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Hemagglutination with Simian Papovavirus SA12

KEERTI V. SHAH,* JANET D. VALIS, AND RICHARD W. DANIEL

Department of Pathobiology, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205

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Simian papovavirus SA12 agglutinated human, guinea pig, and chicken erythrocytes. SA12 hemagglutinin was most effectively released from debris of infected tissue culture cells at an alkaline pH.

SA12 is a newly recognized, immunologically distinct, simian papovavirus of the simian virus 40-polyoma subgroup (13). The virus was originally recovered from a kidney culture of the vervet monkey *Cercopithecus pygerythrus* (8), but serological studies suggest that its principal natural host is the South African chacma baboon (*Papio ursinus*) (13). Papovaviruses of this subgroup of human (BK virus [BKV] and JC virus), rabbit (rabbit kidney vacuolating virus), and mouse (polyoma and K virus) origin agglutinate erythrocytes of one or more species (2, 5, 6, 9). We have therefore tested the two newly recognized simian papovaviruses SA12 and the stumptailed macaque virus (STMV) (7), as well as simian virus 40 (SV40), for this property. We report here that SA12 agglutinates human, guinea pig, and chicken erythrocytes and that, like polyoma virus (1), viral hemagglutinin from debris of infected tissue culture cells is released most efficiently at an alkaline pH. No hemagglutination (HA) was observed with SV40 and STMV. Further, results of hemagglutination-inhibition (HAI) tests with SA12 and BKV indicate that the two simian papovaviruses SA12 and SV40 are less closely related antigenically than human papovavirus BKV is to simian papovavirus SV40.

HA tests were performed with the microtiter technique (11). Erythrocytes from the following sources were tested: human (O blood group), White Leghorn chicken, Hartley strain guinea pig, rhesus macaque, Guinea baboon (*Papio papio*), and sheep. Erythrocytes were collected in Alsever solution, washed three times in 0.85% NaCl solution, and used at a concentration of 0.5%. Tests were performed at 37, 22, and 4°C with 0.85% saline (pH 7.0) as diluent for both the viruses and the erythrocytes. The virus preparations tested were plaque-purified SA12 grown in rhesus embryonic kidney cell line MA-104 (13), SV40 grown in BSC-1 cells, and STMV grown in early-passage rhesus kidney cells (7). Their infectivity titers were, respectively, $10^{7.0}$, $10^{8.0}$, and $>10^{6.0}$ 50% tissue culture infectious doses per ml. Each preparation contained about $10^{9.0}$ particles/ml as determined by electron microscopy after negative staining. The viruses were tested untreated or after treatment with receptor-destroying enzyme (RDE; Microbiological Associates, Bethesda, Md.) (4). Sera were extracted in acetone (3) for HAI tests.

SA12 virus agglutinated human, guinea pig, and chicken erythrocytes but not erythrocytes of rhesus macaque, Guinea baboon, and sheep (Table 1). Hemagglutination was observed only when tests were performed at 4°C. To obtain reproducible results, it was necessary to hold the microtiter plates with antigen dilutions at 4°C for 15 to 20 min before addition of erythrocytes. There was a difference in HA titers with erythrocytes of different species as well as with erythrocytes of different members of the same species. The hemagglutination of chicken erythrocytes was not related to the sex and age of the donor as it is with agglutination by arboviruses (10). All further work with SA12 was done with human erythrocytes because they gave consistently high titers and were readily available. Hemagglutination occurred over a wide range of pH; use of diluents over a pH range between 6.0 and 9.0 resulted in only a twofold variation in titer. Treatment of the virus with RDE resulted in a two- to eightfold increase in HA titer. In tests with untreated and RDE-treated STMV and SV40 viruses at the three temperatures, hemagglutination was not observed with any of the erythrocytes.

Crawford (1) showed that polyoma virus hemagglutinin is released from infected mouse embryo cell debris at an alkaline pH. A similar experiment was performed with SA12 virus. SA12-infected MA-104 cells were scraped from petri dishes 3 days after infection, when there was early viral cytopathic effect and the supernatant fluid was negative for viral hemagglutinin. The cells were pelleted by low-speed centrifugation (500 × *g*, 10 min), washed, and sus-

pended in tris(hydroxymethyl)aminomethane saline, and then ultrasonically disrupted. The cell debris was spun down (100,000 × *g*, 1 h) and suspended in saline. Quantities (0.1 ml) of this suspension were diluted with 0.9 ml of phosphate buffer (0.1 M) of the appropriate pH value and incubated at 37°C for 2 h. The cell debris was then spun down (500 × *g*, 10 min), and the supernatant fluids were tested for HA titers (Fig. 1). There was a marked increase in the release of virus with increase in pH; titers ranged from negative at pH 6.0 to 1:512 at pH 8.5 and 9.0. This pattern of greater virus release at alkaline pH was very similar to that described for polyoma virus (1).

