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Ficoll and Dextran Enhance Adhesion of Sendai Virus to Liposomes Containing Receptor (Ganglioside G_{D1a})†

Anne M. Haywood* and Bradley P. Boyer‡

Departments of Pediatrics, Medicine, and Microbiology, University of Rochester Medical Center, Rochester, New York 14642

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ABSTRACT: Previous work has shown that high-speed centrifugation (300000*g*) of Sendai virus and liposomes in 40% (w/v) sucrose layered under a discontinuous *sucrose* gradient removes Sendai virus bound to liposomes containing the ganglioside G_{D1a}, a Sendai virus receptor. Centrifugation also removes virus bound to liposomes containing other negatively charged lipids. This work shows that centrifugation of virus through a discontinuous *ficoll* gradient does not remove virus bound to liposomes containing G_{D1a} but does remove virus from liposomes containing various other negatively charged lipids including the ganglioside G_{M1}, which is not a Sendai virus receptor. The amount of virus that adheres to liposomes increases with increasing content of G_{D1a} in the liposomes. The adhesion of virus to receptor-containing liposomes during centrifugation through a ficoll gradient results from the presence of ficoll and increases with increasing ficoll concentration. Virus also adheres to receptor-containing liposomes during centrifugation in the presence of dextran. These data indicate that caution should be used in interpreting associations demonstrated by centrifugation through dextran and ficoll gradients. They also indicate that binding of virus by ganglioside receptors can be modulated by carbohydrate polymers, which are thought not to have any specific interaction with either viruses or gangliosides.

Sendai viruses bind to liposomes and cells at 0–4 °C but only penetrate them by endocytosis or by membrane fusion at higher temperatures. This fact means that binding can be studied at low temperatures without the complication of concurrent viral membrane fusion. In previous work it has been shown that high-speed centrifugation through a discontinuous sucrose gradient removes Sendai virus from liposomes containing G_{D1a} or other negatively charged lipids (Maeda et al., 1981; Haywood & Boyer, 1982, 1984). Lyles and Landsberger (1979) noted that centrifugation of Sendai virus bound to red cells through a dextran gradient did not remove bound virus. This work was undertaken to resolve the differences between these results. One reason for these differences turned out to be that adhesion of virus to receptors is greater in ficoll or dextran than in sucrose.

Materials and Methods

Virus. Virus was grown and radiolabeled in embryonated eggs, purified, and dialyzed against PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4) as previously described (Haywood & Boyer, 1982). The protein content of the virus preparation was measured by a modification of the Lowry procedure (Markwell et al., 1978). The virus preparation used in Tables I and II had a specific activity of 1040 cpm/μg of protein. The virus preparation used in Tables III and IV had a specific activity of 132 cpm/μg of protein.

Ficoll, Dextran, and Fetuin. Ficoll 400, M_w 400 000 ± 100 000, and ficoll 70, M_w 68 000 and M_n 25 000, were obtained from Pharmacia Fine Chemicals. Dialyzed ficoll, dextran, M_w 9000, and fetuin, type III lyophilized from fetal calf serum, were obtained from Sigma Chemical Co.

Lipids. Egg phosphatidylcholine (PC), egg phosphatidylethanolamine (PE), egg phosphatidic acid (PA), bovine brain sphingomyelin, bovine brain phosphatidylserine (PS), and bovine brain phosphatidylinositol (PI) were obtained from Avanti Biochemicals. Globoside was obtained from Supelco. Cholesterol, chromatography standard grade, and dicetyl phosphate were obtained from Sigma Chemical Co. When 0.8 μmol of these lipids was chromatographed with chloroform/methanol/concentrated NH_4OH (60:25:4 v/v) and with chloroform/methanol/acetic acid/water (65:25:2:4 v/v) on silica gel G thin-layer chromatography plates, only one spot was observed. Ganglioside G_{D1a} was also obtained from Supelco, Inc. Only one spot was obtained when 0.3 μmol was chromatographed with chloroform/methanol/2.5 M NH_4OH (60:35:8 v/v) plus 20 mg of KCl/100 mL. The concentrations of the phospholipids were determined by the phosphate assay of Ames and Dubin (1960), and the concentration of G_{D1a} was determined by the sialic acid assay of Miettinen and Takki-Luukkainen (1959).

