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Simian Enterovirus SV2 Hemagglutination Studies. II. Effect of Various Treatments on the Hemagglutinin and Its Cell Receptors.* (30465)

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As a part of a continuing study of the characteristics of the simian enteroviruses, we have demonstrated that hemagglutinating activity (HA) is closely associated with the infectious virus particle of SV2(1) and appears to have the properties of hemagglutinins found associated with human enteroviruses(2). Since considerable work has been done on the cell receptors for the human enteroviruses, both hemagglutinating(3,4) and non-hemagglutinating(4-7), it was decided to extend our comparison by studying the receptors for SV2 HA on RBC and primary rhesus monkey kidney cells (PMK). This has been done by determining the effect of various treatments on the ability of SV2 HA to adsorb and elute from these cells.

Materials and methods. The virus and methods for its growth and assay have been described(1). All HA tests were done at 4°C.

Adsorption of HA and infectivity to PMK. Monolayers of PMK were washed 1× with PBS and inoculated at an approximate multiplicity of 1. At intervals the monolayers were washed 4× with PBS and overlaid with 0.25 ml of 1% α-chymotrypsin for 15 minutes at 37°C. HA and infectivity were then determined.

Treatment of cell receptors. Chemical. One-tenth milliliter amounts of packed RBC or 50% PMK in PBS were treated with 0.9 ml of the chemicals at the concentration indicated in Table I. After ½ hour at room temperature the mixtures were dialyzed overnight against cold PBS. In the case of chloroform, ether, butanol or genetron, 0.9 ml of PBS was added to the cells followed by 2 ml of solvent. After homogenization on a vortex mixer, the mixture was centrifuged to separate the phases and the solvent phase discarded.

Physical. Cells suspended in PBS were rapidly frozen and thawed 3× or disintegrated in a 10 Kc Raytheon sonic oscillator.

Enzymatic. A mixture of 0.9 ml of enzyme solution at the concentration indicated in Table II and 0.1 ml cell suspension was incubated at 37°C for 1 hour. The trypsin was obtained from Difco (1:250) and receptor destroying enzyme (RDE) from Microbiological Associates, Inc. All other enzymes came from Worthington Biochemical Corp. The pH of the reaction mixtures was 7.2-7.4 except for elastase (pH 8.0) and pepsin (pH 4.0).

Test for adsorption of HA. After treatment of the cells, 0.1 ml of virus was added and the mixture incubated in an ice bath for ½ hour. The cell debris was then sedimented at 1000 × *g* for 10 minutes and HA determined on the supernatant fluid.

Release of cell bound HA. Equal volumes of virus and packed RBC or a suspension of PMK containing approximately 5 × 10^6 cells per milliliter were mixed and incubated ½ hour at 2°C. The cells were then sedimented and washed 3× with PBS. One-tenth milliliter aliquots of the washed RBC or a

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50% suspension of PMK in PBS, with virus adsorbed, were then subjected to the treatments described under the previous headings and, after centrifugation at 1000 × *g* to sediment the cell debris, eluted HA in the supernatant fluid was determined.

Inactivation of HA. 0.1 ml virus suspension was mixed with 0.9 ml of chemical or enzyme and incubated ½ hour at room temperature (1 hour at 37°C with enzymes). The mixture was then diluted for HA titration.

Results. Adsorption of HA and infectivity to PMK. Adsorption studies were carried out to determine whether HA and infectivity adsorbed to PMK cells at similar rates, as was demonstrated for RBC(1). As can be seen in Fig. 1 both activities were adsorbed at the same rate. This provides further proof of the close association of HA with infectivity.

Effect of various chemical and physical treatments on cell receptors and HA. It can be seen in Table I that, with few exceptions,

TABLE I. Effect of Chemical and Physical Treatments on RBC and PMK Receptors.

