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Binding sites for rubella virus on erythrocyte membrane

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Summary. To identify molecule(s) with the properties of rubella virus (RV) receptor, goose erythrocyte membranes were isolated and tested for their ability to compete with whole cells for viral binding and fusion. Solubilized membranes showed a dose-dependent inhibiting activity on either rubella virus attachment or its fusion with erythrocytes at acidic pH. The inhibitory activity was enhanced by trypsin and neuraminidase, and inactivated by phospholipase A2 digestion, pointing towards the involvement of lipid structures as receptor sites for RV. After isolation of the different membrane components, only the lipid moiety, specifically phospholipids and glycolipids, was found to inhibit viral biological activities. When the major membrane lipids were examined separately, phosphatidylserine and cerebroside sulfate showed a strong inhibiting activity on viral hemagglutination and subsequent hemolysis. The capacity of several pure phospholipids (phosphatidylinositol, phosphatidylcholine and sphingomyelin) to inhibit the hemolysis but not the binding of the virus to the erythrocytes indicated that different membrane lipid components are involved in the attachment and the fusion step. Enzymatic and chemical modifications of whole erythrocytes confirmed the role of membrane lipid molecules in the cell surface receptor for RV.

Introduction

Rubella virus consists of a single positive stranded RNA enclosed within a capsid, which is surrounded by an envelope containing stubby spike proteins. The virus contains [17] three structural glycosilated proteins (E1, E2a, and E2b), and a non-glycosilated capsid protein (C).

Using monoclonal antibodies it has been demonstrated that E1 possess at least six non-overlapping antigenic sites [23, 24]. Four sites are associated with hemagglutination; two of these are involved in the hemolytic activity of the virus, while one appears to be associated with neutralization. A fifth antigenic domain is also associated with neutralization, but not with hemagglutination.

The E2 glycoproteins contain at least one epitope which is involved in neutralizing viral infectivity [8]. Rubella virus is able to bind to goose erythrocyte membrane producing hemagglutination, hemolysis and fusion in a mildly acidic pH [21]. Although the viral structure responsible for the attachment and fusion activity is now well characterized [23, 24], the erythrocyte membrane receptor for rubella virus has not yet been identified. The extreme sensitivity of hemagglutination to nonspecific lipid and lipoprotein inhibitors [19] suggests that direct interaction between the virus and membrane lipid moiety causes the hemagglutination of the red cells. Furthermore it has been demonstrated that trypsin digestion increases the sensitivity of human and animal erythrocytes to rubella virus hemagglutination [16].

We have studied the nature of membrane receptors for rubella virus using competing molecules prepared from goose erythrocytes. Cell membranes were solubilized with Triton X-100, Tween 80 and octyl-β-D-glucopyranoside. The chemical nature of the active components was investigated by enzyme treatment of solubilized membranes (SEM). In addition, we isolated protein, lipid, phospholipid and glycolipid components of membranes and studied their inhibitory activity on either rubella virus attachment or its fusion with erythrocytes. Finally the role of the various membrane components was verified by studying the effect of enzymatic and chemical modifications of whole erythrocytes on the binding and fusion activities of the virus.

Materials and methods

Virus

Rubella virus (RV), Therien strain, was grown in Vero cells in minimum essential medium (MEM) containing 0.2% bovine serum albumin (BSA) and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.3. Semiconfluent monolayers were inoculated with RV at a multiplicity of infection of 0.5 PFU/cell and incubated at 37 °C for 3 days. High titer RV (1.5×10^7 PFU/ml) was prepared after three consecutive plaque purification steps from a single plaque according to Oker-Blom [18]. Virus was concentrated and purified as described [18].

Hemagglutination

HA studies were carried out in 96-well (V-shaped bottom) microtitre plates. Twofold serial dilutions of RV in bovine albumin borate saline buffer pH 9.0 containing 1 mM $CaCl_2$ (BABS Ca^{++}) were mixed with an equal volume of 0.125% goose erythrocyte suspension in 0.2 M phosphate saline buffer, 1 mM $CaCl_2$ pH 5.0. The plates were kept at 4 °C over crushed ice for 4 h, before titres were recorded. In HA inhibition test twofold dilutions of inhibitor in BABS Ca^{++} were mixed with an equal volume of virus containing 4 HA units (HAU). After 1 h incubation at 4 °C erythrocytes were added.