Formation of Liposomes. Multilamellar liposomes were made in PBS as previously described (Haywood & Boyer, 1982). Virus and liposomes were usually combined in 0.3-mL total volume.

Scintillation Counting. Ficoll forms a precipitate in many scintillation fluids. This was initially circumvented by pre-

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‡Present address: American Red Cross, Rochester, NY 14607.

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cipitating the proteins with trichloroacetic acid (TCA) (Tables I and II). To the 1-mL fractions collected from the gradients, 0.1 mL of 2% sodium deoxycholate (DOC) was added. This both lyses the liposomes and helps solubilize the ficoll. Then, 1 mL of cold 20% TCA was added, and the samples were left in an ice bucket 1 h. The samples were put on a Whatman GF/A glass filter, which was washed twice with 5 mL of cold 5% TCA and then once with 5 mL of 99% ethanol. After being dried, the filter was counted in 5 mL of ScintiPrep 2 scintillation fluid (Fisher Scientific Co.). Other samples (Tables III and IV) in ficoll were counted by adding 1 mL of 10% sodium dodecyl sulfate (SDS) and then 10 mL of ACS II scintillation fluid (Amersham) to each fraction.

Centrifugation of Virus–Liposome Complexes through 14% Ficoll/12% Ficoll or 10% Ficoll/7.5% Ficoll Gradients. To look at bound virus in ficoll gradients, 20% ficoll 400 in PBS was added to the samples containing viruses and liposomes to yield a final concentration of 14 or 10% (w/v) ficoll, which was layered over 0.25 mL of 60% (w/v) sucrose in PBS. For the 14%/12% ficoll gradients, the sample in 14% ficoll was layered under 2.6 mL of 12% ficoll, and for the 10%/7.5% gradient, the sample in 10% ficoll was layered under 7.5% ficoll. PBS (0.3 mL) was layered on top of the ficoll, and the samples were centrifuged at 54 000 rpm for 40 min in an SW60 rotor at 4 °C. The liposomes and bound virus floated to the interface between the ficoll and PBS, while the free virus went to the interface between the 60% sucrose and ficoll.

Centrifugation of Virus–Liposome Complexes through 40%/30% Sucrose Gradients. The viruses and liposomes were mixed with a volume of 70% sucrose (w/v) to bring the final concentration to 40% sucrose. This was layered on 0.25 mL of 60% sucrose and under 2.7 mL of 30% sucrose and 0.3 mL of PBS and centrifuged at 54 000 rpm for 40 min at 4 °C in an SW60 rotor. The fraction at the 30% sucrose–PBS interface contained the liposomes and any bound virus, and the fraction at the 30% sucrose–70% sucrose interface contained the free virus.

Centrifugation of Virus–Liposome Complexes That Have Been Layered over 20% Sucrose. The mixture of virus and liposomes with ficoll or dextran at the concentrations indicated was in a volume of 0.3 mL during the experiment and just before centrifugation was brought to a volume of 1.5 mL by the addition of PBS containing the same concentration of ficoll or dextran. The sample was then layered over 1.95 mL of 20% (w/v) sucrose and 0.25 mL of 60% sucrose and under 0.3 mL of PBS and centrifuged at 54 000 rpm for 40 min at 4 °C in an SW60 rotor. The liposomes and bound virus were above the 20% sucrose, and the free virus was at the 60% sucrose–20% sucrose interface.

