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Hemagglutination by Simian Rotavirus

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A simian rotavirus (SA-11) was shown to hemagglutinate human group "O" and guinea pig erthrocytes. The hemagglutinin appeared to be associated with the outer capsid of the SA-11 virus and was inhibited by specific hyperimmune anti-SA-11 guinea pig serum.

It has recently been reported that a calf rotavirus recovered in Canada possesses the ability to agglutinate human group "O" erythrocytes, and this hemagglutinin (HA) was inhibited by calf rotavirus antibodies (M. Fauvel, L. Spence, L. A. Babiuk, and R. Petro, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, RT30, p. 359; 6). When tested against hyperimmune guinea pig anti-human rotavirus serum, a low level or absence of HA inhibition (HAI) was observed; in addition, with calf rotavirus antigens, the HAI test was less efficient than the complement fixation test in detecting seroconversions with human acute- and convalescent-phase sera.

Stimulated by the finding of a calf rotavirus HA, we have studied the HA ability of various rotaviruses, with only limited success. In this report, we describe hemagglutination by a simian rotavirus, SA-11, which was originally isolated from a vervet monkey and is readily propagated in cell culture (3). Also described is a radioimmunoassay (RIA) test for detection of this simian agent.

SA-11-infected African green monkey kidney cell culture fluid (100 ml) was centrifuged at approximately 1,000 × *g* for 15 min to pellet the cells. The cell pellet was resuspended in 10 ml of 0.01 M tris(hydroxymethyl)aminomethane buffer (pH 8.0) and extracted with an equal volume of Genetron 113 (Allied Chemical Corp.), and the extract and supernatant fluid from the low-speed spin were combined. Virus was concentrated to 2.0 ml by first pelleting at 82,500 × *g* for 1 h in an SW27 rotor and resuspending the resultant pellet in 0.2 M tris-(hydroxymethyl) aminomethane-hydrochloride buffer at pH 8.0. The 2.0 ml of resuspended virus was layered onto a preformed 40 to 55% (wt/vol) CsCl density gradient (5) and centrifuged for 18 h at 152,000 × *g* in an SW40 rotor. Fractions (0.5 ml) were collected from the bottom of the tube, and their density was determined by relating refractive index to CsCl density.

A 1-to-100 dilution of a sample from each fraction was used for assay by RIA. The RIA test for antigen was the same as that described previously for human rotavirus (2). Briefly, 75 μl of a 1:1,000 dilution of hyperimmune anti-SA-11 guinea pig serum was used for precoating microtiter wells. The wells were then washed, and the diluted fractions were added and allowed to incubate for 2 h. The wells were washed, and 50 μl of guinea pig anti-SA-11 immunoglobulin G labeled with ^{125}I and adjusted to contain approximately 2×10^5 cpm was added. Following a 2-h incubation, the wells were washed, cut out, placed in tubes, and assayed for radioactivity. A positive over negative ratio was calculated for each fraction, with the residual counts present in wells containing saline as the negative control. The positive over negative ratio was plotted for each fraction.

A sample from each fraction was also assayed for HA by a conventional microtiter method. The samples were diluted twofold in phosphate-buffered saline, pH 7.4, supplemented with 0.5% bovine serum albumin. Fifty microliters of a 0.5% suspension of human group O erthyrocytes was added, and the reagents were mixed on a Micro Shaker (Dynatech Corp) for 5 s. The tests were recorded after 1 to 1.5 h, and the number of HA units was plotted for each fraction.

Hemagglutination of human O erythrocytes by the SA-11 virus was clearly demonstrated. The results from density, RIA, and HA determinations plotted against fraction number are shown in Fig. 1. The RIA demonstrated four distinct peaks with densities of 1.387, 1.369, 1.316, and 1.272 g/cm^3, and of these four, only one peak (1.369 g/cm^3) had a corresponding HA activity peak. One RIA peak with a density of 1.316 g/cm^3 had low-level HA activity (positive at 1:2). The starting SA-11-infected cell culture fluid was HA positive, but with a titer of 1:4.