Hemolysis

Concentrated viral suspensions were subjected to 20 cycles of freezing and thawing according to Kobayashi and Suzuki [11]. Purified virus was twofold diluted in BABS Ca^{++} and 250 µl of each dilution were mixed with an equal volume of 1% goose erythrocyte suspension

in phosphate buffer pH 5.0 containing 1 mM $CaCl_2$. The mixtures were incubated at 4 °C for 90 min and then made 1.5 mM in EDTA. After incubation at 37 °C for 90 min the samples were centrifuged at 170 **g** for 10 min at 4 °C, and the absorbance of the supernatant was measured at 540 nm. The results were expressed as % He compared with the maximal 100% obtained by treatment with 0.1% NP40. One hemolytic unit (HeU) was the viral dilution giving 50% He. In the He inhibition (HeI) test 125 µl of twofold dilutions of inhibitors were mixed with 125 µl of virus containing 8 HeU/ml. Goose erythrocytes were added after 1 h incubation at 4 °C. The results were expressed as percent of the maximum He obtained without inhibitor. The percent inhibition (%HeI) was thus 100% minus percentage He obtained.

Isolation and solubilization of goose erythrocyte membranes

Goose erythrocyte membranes were prepared by the method of Ginsberg et al. [6] as previously described [15]. Briefly, red cells, lysed by hypotonic buffer and washed several times to remove the hemoglobin, were homogenized in a Dounce homogenizer (tight pestle). The purified membrane fraction obtained after sedimentation at 15,000 **g** for three times was resuspended in PBS and solubilized according to the method of VandenBerg et al. [22]. 4% octyl-β-D-glucopyranoside, 3% Triton X-100 and 1% Tween 80 in PBS were used. Detergents were removed from the supernatant obtained after centrifugation at 100,000 **g** for 1 h by overnight dialysis against PBS. Residual detergent was then removed by treatment with Bio-Beads SM_2 at 4 °C by using 0.64 g of beads per ml of dialyzed preparation. After mixing for 2 h, an additional 0.5 g/ml of beads were added for 1.5 h. Following removal of detergent, a membranous protein-lipid particle (membrane liposome) was obtained. As a control, PBS treated with the same detergents and removal procedures was found free of any inhibiting action on viral biological activities.

Isolation of membrane proteins

Proteins were extracted from isolated plasma membranes (12 mg/ml protein) using n-butanol according to the method of Maddy [13].

Isolation of membrane lipids

Total lipids were extracted from membrane with chloroform-methanol as previously described [15]. To obtain phospholipids and glycolipids, total lipids in chloroform : methanol 2 : 1 v/v were partitioned by adding one-fifth volume of distilled water according to Folch et al. [4]. Lipids were solubilized by sonication in PBS before use.

Enzyme treatment of solubilized membranes

Neuraminidase, phospholipases A2 and C were dissolved in PBS. Trypsin and pronase were dissolved in 50 mM Tris-HCl pH 8.2 and 7.4 respectively. Enzymatic digestion was carried out by incubating the membranes with enzymes for 1 h at 37 °C. The enzymes were then inactivated by heating the samples to 100 °C for 15 min.

Enzyme treatment of erythrocytes

Treatments were carried out on a 10% suspension of goose erythrocytes washed three times in PBS. Control and treated cells were incubated for 1 h at 37 °C (neuraminidase, phospholipases) or 23 °C (trypsin, α-fucosidase, β-galactosidase, α-mannosidase). They were collected by centrifugation and washed three times with PBS before being used for virus HA and He studies. After phospholipase A2 digestion, erythrocytes were incubated for 5 min at 23 °C with 100 mg/ml BSA to extract the reaction products [9].

Tetrathionate treatment of erythrocytes

Incubation procedure was essentially as described by Haest et al. [10]. Briefly, goose erythrocytes washed three times in PBS were suspended in 10 volumes of medium A (KCl 90 mM; NaCl 45 mM; Na_2HPO_4/NaH_2PO_4 10 mM; sucrose 44 mM) and incubated at 37 °C and pH 8 for 1 h with 20 mM tetrathionate. Erythrocytes washed three times were incubated for another 2 h in 10 volumes of medium A without tetrathionate at 37 °C and pH 7.4. After three washings in PBS they were used for virus HA and He studies.