Results

To investigate whether bound viruses are removed from liposomes during centrifugation when the gradient is made from a polymer rather than from sucrose, Sendai viruses were bound at 0 °C for 1 h to liposomes containing PC and ganglioside G_{D1a}, which has receptor activity for Sendai virus. The viruses and liposomes were then mixed with ficoll, placed under a layer of ficoll of lower concentration and a layer of PBS, and centrifuged at 54 000 rpm as described under Materials and Methods. At the starting position of the virus and liposomes, this speed gives a centrifugal field of about 300000*g*. The percent of viruses that moved up with the liposomes to the ficoll–PBS interface was measured. In initial experiments, the virus and liposome mixture was brought to a final concentration of 10% ficoll and placed under 7.5% ficoll. In subsequent experiments, the virus–liposome mixture was brought to a final concentration of 14% ficoll and placed under 12% ficoll. Virus did remain bound to G_{D1a}-containing liposomes in these ficoll gradients and moved with the liposomes up to the ficoll–PBS interface. The binding of virus to liposomes was proportional to the amount of G_{D1a} present in the liposomes (Table I).

Table I: Binding of Sendai Virus to Liposomes Containing Different Amounts of Ganglioside G_{D1a} after Centrifugation through Different Ficoll Concentrations[a]

G_{D1a} contained by liposomes (μmol)	% virus bound to liposomes after centrifugation through	
	10%/7.5% ficoll	14%/12% ficoll
0	2.2 ± 0.4	3.6 ± 0.2
0.0025	2.0 (2.1; 1.9)	5.0 ± 0.3
0.005	2.7 (2.7; 2.7)	8.6 ± 0.6
0.010	5.4 (4.1; 6.8)	10.8 ± 1.0
0.020	7.5 (7.5; 7.6)	14.4 ± 0.2
0.050	15.4 (15.7; 15.1)	24.3 ± 1.4
0.10		25.1 ± 2.3

[a] ^{35}S-Labeled Sendai viruses (5 μg of protein) were added to liposomes containing 1 μmol of PC and the indicated amounts of ganglioside G_{D1a}. After 1 h at 0 °C, the virus–liposomes complexes were centrifuged through 14%/12% ficoll or 10%/7.5% ficoll gradients. The percent of counts in virus bound to liposomes was determined and is presented as the mean ± SE when there were three or four experiments and as the mean with the individual values when there were duplicate experiments.

The amount of binding, however, also varied according to the concentration of ficoll in the gradient (Table I). The densities of the liposomes and Sendai virus in ficoll were determined by equilibrium centrifugation on continuous ficoll gradients to check that there were not variations in the density that could account for the differences observed when the two gradients were used. Sendai viruses are pleomorphic and have a density distribution in ficoll of 1.068–1.103 g/cm^3 (20–30% ficoll). This density is lower than the density measured in sucrose, which is 1.19 g/cm^3 (Kingsbury et al., 1978; A. M. Haywood and B. P. Boyer, unpublished observations). Liposomes containing only PC band at 2.5% ficoll and those containing PC and 5 mol % G_{D1a} band near 2.8% ficoll. Therefore, density does not account for the differences observed with the two different gradients. As is described below, these differences can be explained by the fact that the adhesion of Sendai virus to liposomes is related to the concentration of ficoll present.

Liposomes containing PC and 20 mol % G_{D1a} and liposomes containing only PS, however, when centrifuged to equilibrium in ficoll, distribute in several bands including a dense band near 10% ficoll. Therefore, 14%/12% ficoll gradients are necessary for use with liposomes of some compositions.

Sendai viruses not only fuse with liposomes containing receptor gangliosides but also fuse with liposomes containing 5 mol % of a variety of negatively charged lipids (Haywood & Boyer, 1984). Therefore, there must be some binding to these liposomes, although it seems likely the binding is nonspecific. To see if ficoll has an effect upon virus adhesion to these liposomes, Sendai virus was left at 0 °C for 1 h with liposomes containing 5 mol % of a variety of negatively charged lipids. The virus and liposomes were then centrifuged through a 14%/12% ficoll gradient. As shown in Table II, only 3–5% of Sendai virus was associated with these liposomes after centrifugation through ficoll, and this amount is probably due to trapping. The negatively charged lipids used included the ganglioside G_{M1}, which is a ganglioside that does not have receptor activity for Sendai virus (Haywood, 1975; Holmgren et al., 1980; Markwell et al., 1981). Therefore, the combination of ficoll and of receptor activity appears to be necessary