Treatment	Inactivation of HA	RBC		PMK	
		Inactivation of cell receptor	Release of cell bound HA	Inactivation of cell receptor	Release of cell bound HA
A. Chemical					
Urea, 8M	+*	—	ND	—	ND
Guanidine, 5M	—†	+	+	+	+
Phenol, 5% in PBS	+	+	ND	+	ND
Formaldehyde, 5% in PBS	+	—	ND	+	ND
Sodium deoxycholate, 0.5%	—	—	+	—	+
Potassium periodate, 10^{-2}M	—	—	ND	—	ND
p-Chloromercuribenzoate, 10^{-4}M	+	—	ND	—	ND
Versene, 5×10^{-2}M	—	—	—	—	—
pH 2.5 McIlwaine buffer	—	—	+	—	+
Lithium chloride, 6M	—	—	+	ND	+
Chloroform, 2 min extraction	—	+	—	+	—
Ether, 2 min extraction	—	—	—	—	—
5 " "	—	+	ND	+	ND
n-Butanol, 5 min extraction	—	—	—	—	—
Genetron, 5 " "	—	+	—	+	—
B. Physical					
Freeze and thaw, 3×	—	—	±‡	—	—
Sonication, 10 min	—	+	±	—	—
PBS control	—	—	—	—	—

* + = Treatment 50-100% effective. † — = Treatment <10% effective. ‡ ± = Inconsistent results, 10-50% effective. ND = Not done.

TABLE II. Effect of Enzymes on RBC and PMK Receptors.

Enzyme	Inactivation of HA	Rhesus RBC		Primary rhesus kidney cells	
		Inactivation of cell receptor	Release of cell bound HA	Inactivation of cell receptor	Release of cell bound HA
PBS control	—*	—	—	—	—
Carboxypeptidase A, 0.25%	—	—	ND	ND	ND
" B, 0.5%	—	+†	+	+	+
Papain, 0.5%	—	+	+	+	+
Elastase, 0.5%	—	+	+	+	+
Pepsin, 0.5%	—	—	ND	ND	ND
Trypsin, 0.5%	—	+	+	+	+
Lipase, 0.5%	—	—	—	—	—
Phospholipase C, 0.5%	—	—	—	—	—
α-Chymotrypsin, 0.5%	—	+	+	+	+
Hyaluronidase, 0.5%	—	—	—	—	—
RDE (titer = 1000)	—	—	—	—	—

* — = Treatment <10% effective. † + = Treatment 50-100% effective. ND = Not done.

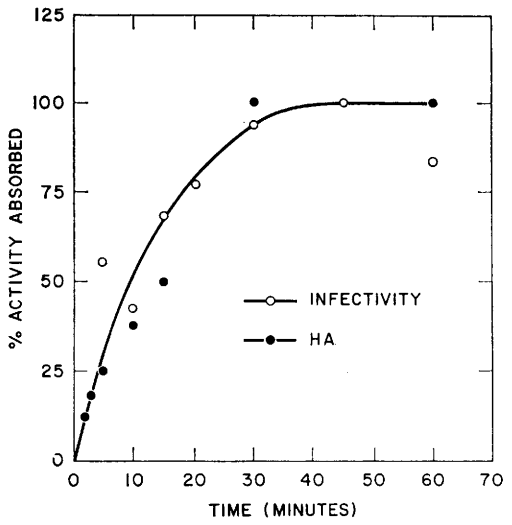

FIG. 1. Adsorption of SV2 virus hemagglutinating activity and infectivity to PMK.

the receptors on both cells reacted similarly to the various chemical and physical treatments to which they were exposed. Guanidine, phenol, chloroform, ether and genetron destroyed receptor activity on both types of cells. Formaldehyde destroyed receptor activity on PMK but not RBC, while prolonged sonication inactivated the RBC receptors but not those on PMK. Treatment with chloroform, ether and genetron, while destroying receptor activity, failed to release HA bound to either cell type. On the other hand, sodium deoxycholate (SDC), low pH and high concentrations of lithium chloride released cell bound HA without destroying receptor activity. Periodate showed no effect on the receptors.

HA was destroyed by phenol, formaldehyde, urea and p-chloromercuribenzoate (pCMB). It can be seen the latter two had no effect on the cell receptors under the conditions employed.

Effect of enzymes on cell receptors and HA. Table II demonstrates that SV2 HA was not inactivated by any of the enzymes tested. On the other hand, receptor activity was destroyed on, and bound HA released from, both RBC and PMK by carboxypeptidase B, papain, elastase, trypsin and α-chymotrypsin.