Chemical determinations

Protein concentration was determined by the method of Lowry et al. [12] using BSA as a standard. Lipid concentration was determined by the procedure of Frings et al. [5] using olive oil as a standard. Sialic acid was determined by the thiobarbituric acid method of Aminoff [1].

Chemicals and enzymes

Octyl-β-D-glucopyranoside was purchased from Calbiochem. Triton X-100 and Tween 80 were obtained from Sigma. Neuraminidase (from *Vibrio cholerae*) was obtained from Behring. α-L-fucosidase (from beef kidney), β-galactosidase (from *E. coli*) and α-mannosidase (from *Canavalia ensiformis*) were from Boehringer Mannheim). Phospholipases A2 (from Bee venom) and C (from *Bacillus cereus*), pronase (from *Streptomyces griseus*) and trypsin (from bovine pancreas) were obtained from Sigma. Tetrathionate was from Merck.

Cholesterol, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, cerebrosides, phosphoserine, sialic acid, palmitic acid, and oleic acid were obtained from Sigma.

Results

Relationship between hemagglutination and hemolysis activity of RV

The correlation between per cent hemolysis and the hemagglutinating antigen dilution is reported in Fig. 1. A 100% hemolysis was obtained with a viral preparation possessing a HA titre of 256 and a protein concentration of 20 μg/ml. The range of maximum sensitivity of hemolysis was between 16 and 64 HA titre corresponding to 30–80% of hemolysis. For this reason in the He and HeI studies we used preparations giving approximately 50% hemolysis.

Inhibiting activitiy of solubilized erythrocyte membranes (SEM) and isolated membrane components

To identify molecule(s) with the properties of RV receptor, goose erythrocyte membranes were solubilized and used to compete with whole cells for viral binding and fusion. The receptor activity of SEM was assessed by verifying their capacity to inhibit viral HA and He. Results from Table 1 show that the inhibitory effect of SEM was potent on both viral biological activities. To evaluate the role of different membrane components, protein, lipid, phospholipid and glycolipid were extracted from erythrocyte membranes and tested for their inhibiting activity. Membrane proteins were unable to modify the viral HA and He at concentration up to 1.5 mg/ml. On the contrary, membrane

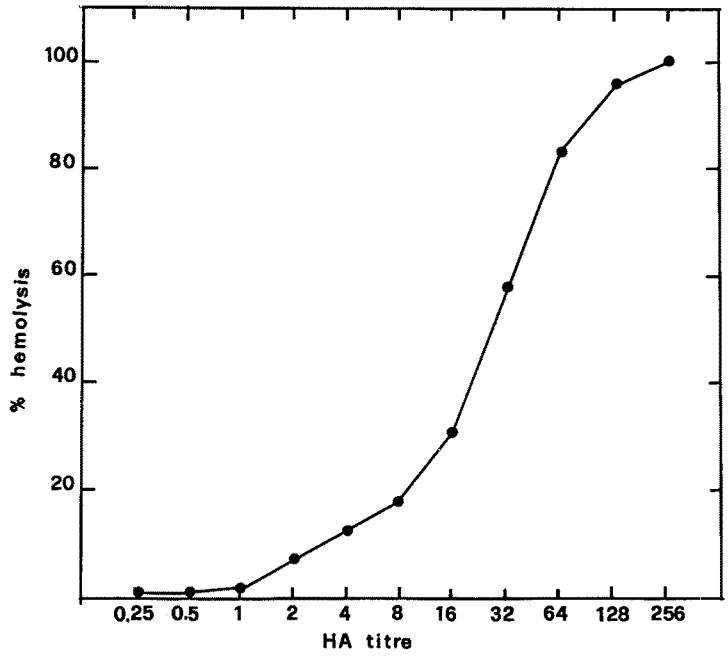

Fig. 1. Correlation between percent hemolysis and hemagglutination titre of RV

Table 1. Inhibiting activity of solubilized erythrocyte membrane (SEM) and extracted membrane components towards hemagglutinating and hemolytic activity of RV

	Concentration (μg/ml) for 50% inhibition	
	HA	He
SEM lipid	19	4.4
SEM protein	13	3.1
Protein	>1,500	>1,500
Total lipid	15.6	6.2
Phospholipid	21	7.7
Glycolipid	21	6.9

Values reported are the average of three different experiments

extracted lipids showed a strong inhibiting activity similar to that of whole solubilized membrane. To clarify the participation of the different lipid molecules in the erythrocyte membrane receptors for RV, total extracted lipids were separated into their phospholipid and glycolipid components. Both classes, obtained after Folch procedure [4], strongly reduced RV attachment and fusion.

