10 min at 22°C with ferritin-lectin (equivalent to 5 μg/ml *R. communis* I agglutinin) appear as rounded cells with few distinctive surface features. High concentrations of ferritin-lectin molecules are seen between adjacent cells indicating the presence of multiple lectin cross-bridges that are probably responsible for initially holding cells together (Fig. 4). Agglutination of S49 cells by ferritin-*R. communis* I agglutinin for 10 min followed by immediate addition of 50—100 mM lactose results in removal of cell-bound ferritin

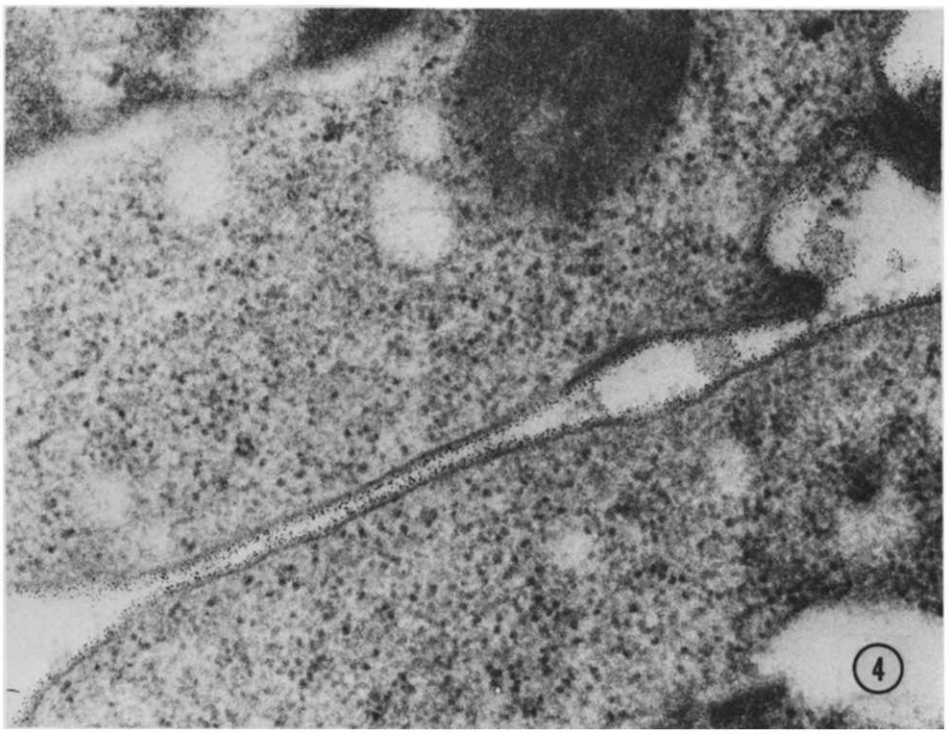


Fig. 4. Cell contact morphology in a multicell aggregate of S49 lymphoma cells agglutinated for 10 min with ferritin-*R. communis* I agglutinin (equivalent to 5 μg/ml lectin) at 22°C. ×42 500.