Interaction of HA with RBC receptors. We previously showed that SV2 HA adsorbed

TABLE III. Elution of SV2 HA from RBC.

Time (hr)	HA titer of eluted virus	HA titer of virus released by chymotrypsin
0	<2	64
1	<2	128
2	<2	128
3	<2	64
4	<2	256

Titer of virus before adsorption—128.

to RBC at 0°C at the same rate as at 37°C in spite of the fact that hemagglutination occurred at 0°C but not at 37°C. Table III shows the results of an experiment designed to determine whether SV2 HA was eluting from RBC at 37°C or penetrating into the cell. This was accomplished by measuring elution of virus and by using chymotrypsin to release bound virus which had not penetrated into the cell(3).

It can be seen that HA failed to elute from RBC at 37° during the 4 hours of observation. As evidenced by the release of bound virus by chymotrypsin, this virus also failed to penetrate into the cell. In other experiments chymotrypsin eluted virus from RBC as long as 120 hours after adsorption.

Discussion. It has previously been shown that the receptors for hemagglutinating human enteroviruses are similar on human RBC and HeLa cells(3). In addition, poliovirus receptors on HeLa cells have been shown to be different from those for the hemagglutinating enteroviruses(3).

The receptors for SV2 virus on rhesus RBC and PMK are destroyed by papain, chymotrypsin, trypsin, elastase and carboxypeptidase B but none of the non-proteolytic enzymes. In addition receptor activity is destroyed by guanidine, ether, chloroform, genetron and phenol. Periodate failed to effect receptors on either cell type. The only differences noted were that prolonged sonication destroyed RBC receptors but not those found on PMK, whereas, formaldehyde destroyed PMK receptors but not RBC receptors. Therefore, one may conclude that, as with the human enteroviruses, the receptor material for SV2 virus on rhesus RBC is lipoprotein and similar in composition and structure to that on PMK.

As with the poliovirus-HeLa cell system, low pH and high salt concentration dissociate the virus-receptor complex. It is interesting that lipid solvents destroy receptor activity without releasing receptor bound virus. This indicates that, once receptor protein is bound to the virus, receptor lipid can be dissolved without effecting the receptor-virus complex. Therefore, lipid may not function as receptor material, except to preserve the integrity of the protein moiety in the membrane structure. SDC, on the other hand, releases bound virus without destroying receptor activity. Preliminary experiments suggest that SDC may be solubilizing RBC receptor but further work is required to confirm that the observed inactivation of HA is due to receptor activity.

The receptor material reported on here appears to possess properties common to the hemagglutinating and non-hemagglutinating human enterovirus receptors(3-8), *i.e.*, it resembles poliovirus receptors in its sensitivity to lipid solvents, hydrogen bond disruptors and trypsin, and ECHO 7 receptors in its sensitivity to chymotrypsin. This intermediate position may merely be a reflection of the different cell species used or differences in experimental technique and therefore lack taxonomic significance for the viruses involved. It is of interest that SV2 HA was inactivated by pCMB in a manner similar to human enteroviruses(9,10), *i.e.*, HA was inactivated but no effect was observed on cell receptors.

The bond formed between SV2 and RBC appears to be quite stable, as evidenced by its failure to elute at 37°C even though there is no hemagglutination at this temperature. In this respect it resembles ECHO 13 and 19(11) which also fail to elute or cause hemagglutination at 37°C. The reason for this remains obscure but it may be that the bond formed between SV2 and RBC at 37°C is different from the bond which causes hemagglutination, the former being electrostatic and the latter a temperature dependent bond.

Summary. A study was made of the effect of various chemical, physical and enzymatic treatments on the receptor activity of rhesus RBC and PMK for SV2 virus. The results indicate that the receptors are lipoprotein in nature and similar to one another on both types of cell.

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Assay of Papain Using I^{131}-Labeled Albumin.* (30466)

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Albumin and casein, labeled with I^{131}, have been reported to be suitable substrates for measurement of the activity of papain and other proteolytic enzymes(1-6). Radiochemi-

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