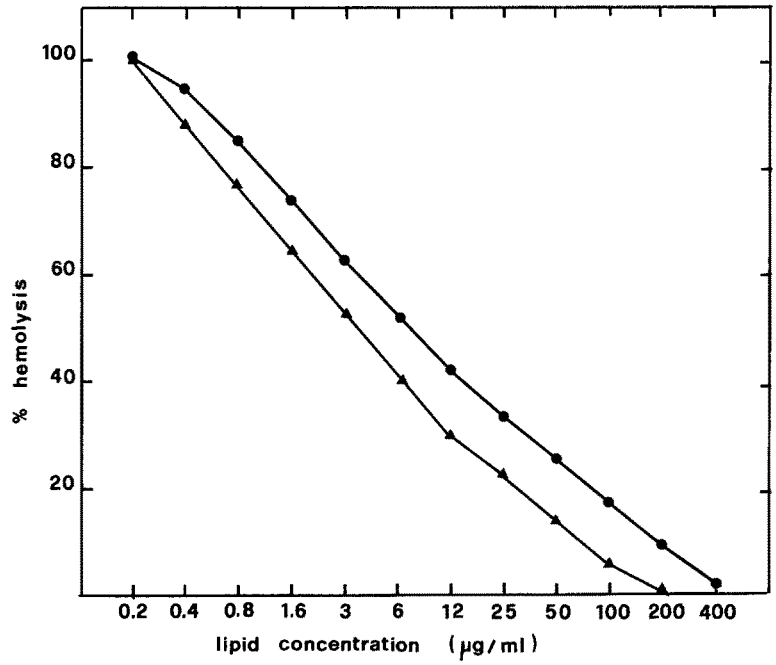

Fig. 2. Dose-dependent inhibition of RV hemolysis by SEM and total lipid extracted from goose erythrocyte membranes. RV was incubated for 1 h at 4 °C with twofold dilutions of SEM (▲) or total lipid (●) extracted from membranes before the addition of erythrocytes

The half-maximal inhibitory concentrations were very similar for all the active membrane components. All the inhibitors were more effective towards the hemolysis than the hemagglutination, despite the fact that in the hemolysis inhibition a seven fold concentrated viral preparation was used (Table 1). The inhibiting activity was due to direct action on the virus, since preincubation of erythrocytes with SEM or lipids did not modify their susceptibility to RV-induced HA and He (data not shown).

The dose-dependent inhibition of RV He produced by solubilized membranes and lipid components is recorded in Fig. 2. SEM and extracted lipid gave a complete inhibition of the hemolytic activity at a concentration of 200 and 300 µg/ml respectively.

Inhibiting activity of isolated membrane lipids

The major plasma membrane lipids were examined separately for their ability to inhibit RV induced hemagglutination and hemolysis in order to gain insight into the chemical nature of the active components. The results reported in Table 2 demonstrated that phosphatidylserine was the only phospholipid effective in the inhibition of both viral biological activities. Phosphatidylinositol, phosphatidylcholine, and sphingomyelin showed a strong inhibitory action only on the hemolytic activity, without any effect on the binding of the virus to

Table 2. Effect of individual membrane components on hemagglutinating and hemolytic activities of RV

	Concentration (μg/ml) for 50% inhibition	
	HA	He
Phosphatidylserine	62	97
Phosphatidylinositol	>1,000	137
Phosphatidylethanolamine	>500	>500
Phosphatidylcholine	>1,000	118
Sphingomyelin	>500	101
Cholesterol	>1,000	>1,000
GM3 ganglioside	>1,000	1,060
Neutral glycolipids	>1,000	>1,000
Cerebroside sulfate	31	52
Galactocerebroside type I	>1,000	810
Galactocerebroside type II	>1,000	810
Glucocerebroside	>1,000	>1,000
Phosphoserine	>1,000	>1,000
N-Acetylneuraminic acid	>1,000	>1,000
Palmitic acid	>250	154
Oleic acid	>250	>250