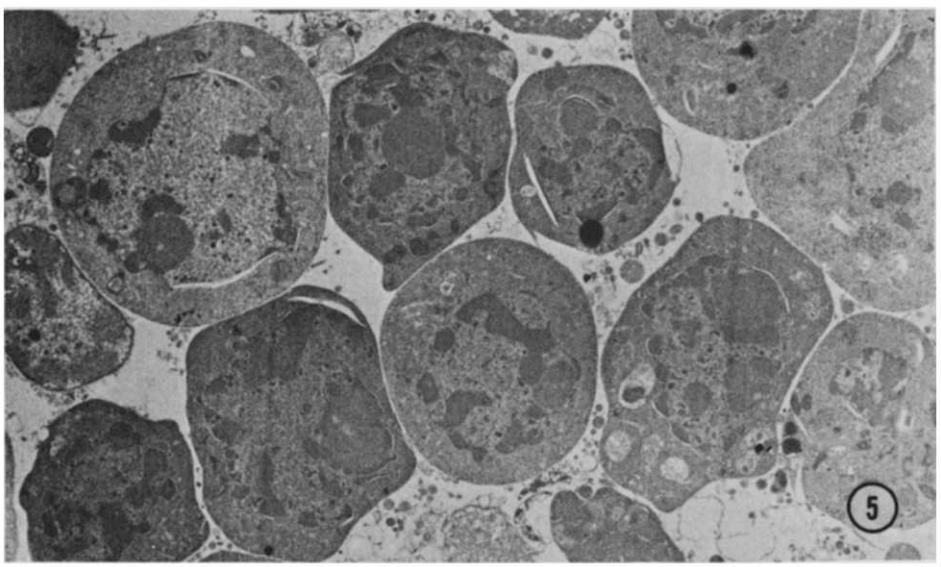


Fig. 5. Legend is the same as in Fig. 4 except that S49 cells were agglutinated by ferritin-*R. communis* I agglutinin, washed and incubated for 90 min at 37°C prior to addition of 50 mM lactose for 10 min. ×3600.

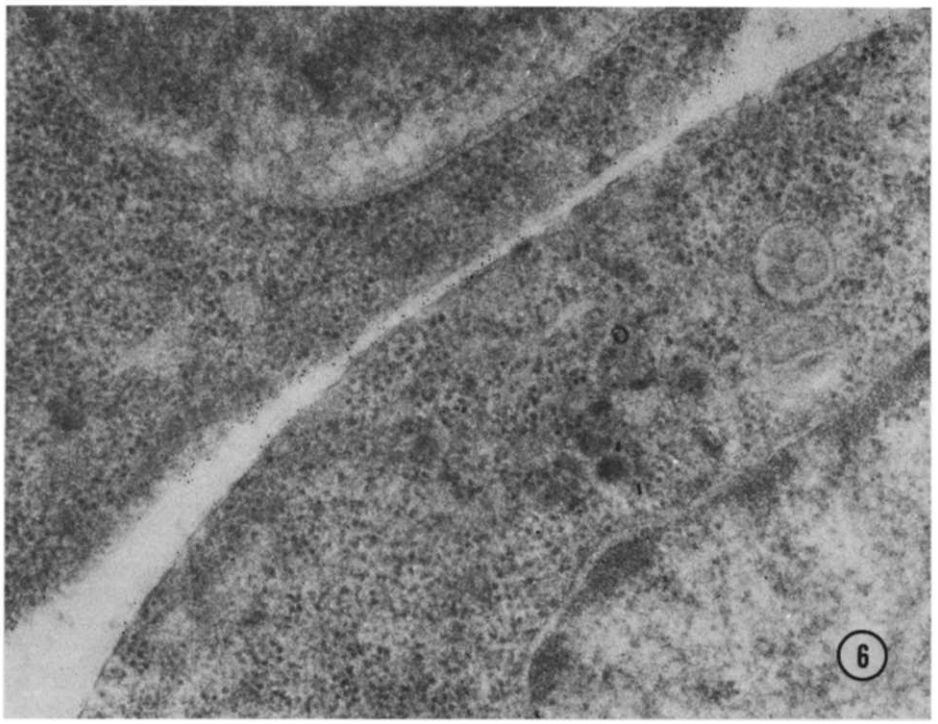


Fig. 6. Legend is the same as in Fig. 5. Note that some ferritin-*R. communis* I agglutinin molecules still remain on the surface of the cells after lactose addition. X42 500.

and complete dispersal of multicell aggregates similar to the quantitative data in Fig. 2. However, subsequent incubation of the agglutinated cells for 90 min at 37°C results in stable cell aggregates which cannot be dispersed by 50—100 mM lactose. Examination of these non-dispersible S49 cell aggregates reveals that many of the cells are more deformed and flattened in appearance and the cell surface areas in close proximity on adjacent cells are increased (Figs. 5 and 6). Although at this time lactose addition still removes most of the cell surface-associated ferritin-lectin, the S49 cell aggregates are unaffected and are not dispersed (Fig. 6).