Sera from chacma baboons, humans, and rhesus macaques were tested for HAI antibodies to SA12 and BKV viruses to check the specificity of the test and to examine the immunological relationship of SA12 to SV40 and BKV. Twofold dilutions of acetone-extracted sera, beginning at 1:20, were tested with 4 to 8 units of SA12 or

TABLE 1. *Hemagglutination tests with simian papovavirus SA12*

Erythrocyte donor	Reciprocal of SA12 titer at 4°C[a]
Human, O blood group	1,024, 1,024
Guinea pig, Hartley strain	512, 4,096, 1,024
White Leghorn	
Hen, 6 weeks old	256, 256
Rooster, 6 months old	4,096, 512
Chicks, 1 day old	256
Rhesus macaque	N,[b] N
Guinea baboon	N
Sheep	N

[a] Virus was treated with RDE. No hemagglutination was seen at 22 and 37°C. Each figure in this column represents an individual donor.

[b] Negative; lowest dilution tested 1:2.

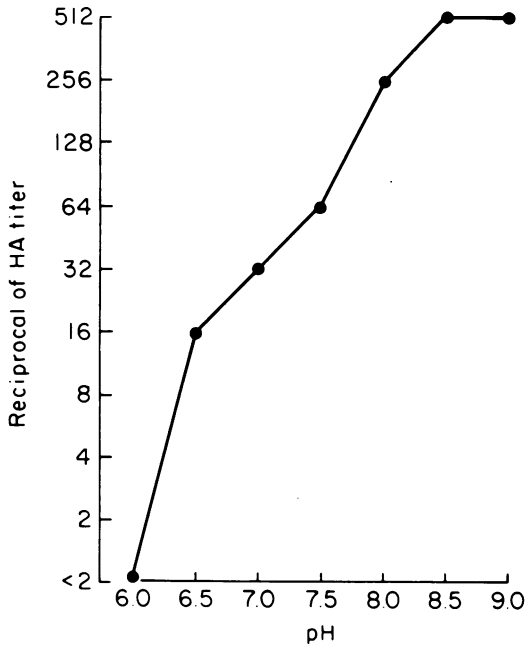

FIG. 1. *Release of SA12 hemagglutinin from infected MA-104 cell debris at different pH.*

TABLE 2. *Hemagglutination-inhibiting antibodies to SA12 and BKV in primate sera*

Serum donor	No. tested	No. of sera with HAI antibody titers:											
		SA12					BKV						
		N[a]	1:20	1:40	1:80	1:160	N	1:20	1:40	1:80	1:160	1:320	1:640
Chacma baboon[b]													
With SA12 neutralizing antibody	31	8	5	9	6	3	31						
Without SA12 neutralizing antibody	28						28						
Human[c]													
With BKV HAI antibody	26	26						1	6	2	4	11	2
Without BKV HAI antibody	15	15					15						
Rhesus[d]													
With SV40 neutralizing antibody	40	40					24	12	4				
Without SV40 neutralizing antibody	36	36					36						

[a] Negative; lowest dilution tested 1:20.

[b] These were selected from sera described in reference 13.

[c] From normal Maryland children (12).

[d] SV40-neutralizing antibody titers of positive sera ranged from 1:64 to 1:1,024 in tube neutralization tests.

BKV hemagglutinin. Serum-virus mixtures were incubated at room temperature for 1 h and then held at 4°C for 20 min. After addition of erythrocytes, the plates were again held at 4°C until settling or agglutination was observed.

SA12 HAI antibodies were detected in 23 of 31 (74%) baboon sera which were previously shown to have SA12 neutralizing antibodies (13) and in none of 28 sera which were negative for SA12 neutralizing antibodies (Table 2). The pattern of SA12 hemagglutination in wells with 1:20 and 1:40 dilutions of some of the negative sera was transient, so it was necessary to read the test as soon as possible. Thus, if the neutralization test is taken as the standard, the HAI test was completely specific and 75% sensitive. The HAI antibody titers of the positive sera ranged between 1:20 and 1:160. None of the baboon sera inhibited BKV hemagglutinin and, conversely, none of the human sera, whether they were with or without BKV HAI antibodies, inhibited SA12 hemagglutinin. These results confirmed the lack of cross-reactivity between SA12 and BKV which was previously reported on the basis of results of neutralization, immunofluorescence, and immune electron microscopy tests (13). All of the 40 rhesus sera with SV40-neutralizing antibodies were completely nonreactive with SA12 antigen, but 16 of these (40%) exhibited low levels of antibodies to BKV antigen; SV40 antibody-negative rhesus sera were negative with both antigens. These results suggest that SV40 is more closely related to BKV than to SA12. This finding was somewhat surprising because the natural hosts of SV40 and SA12 have a much closer phylogenetic relationship than the natural hosts of SV40 and BKV. Deoxyribonucleic acid homology studies have also shown a closer relationship between the deoxyribonucleic acid species of SV40 and BKV than between the deoxyribonucleic acid species of SV40 and SA12 (N. Newell, personal communication).

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