Ganglioside and neutral glycolipids were extracted from goose erythrocyte membrane. All other lipids were from Sigma. Lipids were tested at the highest non-hemagglutinating concentration

erythrocytes. Conversely, phosphatidylethanolamine was completely inactive. Cholesterol and oleic acid also failed to inhibit HA and He. Plamitic acid showed He inhibitory effect only at a relatively high concentration. Among glycolipids, cerebroside sulfate showed very potent HAI and HeI activity, whereas the corresponding neutral molecules (galactocerebrosides) were a very poor inhibitor of RV-induced hemolysis. In addition GM3 ganglioside, the major sialic acid containing glycolipid of goose erythrocyte membrane [15], had moderate HeI activity.

Enzyme treatment of SEM

To verify the role of different components in the interaction with rubella virus when arranged in a liposome membrane, SEM preparations were digested with several enzymes, as reported in Table 3. After the treatment, the enzymatic activity was destroyed by heating the samples to 100 °C for 15 minutes before determining their inhibitor effect. We previously verified that heating to 100 °C did not modify the initial inhibitory activity of SEM.

Table 3. Effect of enzyme treatments on inhibitory activity of solubilized erythrocyte membrane (SEM) towards hemagglutination and hemolysis by RV

	Enzyme[a] concentration	50% HAI[b]	% HeI[c]
SEM	0	19	45
+ freeze-thaw	0	19	48
+ 100 °C, 15 min	0	19	47
+ trypsin	5	38	72
+ pronase	5	28	56
+ phospholipase A2	50	76	2
+ phospholipase C	50	19	51
+ neuraminidase	0.5	28	64

[a] Protease concentrations are in mg/ml. Phospholipase and neuraminidase concentrations are in units/ml

[b] Values reported represent lipid concentration (μg/ml) of SEM giving 50% HA inhibition

[c] Lipid concentration of SEM was 4.7 μg/ml

Table 4. Effect of enzyme and chemical treatments of goose erythrocyte on the attachment and fusion of RV

Treatment	Concentration[a]	HA titre	% He
Neuraminidase	0	16	52
	0.1	64	81
	0.5	128	94
Trypsin	0	16	54
	1	96	77
Phospholipase A2	0	12	46
	10	16	54
	50	24	60
	250	48	71
Phospholipase C	0	16	48
	0.1	16	47
	1	16	48
	10	16	46
Untreated	0	16	44
α-L-fucosidase	0.094		
β-galactosidase	82.5	16	34
α-mannosidase	2.8		
Tetrathionate	0	16	58
	20	96	82

[a] Phospholipase, neuraminidase and glycosidase concentrations are in units/ml. Trypsin concentration is in mg/ml. Tetrathionate concentration is in mM

Trypsin and neuraminidase treatment produced an enhancement of the hemolysis inhibiting activity of SEM; on the contrary phospholipase A2 digestion produced a strong reduction in the capacity of membranes to inhibit viral attachment and fusion.

Enzyme and chemical modifications of whole erythrocyte membrane

In another series of experiments, whole cells were treated with enzymes and the sensitivity of modified erythrocytes to RV attachment and fusion was studied. Results obtained, reported in Table 4, demonstrated that removal of sialic acid from the cell surface by action of neuraminidase produced a strong increase in the binding and the hemolysis by the virus. This effect was dose-dependent. Trypsin and phospholipase A2 digestion of erythrocytes gave similar results. After treatment with phospholipase C the capacity of erythrocytes to bind RV and to undergo to lysis was unaffected. Glycosidases produced a slight reduction in the sensitivity of cells to viral hemolysis. Since some phospholipids (phosphatidylserine and phosphatidylethanolamine) are supposed to be located mainly in the inner lipid layer of erythrocyte membrane [25] we used tetrathionate to modify phospholipid asymmetry. It has been demonstrated that after SH oxidation by tetrathionate a reorientation of phosphatidylserine and phosphatidylethanolamine from the inner to the outer lipid monolayer of the membrane was obtained [10]. After treatment of erythrocytes with 20 mM tetrathionate a great enhancement in the agglutinability and hemolysis by RV was observed (Table 4).