Drug effects on lectin agglutination and reversibility

In order to determine the possible mechanism(s) responsible for the irreversibility of lectin agglutination we next assessed the effects of various drugs known to perturb lectin agglutination. Cytochalasin B (10—20 μg/ml) enhances S49 agglutination by *R. communis* I agglutinin while vinblastine sulfate (1—10 μM) produces essentially no effect (Fig. 7). Combinations of vinblastine sulfate (1 μM) and cytochalasin B (10 μg/ml) increase lectin-mediated agglutination slightly above the level for cytochalasin B alone, but only when higher (1 μg/ml) agglutinin concentrations are used (Fig. 7). Pre-incubation of S49 cells in sodium fluoride (5 mM) plus sodium azide (2 mM) has no effect on *R. communis* I-mediated agglutination. Cytochalasin B, vinblastine sulfate, a combina-

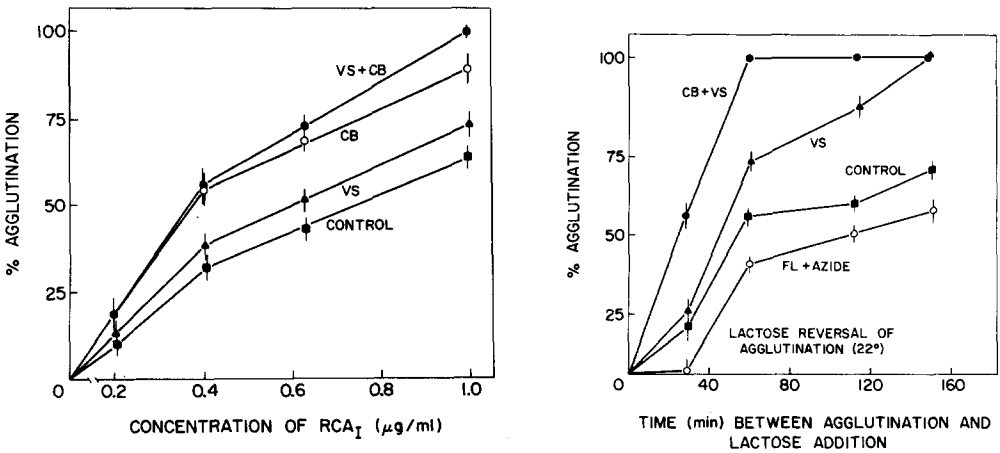


Fig. 7. Effects of various drugs on *R. communis* I-mediated agglutination of S49 cells. Cells (4—8 · 10^6/ml) were incubated with cytochalasin B (CB, ○) (10 μg/ml final concentration); vinblastine sulfate (VS, ▲) (1 μM final concentration); or combinations of vinblastine sulfate plus cytochalasin B (VS + CB, ●) for 15 min at 37°C and then agglutinated with various concentrations of lectin for 10 min at 22°C in the presence of the drug(s). Controls (■) were incubated and agglutinated without drug(s). Standard deviations are indicated by bars.

Fig. 8. Lactose dispersal of *R. communis* I-agglutinated S49 cells at 22°C. Cells (4—8 · 10^6) were incubated with 10 μg/ml cytochalasin B plus 1 μM vinblastine sulfate (CB + VS, ●), 1 μM vinblastine sulfate (VS, ▲) or 5 mM sodium chloride plus 2 mM sodium azide (Fl + azide, ○) for 15 min at 37°C. Drug-treated cells and controls (■) were agglutinated with 5 μg/ml lectin for 10 min at 22°C, washed and incubated for various times at 22°C in the presence or absence of drug(s) prior to addition of 50 mM lactose for 10 min at 22°C.

tion of these two agents, or sodium fluoride plus sodium azide did not affect the quantitative binding of ^{125}I-labeled lectin at any lectin concentration tested (data not shown). When hapten reversibility experiments are performed after agglutination by *R. communis* I, followed by washing and incubation at 22°C for various times without lectin, drug effects are more pronounced. Vinblastine sulfate or vinblastine sulfate plus cytochalasin B inhibits lactose dispersal of the S49 multicell aggregates, while sodium fluoride plus sodium azide enhances hapten reversibility (Fig. 8).