In a second CsCl gradient, similar results for RIA, density, and HA were obtained, and all four RIA peak fractions were also examined by

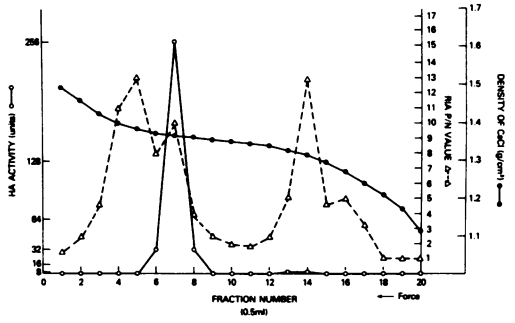

FIG. 1. *Cesium chloride gradient of SA-11 virus showing HA, density, and RIA results for each fraction.*

negative-stain electron microscopy using 3% phosphotungstic acid with 100 μg of bacitracin per ml at pH 7.4. Both higher-density RIA-positive peaks (densities of 1.379 and 1.359 g/cm^3) contained rotavirus particles and had no obvious particulate-contaminating material. In the first of these fractions, 70% of the rotavirus particles observed had all or most of their outer capsid missing. Ninety percent of the rotavirus particles observed in the second peak had intact outer capsids; this was the only RIA peak with a corresponding HA peak. The remaining two peaks (densities of 1.302 and 1.251 g/cm^3) contained much contaminating particulate debris. Rotavirus particles with both single and double capsids were present in both of these fractions, and full and empty particles of both types were seen in about equal proportions. In the less dense of these two fractions, many of the full particles were adsorbed to large irregular pieces of debris which were roughly several thousand nanometers in diameter.

Earlier workers (4) were unsuccessful in demonstrating HA activity with SA-11 virus. Such results may have been due to a low concentration of HA-positive particles in their preparations. HA activity in the starting SA-11 material was present at levels which at times were at the limits of detectability. The concentration and selection of HA-positive SA-11 particles resulting from techniques used in the present study removed the difficulties which existed when crude, untreated material was used.

The specificity of SA-11 virus HA activity was demonstrated by inhibition with guinea pig anti-SA-11 serum. This guinea pig had pre- and post-inoculation neutralization titers of 1:20 and 1:5120, respectively, and complement-fixation titers of 1:4 and 1:1024, respectively. Thirty-two 50% tissue culture infectious doses and 4 complement-fixation units of SA-11 virus were used in the respective tests. When tested for HAI activity with 4 to 8 HA units of SA-11 virus, the titers of the pre- and postsera were 1:32 and 1:4096, respectively. The specificity of the HAI activity in the preserum was investigated. The globulin fraction from the pre- and post-guinea pig serum was precipitated twice with saturated ammonium sulfate (pH 6.5), and the resuspended (in water) globulins were assayed for HAI activity. By this procedure, the HAI activity seen in the preserum was removed (titer <1:4), whereas the titer of the post-serum remained unaffected.

The SA-11 virus was also tested for HA activity using 0.5% suspensions of hamster, guinea pig, and chicken erythrocytes. The guinea pig cells hemagglutinated as well as did the human group O cells. The hamster and chicken cells were not agglutinated by SA-11 virus.

Thus the SA-11 virus which was shown to hemagglutinate guinea pig and human group O cells is the second rotavirus to demonstrate hemagglutination. The HA appears to reside on the outer capsid of SA-11 virions, since the HA-positive peak has a density of 1.359 g/cm^3, and 90% of the particles present in this peak had the outer capsid present. The RIA also detected virus particles in three other regions of the gradient, but in each case, at least half of the particles present in these regions were incomplete and HA was low or absent. Since type-specific antigens may reside in the outer capsid (1), the HA of rotaviruses may prove useful in defining rotavirus types.

ADDENDUM

After this report was submitted for publication, Fauvel et al., in a brief addendum to a report on hemagglutination by calf rotavirus (Intervirology 9:95–105, 1978), stated that they had prepared an HA from the simian rotavirus, but did not give the details of their studies with this virus.

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