Discussion

In this study the chemical nature of rubella virus receptor on goose erythrocytes was investigated by means of two different experimental approaches: a) by using natural liposomes extracted from membranes to compete with cells for virus binding and b) by enzyme treatment of whole cell membrane.

The solubilization of erythrocyte membranes by means of octyl-β-D-glucopyranoside, Triton X-100 and Tween 80 yielded an extract which reduced both binding and fusion of RV. The isolation of protein and lipid components from membranes showed that only the lipid moiety inhibited the viral activities, pointing towards the involvement of lipid structures as receptor sites for RV. In a more detailed analysis we ascertained that both phospholipids and glycolipids were responsible for the inhibition of virus attachment and fusion. The role of membrane lipids in the interaction with RV was further supported by the susceptibility of SEM to phospholipase A2 digestion.

There are four lines of evidence that proteins are not involved in the rubella virus receptor: a) membrane extracted proteins were devoid of any inhibitory effect; b) trypsin treatment of SEM produced an enhancement of the inhibiting activity; c) the activity of SEM was resistant to denaturing conditions; d) trypsin digestion of whole cells strongly enhanced their susceptibility to RV induced hemagglutination and hemolysis.

Neuraminidase treatment of erythrocytes increased the sensitivity of cells to attachment and fusion by RV indicating that sialic acid containing glycoproteins and glycolipids are not required for virus binding. The enhancing effect of neuraminidase digestion may be due to different mechanisms: a) the reduction

of the negative charge on the cell surface by the enzyme might decrease the repulsive electrostatic forces between virus particles and red cell receptors; b) removal of sialic acid residues could expose new binding sites for viral envelope glycoproteins; c) neuraminidase treatment could produce a nonspecific effect on virus receptor configuration by causing a rearrangement of cell surface molecules. The increasing effect on virus HA and He observed after trypsin treatment of erythrocytes can be referred to the same mechanisms since trypsin digestion produced a release of sialoglycopeptides containing about 70% of the total cell surface sialic acid (data not shown).

The role of lipids as membrane receptors for RV was further supported by results obtained in competition experiments using purified lipids. When the major membrane lipids were examined separately, it was found that phosphatidylserine and cerebroside sulfate were able very efficiently to reduce viral adsorption on erythrocyte surface and subsequent hemolysis and appeared to represent major binding sites for RV on erythrocyte membrane at acidic pH. Glycolipids are minor components of cellular membranes. Interestingly, cerebroside sulfate was the isolated membrane component most active in the inhibition. Therefore it can be hypothesized that the predominance of sulfatides in cell membrane of the central nervous system may account for the frequent localization of RV in nervous cells [2, 3] in children with congenital rubella syndrome.

The capacity of several pure phospholipids (phosphatidylinositol, phosphatidylcholine, and sphingomyelin) to inhibit the hemolysis but not the binding of virus to erythrocytes indicates that different membrane components are involved in the attachment and the real fusion step. Therefore binding and fusion activity of RV are not strictly correlated; HA is essential but not sufficient for fusion. It is possible to suppose the existance of a second receptor in the cellular membrane which is required for membrane fusion. Furthermore the different sensitivity of HA and He to phospholipids indicates that the hemolytic activity of the virus is located on a surface glycoprotein epitope distinct from, although functionally dependent on, that responsible for the hemagglutination.

As to the absence of reduction in the agglutinability of erythrocytes after phospholipases digestion it must be pointed out that receptor active phospholipid (phophatidylserine) is not accessible to enzymes [10]. It can be hypothesized that after initial loose binding with external membrane components, phosphatidylserine may be critical for a tight bond of RV with the cell surface. The treatment with tetrathionate, which exposes on the outer layer some inner phospholipids (phosphatidylserine, phosphatidylethanolamine) enhanced viral HA and He, confirming that phosphatidylserine actually represents a membrane receptor for RV.

Several reports [7, 14, 20] indicate that fusion at low pH is essential in the replicative cycle of most enveloped viruses, therefore the capacity of membrane lipids to inhibit RV attachment and fusion at acidic pH can suggest a role of these substances in the early stages of infection of susceptible cells by rubella virus.