Examination of drug-treated cells by electron microscopy revealed some changes in cell shape. Pre-treatment with cytochalasin B (10 μg/ml) results in a slightly more irregular surface with the occasional presence of broad surface blebs (similar to Oppenheimer et al. [27]), while the other drugs produce no obvious changes in gross cell morphology. When drug effects on lactose dispersal of cell aggregates are analyzed ultrastructurally, cell deformation and lack of reversal of agglutination after a 90 min incubation appear to be related. Cell aggregates that show lactose reversibility in the presence of drugs such as sodium fluoride plus sodium azide are unchanged or less deformed compared to controls, while agglutinated S49 cells incubated with cytochalasin B (10 μg/ml) plus vinblastine sulfate (1 mM) show greater cell deformation and an increase in cell surface area in close apposition in the multicell aggregates (Fig. 9) compared to controls (Fig. 6).

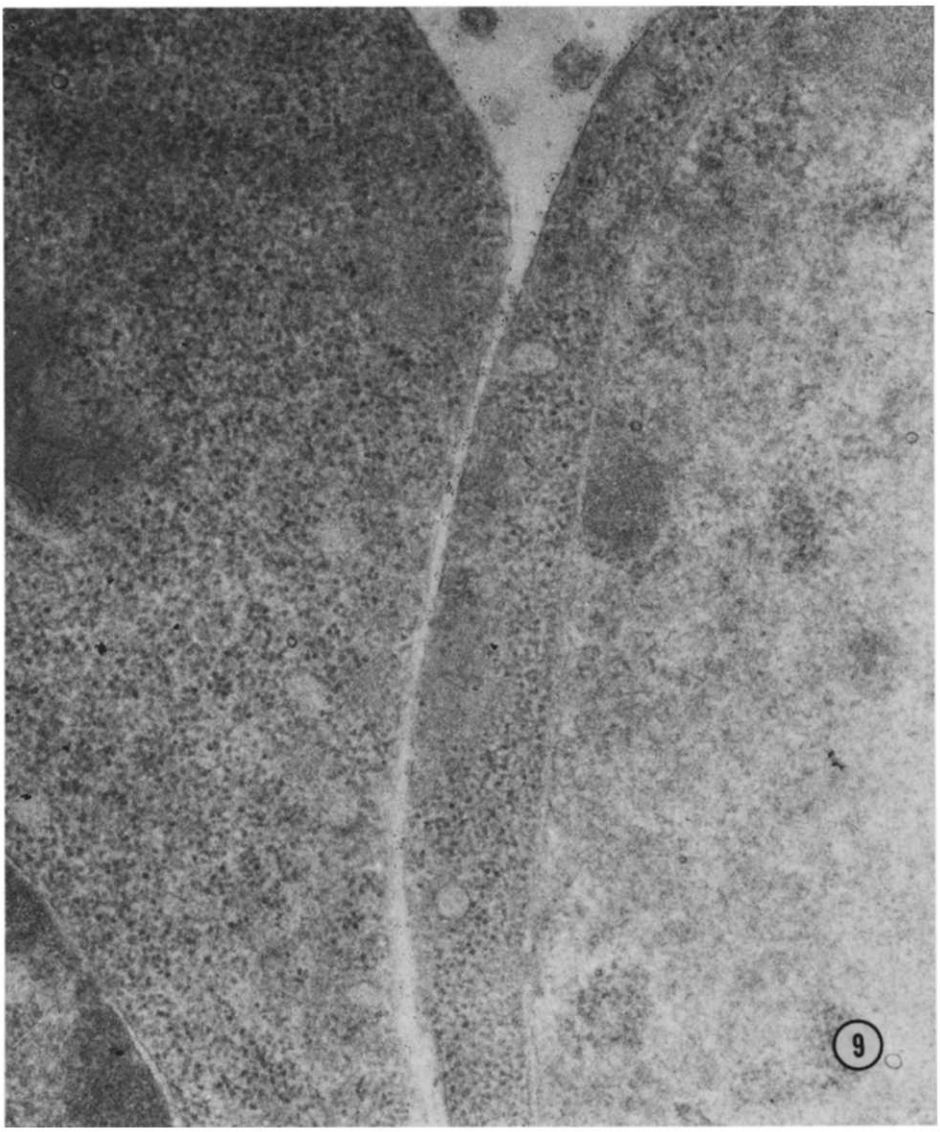


Fig. 9. S49 cells were incubated with cytochalasin B (10 μg/ml) plus vinblastine sulfate (1 μM) for 15 min at 37°C and then agglutinated with ferritin-*R. communis* I agglutinin (equivalent to 5 μg/ml lectin) for 10 min at 22°C. The agglutinated cells were washed and incubated at 22°C for 90 min in the presence of drugs prior to addition of 50 mM lactose for 10 min at 22°C. Remaining cell aggregates were fixed and embedded for electron microscopy. Note the greater surface deformation between adjacent cells in the aggregate. X42 500.

Discussion

Lectin-mediated cell agglutination appears to be governed by a variety of interrelated factors that determine whether agglutination will occur in any given system [2—4,11]. These include: (a) the biochemical nature of the agglutinin molecules; (b) the number of agglutinin molecules bound to cell surfaces