Table II: Binding of Virus to Liposomes of Different Compositions after Centrifugation through Ficoll Gradients[a]

liposome composition	virus bound to liposomes (%)	liposome composition	virus bound to liposomes (%)
PC	3.6 ± 0.2	PC + 5% dicetyl phosphate	5.2 ± 0.4
PC + 5% globoside	5.0 ± 0.2		
PC + 5% PA	3.9 ± 0.3	PC + 5% stearylamine	45.2 ± 1.3
PC + 5% G_{M1}	4.0 ± 0.4	PC + 5% G_{D1a}	24.3 ± 1.4
PC + 5% PS	3.9 ± 0.5	PC + 5% G_{D1a}, fetuin after binding	1.1 ± 0.2
PC + 5% PI	4.1 ± 0.2		

[a] Liposomes were made of the compositions indicated in PBS to make a total of 1 μmol of lipid. ^{35}S-Labeled Sendai viruses (5 μg of protein) were added to the liposomes. After 1 h at 0 °C, the virus–liposome complexes were centrifuged through 14%/12% ficoll gradients. The percent of counts in virus bound to liposomes was determined and is given as the mean ± SE.

for virus to adhere to negatively charged liposomes during high-speed centrifugation. Sendai viruses do, however, remain bound to liposomes containing stearylamine, which gives the liposomes a net positive charge.

Since Sendai virus has previously been found to bind to ganglioside-containing liposomes at 0–4 °C (Haywood, 1974a), but only fuses with such liposomes after incubation at higher temperatures (Haywood, 1974b; Haywood & Boyer, 1981), it is to be expected that the association of virus with G_{D1a}-containing liposomes in the cold in these experiments represents only binding. To check that ficoll does not promote fusion in the cold and that binding but not fusion with G_{D1a}-containing liposomes is being measured in these experiments, fetuin was added at a final concentration of 50 mg/mL after virus had been bound to G_{D1a}-containing liposomes for 1 h at 0 °C. Fetuin is a sialoglycoprotein that binds Sendai virus. It therefore can compete with G_{D1a}-containing liposomes for the virus if the virus is bound but cannot compete if the virus is fused with the liposome. The amount of counts remaining with liposomes after fetuin treatment and centrifugation through ficoll was 1.1 ± 0.2% (Table II), which is consistent with the fact that at 0 °C only binding occurs. As a control, viruses were incubated with liposomes at 40 °C for 2 h, so that the virus membranes fused with the liposomes, and then the virus and liposomes were chilled and treated with fetuin. The amount of counts remaining with liposomes after centrifugation through ficoll was 24.6 ± 0.6%, which is consistent with the amount of fusion expected. This confirms that treatment with fetuin distinguishes between binding and fusion and that only binding is being measured at 0 °C. These results on Sendai virus binding to liposomes of different compositions after centrifugation through ficoll are consistent with the early studies on Sendai virus binding with low-speed centrifugation (Haywood, 1974a, 1975). These studies show that Sendai viruses bind to liposomes containing receptor gangliosides or to liposomes containing a net positive charge but that the binding to liposomes containing other lipids with a net negative charge does not cause enough adhesion to result in cosedimentation of viruses and liposomes.