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References

1. Aminoff D (1959) The determination of free sialic acid in the presence of the bound compound. Virology 7: 355–357
2. Callaghan N, Feely M, Walsh B (1977) Relapsing neurological disorder associated with rubella virus infection. J Neurol Neurosurg Psychiatry 40: 1117–1120
3. Desmond MM, Fisher ES, Vorderman AL (1978) The longitudinal course of congenital rubella encephalitis in non-retarded children. J Pediatr 93: 584–591
4. Folch J, Lees M, Sloane Stanley GM (1957) A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226: 497–509
5. Frings CS, Fendley TW, Dunn RT, Quenn CA (1972) Improved determination of total serum lipids by sulfo-phospho-vanillin reaction. Clin Chem 18: 673–674
6. Ginsberg BH, Kahn CR, Roth J (1976) The insulin receptor of the turkey erythrocyte. Characterization of the membrane bound receptor. Biochim Biophys Acta 443: 227–242
7. Gollins SW, Porterfield JS (1986) The uncoating and infectiviy of the flavivirus West Nile on interaction with cells: effect of pH and ammonium chloride. J Gen Virol 67: 1941–1950
8. Green KY, Dorsett PH (1986) Rubella virus antigens: localization of epitopes involved in hemagglutination and neutralization by using monoclonal antibodies. J Virol 57: 893–898
9. Haest CWM, Plasa G, Deuticke B (1981) Selective removal of lipids from the outer membrane layer of human erythrocytes without haemolysis. Consequences for bilayer stability and cell shape. Biochim Biophys Acta 649: 701–708
10. Haest CWM, Plasa G, Kamp D, Deuticke B (1978) Spectrin as a stabilizer of the phospholipid asymmetry in the human erythrocyte membrane. Biochim Biophys Acta 509: 21–32
11. Kobayashi N, Suzuki M (1982) Characterization of rubella virus-induced hemolysis. Arch Virol 71: 149–154
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
13. Maddy AH (1966) The properties of the protein of the plasma membrane of ox erythrocytes. Biochim Biophys Acta 117: 193–200
14. Marsh M, Bolzau E, Helenius A (1983) Penetration of Semliki forest virus from acidic prelysosomal vacuoles. Cell 32: 931–940
15. Mastromarino P, Conti C, Goldoni P, Hauttecoeur B, Orsi N (1987) Characterization of membrane components of the erythrocyte involved in vesicular stomatitis virus attachment and fusion at acidic pH. J Gen Virol 68: 2359–2369
16. Negro Ponzi A, Pugliese A, Pertusio P (1978) Rubella virus hemagglutination with human and animal erythrocytes: effect of age and trypsinization. J Clin Microbiol 7: 442–447
17. Oker-Blom C, Kalkkinen N, Kääriäinen L, Pettersson RF (1983) Rubella virus contains one capsid protein and three envelope glycoproteins, E1, E2a, and E2b. J Virol 46: 964–973

18. Oker-Blom C, Veman I, Kääriäinen L, Petterson R (1983) Rubella virus 40 S genome RNA specifies a 24 S subgenomic mRNA that codes for a precursor to structural proteins. J Virol 9: 403–408
19. Shortridge KF, Ho WKK (1976) Comparison of the activities in inhibition of haemagglutination by different togaviruses for human serum lipoproteins and their constitutents. J Gen Virol 33: 523–527
20. Talbot PJ, Vance DE (1982) Biochemical studies on the entry of Sindbis virus into BHK 21 cells and the effect of NH_4Cl. Virology 118: 451–455
21. Väänänen P, Kääriäinen L (1980) Fusion and haemolysis of erythrocytes caused by three togaviruses: Semliki Forest, Sindbis and rubella. J Gen Virol 46: 467–475
22. VandenBerg SR, Allgren RL, Todd RD, Ciaramello RD (1983) Solubilization and characterization of high-affinity (3 H)serotonin binding site from bovine cortical membranes. Proc Natl Acad Sci USA 80: 3508–3512
23. Waxham MN, Wolinsky JS (1985) Detailed immunologic analysis of the structural polypeptides of rubella virus using monoclonal antibodies. Virology 143: 153–165
24. Waxham MN, Wolinsky JS (1985) A model for the structural organization of rubella virions. Rev Infect Dis [Suppl] 7: 133–139
25. Zwaal RFA, Roelofsen B, Comfurius P, Van Dehen LLM (1975) Organization of phospholipids in human red cell membranes as detected by the action of various purified phospholipases. Biochim Biophys Acta 406: 83–96

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