and the nature of their cell surface-binding sites; (c) the correct mobility and topographic arrangement of cell surface agglutinin receptors; (d) the presence of cell surface structures such as blebs, microvilli and pseudopodia; (e) repulsive and attractive nonspecific charge and non-charge forces between cells; (f) inner and outer membrane surface peripheral or membrane-associated components such as cytoskeletal assemblages and cell coat mucopolysaccharides; (g) specific adhesive forces between cells; and (h) cell surface rigidity or deformability. These various factors can affect lectin-mediated cell agglutination differently, and cell agglutination probably occurs when the sum of the factors favoring agglutination outweigh opposing forces [2,11].

Little is known concerning the factors that affect the reversal of lectin-mediated agglutination by specific saccharides. Lectin-mediated agglutination in certain cell systems such as human erythrocytes is almost completely reversible by specific saccharides as much as several hours after agglutination [8], while others (usually nucleated cells) can only be partly reversed or are completely irreversible within less than an hour after initial agglutination has begun [9,10,28]. The irreversibility of lectin-mediated agglutination has been attributed to cell-cell adhesive interactions that stabilize the multicell aggregates [9, 10] and/or nonspecific secondary binding of lectin molecules to cell surfaces [3].

In the present studies the role of cell adhesion in maintaining multicell aggregates of *R. communis* I lectin-agglutinated S49 lymphoma cells after lactose elution of lectin molecules could not be assessed. S49 lymphoma cells grown singly in suspension culture do not normally form homotypic adhesions. This contrasts with other cell systems which readily form homotypic adhesions often at significant rates in the absence of lectin-mediated agglutination [9,10, 16,17]. It is considered unlikely that the formation of adhesions between S49 cells occurred because the lectin molecules were able to overcome cell charge repulsive forces, because enzymatic removal of most of the cell surface sialic acid molecules decreases surface charge density but does not result in spontaneous homotypic aggregation (Nicolson, G.L., unpublished data).