In contrast to these experiments, which use ficoll gradients, previous experiments have shown that virus bound to liposomes containing PC and either 5 mol % G_{D1a} or 5 mol % of other negatively charged lipids is released by centrifugation through a discontinuous *sucrose* gradient. The experiments using a sucrose density gradient were repeated for liposomes containing PC and 5 mol % G_{D1a} and for liposomes containing PC and 5 mol % PS. Consistent with previous findings (Haywood & Boyer, 1982, 1984), only 1% of the virus remained bound. Whether centrifugation through sucrose could release virus from liposomes containing a net positive charge had not been previously investigated. When viruses were bound to liposomes containing PC and 5 mol % stearylamine and centrifuged up through a discontinuous sucrose gradient, 79.7 ± 0.7% of the viral counts remained with the liposomes. The virus has been shown to have a net negative charge (Haywood, 1974a), so the binding to positively liposomes is presumably due to electrostatic bonds. The fact that the binding of Sendai virus to G_{D1a}-containing liposomes can be released by centrifugation through sucrose suggests that this binding is due to bonds that are weaker than electrostatic bonds. It should be noted that influenza virus cannot be removed from negatively charged liposomes by centrifugation through sucrose at 300000*g* (Maeda et al., 1981; Haywood & Boyer, 1985). Sendai virus has an isoelectric point of 4.0 (A. M. Haywood, unpublished data), whereas, for those influenza strains where the isoelectric point is published, the isoelectric point is considerably higher, e.g., 6.0 for influenza WSN (Lakshmi & Schulze, 1978), so the charge distribution on influenza and Sendai viruses differs and may account for the differences in adhesion to negatively charged liposomes.

Table III: Effect of Concentration of Ficoll upon Adhesion of Sendai Virus to Liposomes Containing G_{D1a}[a]

ficoll (% w/v)	virus bound to liposomes (%)
PBS (no ficoll)	4.7 ± 0.7
2% ficoll, M_w 400 000	4.4 ± 0.9
4% ficoll, M_w 400 000	7.9 ± 0.4
6% ficoll, M_w 400 000	13.4 ± 0.8
7% ficoll, M_w 400 000	18.0 ± 1.9
10% ficoll, M_w 400 000	23.3 ± 0.9
14% ficoll, M_w 400 000	30.2 ± 1.4
14% ficoll, M_w 400 000 (dialyzed Sigma)	27.6 ± 0.9
14% ficoll, M_w 70 000	26.0 ± 0.3

[a] Liposomes containing 0.95 μmol of PC and 0.05 μmol of G_{D1a} were mixed with ^{35}S-labeled Sendai virus (27 μg of protein). Ficoll was added as indicated. After 1 h at 0 °C, the samples were layered over 20% sucrose and centrifuged as described under Materials and Methods. The percent of virus bound to liposomes after centrifugation was measured and is given as the mean ± SE.

The fact that the amounts of Sendai virus released from G_{D1a}-containing liposomes centrifuged through sucrose and from G_{D1a}-containing liposomes centrifuged through ficoll differ could be because the high concentration of sucrose facilitates the release. Alternatively, ficoll could aid the binding in such a way that the binding is not easily released upon centrifugation. To distinguish between these possibilities, the virus and liposomes were layered over 20% sucrose and a 60% sucrose pad, so the virus moved downward in the centrifugal field. Previously, the virus and liposomes had been mixed with sucrose and ficoll and layered below a discontinuous gradient so the liposomes moved upward. The region at the top of the centrifuge tube will be subjected to about 210000*g*–290000*g*. In the absence of ficoll, bound viruses were released from the liposomes, to that only 4.7% of the virus, which probably represents trapping, remained at the top of the tube with liposomes that contained PC and 5% G_{D1a} (Table III). This indicates that the high concentration (40% w/v) of sucrose previously used in order to layer the virus under the gradient is not required for release of virus but rather the forces involved in centrifugation can cause release of virus.

To test whether ficoll causes the virus to remain bound to G_{D1a}-containing liposomes during centrifugation, ficoll was added to virus and liposomes that were left at 0 °C for 1 h. The virus and liposomes in ficoll were layered over 20% sucrose and centrifuged. As also shown in Table III, in the presence of ficoll, virus does remain bound to G_{D1a}-containing liposomes.

When the ficoll concentration is above 2%, the amount of adhesion increases as the ficoll concentration increases. Therefore, it appears that, rather than sucrose decreasing the adhesion of virus to liposomes that contain the ganglioside G_{D1a}, ficoll increases the adhesion.