Deformation of S49 cell surfaces was observed ultrastructurally during the post-agglutination incubation period, and this was temporally correlated with the onset of lactose irreversibility. Low temperature, in part, prevented this irreversible secondary phase of agglutination [8], and this could result from prevention of changes in cell deformability. Some lectin molecules are themselves affected by low temperature [29,30], but this does not seem to be the case with *R. communis* I agglutinin [21]. In other investigations aldehyde fixation was used to lower lectin-mediated agglutinability; this could also be explained, in part, by decreased cell deformability as well as by reduced lectin receptor mobility [12–15,27,31–37].

When S49 cells were treated with various drugs known to disrupt cytoskeletal systems (such as combinations of cytochalasin B plus vinblastine), both the forward and reverse steps of lectin-mediated agglutination were affected. The irreversibility of S49 multicell aggregates by lectin saccharide inhibitors was dramatically enhanced in the presence of cytochalasin B plus vinblastine correlating with an increase in cell deformation seen after treatment with these agents. S49 cells do not have abundant numbers of microfilaments and micro-

tubules which are disrupted by cytochalasin B and vinblastine, respectively; these structures are known to be responsible for controlling shape and movement in a variety of cell types [38]. Also, the fact that cellular poisons such as sodium fluoride plus sodium azide enhance lactose reversal of lectin-agglutinated S49 cells is also consistent with a possible role for cytoskeletal systems in controlling deformability. In several cellular systems sodium azide, sodium fluoride or their combination inhibits lectin-mediated cell agglutination [39, 40], while in other systems it appears to enhance agglutination [23,36]. Although other explanations are possible [36], cell systems which are drug sensitive in terms of their lectin-mediated cell agglutinability may exhibit differing deformabilities under conditions of cellular energy depletion.

In contrast to the reversal of agglutination, a direct role for cell deformability in lectin-mediated S49 cell agglutination remains to be established. Although low temperature and combinations of drugs known to affect cytoskeletal systems and cell shape (cytochalasin B plus vinblastine) did affect the forward rates of agglutination, these effects were not as dramatic as their action on reversal of agglutination after lectin-free incubation.

Each cell and lectin system will have differing properties such that cellular agglutination and reversal of agglutination will probably be determined by several factors [2,3]. In the S49 lymphoma cell system increased cell deformability after agglutination appears to enhance the stability of multicell aggregates, even when most of the lectin molecules are subsequently removed from the aggregates by specific saccharide inhibitors. Either the remaining lectin molecules found between adjacent agglutinated cells are enough to hold these cells together, or the adjacent cell surfaces are now adhesive because of specific and/or nonspecific cell-to-cell interactions. That these interactions can be reversed by low, non-toxic concentrations of chaotropic agents or neutral detergents (Nicolson, G.L., unpublished observations), but not by ionic or osmotic perturbations, suggests that nonspecific secondary forces are involved in maintaining the multicell aggregates.

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References

1 Lis, H. and Sharon, N. (1973) Annu. Rev. Biochem. 43, 541—574
2 Nicolson, G.L. (1974) Int. Rev. Cytol. 39, 89—190
3 Burger, M.M. (1973) Fed. Proc. 32, 91—101
4 Rapin, A.M.C. and Burger, M.M. (1974) Adv. Cancer Res. 20, 1—91
5 Burger, M.M. and Goldberg, A.R. (1967) Proc. Natl. Acad. Sci. U.S. 57, 359—366
6 Burger, M.M. (1969) Proc. Natl. Acad. Sci. U.S. 62, 994—1001
7 Inbar, M. and Sachs, L. (1969) Proc. Natl. Acad. Sci. U.S. 63, 1418—1425
8 Nicolson, G.L. (1973) Ser. Haematol. 6, 275—291
9 Rottmann, W.L., Walther, B.T., Hellerqvist, C.G., Umbreit, J. and Roseman, S. (1974) J. Biol. Chem. 249, 373—380