To test whether possible impurities, particularly salts, in the Pharmacia ficoll play a role in binding, a comparison was made of the amount of virus bound to liposomes when 14% ficoll 400 from Pharmacia and 14% dialyzed ficoll 400 from Sigma Chemical Co. were present. The amount of virus binding was 30.2% and 27.6%, respectively (Table III). To test the effect of the molecular weight of the polymer, the amount of binding when 14% ficoll 70 was present was tested and found to be 26.0% (Table III).

To determine if the ficoll preferentially interacts with the virus, the liposomes, or the virus–liposome complex, the effect of the order of addition of ficoll, virus, and liposomes was tested. Liposomes containing 0.95 μmol of PC and 0.05 μmol of G_{D1a}, Sendai virus (7 μg of protein), and ficoll, M_w 400 000, at a final concentration of 14% were mixed in different orders. The following sequences were used: virus and ficoll, 0 °C, 15 min, then liposomes, 0 °C, 45 min; liposomes and ficoll, 0 °C, 15 min, then virus, 0 °C, 45 min; virus and liposomes, 0 °C, 15 min, then ficoll, 0 °C, 45 min. The mixtures were layered over 20% sucrose and centrifuged, and the percent of virus bound to liposomes was 26 ± 1, 25 ± 1, and 28 ± 2, respectively. Thus, the result was similar regardless of the order of addition. This suggests that there is no requirement that the virus and liposome already be bound for the ficoll to enhance binding. There is also no indication that ficoll must interact with either the virus or the liposome prior to the virus–liposome binding. Also, there is no indication that ficoll is able to selectively act upon the virus or liposome is some way, such as aggregation, that prevents virus–liposome binding.

To determine if the effect of ficoll on binding is reversible, virus and liposomes that had been together in the presence of 14% ficoll at 0 °C for 1 h were diluted 7-fold with PBS before centrifugation so the final concentration of ficoll was 2%. This concentration was shown above to have a minimal effect upon binding. The amount of binding after dilution and centrifugation was 4.7 ± 0.1%, so the effect of ficoll upon binding of Sendai virus to G_{D1a}-containing liposomes is reversible. That the reversibility is not due to the concurrent dilution of virus and liposomes is shown by the fact that in the experiments shown in Table III the virus, liposomes, and ficoll were diluted from 0.3 to 1.5 mL with the same concentration of ficoll but this dilution did not reverse the binding.

Another polymer that is used for density gradients is dextran, a polymer of glucose. Dextran was tested to see if it also causes Sendai virus to remain bound during centrifugation to liposomes and whether a difference can also be detected between adhesion to liposomes containing ganglioside G_{D1a}, which has Sendai virus receptor activity, and adhesion to liposomes containing ganglioside G_{M1}, which does not have receptor activity. Table IV shows that the addition of dextran does indeed result in Sendai virus remaining bound to G_{D1a}-containing liposomes. As a further control that virus does not adhere to liposomes through centrifugation in the absence of a polymer, sucrose was added to virus and G_{D1a}-containing liposomes to give a final concentration of 14%, and the mixture was layered over 20% sucrose and centrifuged. The virus was released from the liposomes (Table IV).

Table IV: Effect of 14% Sucrose, Ficoll, and Dextran upon Adhesion of Sendai Virus to Liposomes Containing PC or PC plus either G_{D1a} or G_{M1}[a]

addition	% virus bound to liposomes containing		
	PC	PC + G_{M1}	PC + G_{D1a}
14% sucrose			2.8 ± 0.3
14% ficoll, M_w 400 000	6.7 ± 0.1	6.4 ± 0.5	30.2 ± 1.4
14% dextran, M_w 9000		4.2 ± 0.2	23.5 ± 0.7

[a] Liposomes containing 1.0 μmol of PC or 0.95 μmol of PC plus 0.05 μmol of either G_{D1a} or G_{M1} were mixed with ^{35}S-labeled Sendai virus (27 μg of protein). Sucrose, ficoll, or dextran was added to give a final concentration of 14%. After 1 h at 0 °C, the mixture was layered over 20% sucrose and centrifuged. The percent of virus associated with the liposomal fraction was determined and is given as the mean ± SE.

Discussion

The receptors for paramyxoviruses, e.g., Sendai virus, and for myxoviruses, e.g., influenza virus, bind to host components that contain sialic acid as shown by the fact that treatment of cells with sialidase (neuraminidase) destroys the ability of the cells to bind and be infected by these viruses (Hirst, 1959; Fidgen, 1975; Markwell & Paulson, 1980) and as shown by the fact that sialoglycoproteins can inhibit hemagglutination by these viruses (Gottschalk et al., 1972). It was assumed that cellular receptors for both myxoviruses and paramyxoviruses were sialoglycoproteins until it was shown that some gangliosides when incorporated into liposomes could also serve as Sendai virus receptors (Haywood, 1974a, 1975). Ganglioside-containing liposomes were shown to cause hemagglutination inhibition and to cause virus to cosediment during centrifugation at 10000*g* for 10 min. To demonstrate cosedimentation, it was necessary first both to centrifuge the liposomes at 6400*g* for 10 min in order to select the large liposomes and to centrifuge the virus at 10000*g* and collect the supernatant to remove aggregates or large virus that sediment at low speeds. Then, the resulting liposomes and viruses were mixed, and the binding was assayed by the ability of the liposomes to cause the virus to cosediment after centrifugation at 8800*g* for 10 min in PBS. Apparently not recognizing the need for the preliminary centrifugation of virus, Wu et al. (1980) later questioned the role of gangliosides as receptors. Holmgren et al. (1980), however, confirmed and extended the work and quantitated the binding of Sendai virus to individual gangliosides. Markwell et al. (1981) showed that addition of gangliosides to sialidase-treated cells made the cells susceptible to Sendai virus infection in proportion to the ability of the added ganglioside to act as a receptor. The precise roles of sialoglycoproteins and gangliosides in virus infection in vivo have not been resolved.

In previous work it has been shown that high-speed centrifugation removes Sendai virus from liposomes containing G_{D1a} or other negatively charged lipids (Maeda et al., 1981; Haywood & Boyer, 1982, 1984). In this work it has been shown that in the presence of the polymers ficoll and dextran Sendai virus adheres though centrifugation to liposomes containing the receptor ganglioside G_{D1a}. Further, the concentrations of polymer required for this effect are low, so it is possible carbohydrate found in cell glycocalyces can supply the same function.

Adhesion involves at least two different parameters, which are the ease of bond formation and the resistance of the aggregate to disruption (Bongrand & Bell, 1984). The strength of the bonding depends upon the nature of the bonds and the number and mobility of the receptors. Dextran is known to cause agglutination between red cells (Brooks, 1973; Buxbaum et al., 1982), between red cells and liposomes (Evans & Kukan, 1983), and between liposomes (Schachter, 1978). Poly(ethylene glycols) (PEG) cause sonicated liposomes to aggregate with the maximum aggregation reached at about 6%

w/w PEG 6000, and a steric exclusion mechanism was suggested (Tilcock & Fisher, 1982). Therefore, it seems possible that polymers such as dextran and ficoll may cause weak nonspecific binding between the virus and G_{D1a}-containing liposomes, which could hold the virus and liposomes together enough to either allow the virus to recruit G_{D1a} and thereby increase the number of bonds or allow rearrangements in the binding of the virus HN protein G_{D1a}, which would increase the strength of the bonds. Also, these polymers might aid adhesion by removing water. A similar mechanism may explain the fact that high-affinity lectin binding to liposomes containing receptor glycoprotein is increased by the presence of dextran or serum albumin (Ketis & Grant, 1982; Grant & Peters, 1984).

Since similar requirements have not been noted for binding to cells, serum components and the glycocalyx may very well influence the receptor activity of gangliosides and glycoproteins in cells in the same manner that macromolecules influence Sendai virus binding to gangliosides and lectin binding to glycoproteins in liposomes.

Registry No. Ganglioside G_{D1a}, 12707-58-3; ficoll, 25702-74-3; dextran, 9004-54-0.

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