10 Walther, B.T. (1976) in Concanavalin A as a Tool (Bittiger, H. and Schnebli, H.P., eds.), pp. 231—248, John Wiley and Sons, London
11 Nicolson, G.L. (1976) Biochim. Biophys. Acta 457, 57—108
12 De Petris, S., Raff, M.C. and Mallucci, L. (1973) Nat. New Biol. 244, 275—278
13 Marquardt, M.D. and Gordon, J.A. (1975) Exp. Cell Res. 91, 310—316
14 Gibson, D.A., Marquardt, M.D. and Gordon, J.A. (1975) Science 189, 45—46
15 Rutishauser, U. and Sachs, L. (1975) J. Cell Biol. 65, 247—257
16 Ukena, T.E., Goldman, E., Benjamin, T.L. and Karnovsky, M.J. (1976) Cell 7, 213—222
17 Ukena, T.E. and Karnovsky, M.J. (1976) in Membranes and Neoplasia: New Approaches and Strategies (Marchesi, V.T., ed.), pp. 261—273, Alan R. Liss, Inc., New York
18 Horibata, K. and Harris, A. (1970) Exp. Cell Res. 60, 61—77
19 Hyman, R., Ralph, P. and Sarkar, S. (1972) J. Natl. Cancer Inst. 48, 173—184
20 Nicolson, G.L. and Blaustein, J. (1972) Biochim. Biophys. Acta 266, 543—547
21 Nicolson, G.L., Blaustein, J. and Etzler, M.E. (1974) Biochemistry 13, 196—204
22 Nicolson, G.L. (1973) J. Natl. Cancer Inst. 50, 1443—1451
23 Oppenheimer, S.B. and Odencrantz, J. (1972) Exp. Cell Res. 73, 475—480
24 Henkart, P. and Humphreys, T. (1970) Exp. Cell Res. 63, 224—227
25 Nicolson, G.L. and Singer, S.J. (1974) J. Cell Biol. 60, 236—248
26 Nicolson, G.L. (1978) in Advanced Techniques in Biological Electron Microscopy (J.K. Koehler, ed.), Vol. II, pp. 1—38, Springer-Verlag, New York
27 Oppenheimer, S.B., Bales, B.L., Brenneman, G., Knapp, L., Lesin, E.S., Neri, A. and Pollock, E.G. (1977) Exp. Cell Res. 105, 291—300
28 Inbar, M. and Sachs, L. (1973) FEBS Lett. 32, 124—128
29 Gordon, J.A. and Marquardt, M.D. (1974) Biochim. Biophys. Acta 332, 136—144
30 Huet, Ch., Lonchampt, M., Huet, M. and Bernadac, A. (1974) Biochim. Biophys. Acta 365, 28—39
31 Inbar, M., Ben-Bassat, H., Sachs, L. and Huet, Ch. and Oseroff, A.R. (1973) Biochim. Biophys. Acta 311, 594—599
32 Nicolson, G.L. (1973) Nat. New Biol. 243, 218—220
33 Noonan, K.D. and Burger, M.M. (1973) J. Cell Biol. 59, 134—142
34 Poste, G. and Reeve, P. (1974) Nature 247, 469—471
35 Rosenblith, J.Z., Ukena, T.E., Yin, H.H., Berlin, R.D. and Karnovsky, M.J. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1625—1629
36 Vlodavsky, I., Inbar, M. and Sachs, L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1780—1784
37 Rittenhouse, H.G. and Fox, C.F. (1974) Biochem. Biophys. Res. Commun. 57, 323—331
38 Goldman, R., Pollard, T. and Rosenbaum, J. (eds.) (1976) Cell Motility, Cold Spring Harbor Conferences on Cell Proliferation, Vol. 3, Cold Spring Harbor Laboratory, New York
39 Loor, F. (1973) Exp. Cell Res. 82, 415—425
40 Kaneko, I., Satoh, H. and Ukita, T. (1973) Biochem. Biophys. Res. Commun. 50, 1087—1094