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ANTIGENIC RELATIONSHIP BETWEEN HUMAN AND SIMIAN ROTAVIRUSES

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PLATE I

ROTAVIRUSES have been shown to be an important cause of infantile gastroenteritis in many parts of the world (Flewett, Bryden and Davies, 1973; Bishop *et al.*, 1974; Cruickshank, Axton and Webster, 1974; Kapikian *et al.*, 1974; Middleton *et al.*, 1974; Orstavik, Figenschau and Ulstrup, 1974; Tan *et al.*, 1974; Konno, Suzuki and Ishida, 1975; Lombardi *et al.*, 1975; Schoub *et al.*, 1975). Although in-vitro replication has been achieved in human foetal intestinal organ culture (Wyatt *et al.*, 1974) and in cell culture (Banatvala *et al.*, 1975; Purdham *et al.*, 1975; Wyatt *et al.*, 1976), the yield of virus has been low. The calf rotavirus, which has been adapted to grow in cell culture, cross-reacts antigenically with the human agent by complement fixation, immunofluorescence, immunoelectronmicroscopy and counterimmunoelectrophoresis, and has thus been widely used for serodiagnosis of human rotavirus infection (Flewett *et al.*, 1974; Kapikian *et al.*, 1974, 1975; Echeverria, Blacklow and Smith, 1975; Spence *et al.*, 1975). Its possible use for immunoprophylaxis has also been suggested (Kapikian *et al.*, 1975). The simian rotavirus SA 11 (Malherbe and Harwin, 1957) can be readily propagated in cell culture, and we have investigated its serological relatedness to the human virus with a view to using it for serodiagnosis and perhaps immunoprophylaxis of acute infantile gastroenteritis. The human and simian viruses were also compared serologically with the murine rotavirus, EDIM (Kraft, 1957).

MATERIALS AND METHODS

Patients. Stool specimens and acute and convalescent sera were obtained from six Black infants admitted to Baragwanath and Kalafong Hospitals in the Witwatersrand-Pretoria area for severe acute infantile gastroenteritis. Control sera were obtained from age-matched

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children attending the surgical out-patient departments, who were without any gastrointestinal symptoms. The ages of the patients and the controls ranged from 2 to 9 months (see table). The stool specimens and acute sera were collected 2-3 days after the onset of diarrhoea and convalescent sera 2 weeks later.

Viruses and antisera. (i) *Human rotavirus* was recovered from patients' stools by clarifying a 10% (w/v) aqueous homogenate of stool by low-speed centrifugation at 2000 *g* for 15 min. and then centrifuging the supernate through a 45% sucrose cushion at 100 000 *g* for 75 min. The virus deposit was resuspended in 0.5 ml of 0.85% saline per 10 ml of original homogenate. (ii) *SA 11 virus* was kindly supplied by Dr H. H. Malherbe, Southwest Foundation for Research and Education, San Antonio, Texas, and propagated on primary vervet-kidney cells. The virus was harvested, 8 days after inoculation, by freeze-thawing the cultures three times and then clarifying and ultracentrifuging the medium, as described above for the human virus. (iii) *EDIM virus*, kindly supplied by Dr T. H. Flewett, Regional Virus Laboratory, East Birmingham Hospital, Birmingham, was fed to randomly bred laboratory mice, previously shown to be rotavirus-free, 3-5 days old. Five days after infection, the gut was removed, homogenised in phosphate-buffered saline (pH 7.2) and extracted three times with fluorocarbon (Freon 113, Du Pont). The aqueous phases were pooled and then ultracentrifuged as for the human and simian viruses. (iv) *Hyperimmune rabbit antisera* to SA 11 and EDIM viruses were prepared by inoculating saline suspensions of the viruses into rabbits intravenously every 2 weeks for 2 months. The sera were absorbed overnight at 4°C with packed uninfected vervet-kidney cells or lyophilised rotavirus-free mouse-gut homogenate, respectively.

Immunofluorescence. SA 11-infected vervet-kidney cells were detached from 75 cm Falcon flasks by means of a rubber-tipped glass rod, washed three times with phosphate-buffered saline (pH 7.2) and suspended in 3 ml of buffer per confluent flask. Drops of cell suspension were placed in the holes of teflon-coated slides, allowed to dry, and then incubated with human serum or rabbit anti-SA 11 antiserum for 1 hr at 37°C, followed by fluorescein-conjugated anti-human or anti-rabbit antiserum (Wellcome Reagents), respectively.

Fluorescent antibody to EDIM virus was demonstrated with cryostat-cut sections of jejunum taken from mice 5 days after infection with EDIM virus. Immunofluorescence tests were performed as for the human and SA 11 agents.

The various tests were always done with controls, namely, uninfected vervet-kidney cells or uninfected mouse gut and serum from non-immunised rabbits.

Immunoelectronmicroscopy. Human rotavirus, SA 11 virus and EDIM virus were each tested against acute, convalescent and control human sera and against anti-SA 11 and anti-EDIM rabbit antisera. Saline suspensions of the viruses, prepared as described above, were mixed with an equal volume of a 1 in 10 dilution of each serum and incubated overnight at 37°C. The mixtures were then diluted 1 in 10 with distilled water and drops were treated with 3% phosphotungstic acid, at pH 6.0, and applied to formvar-coated copper grids; the excess was removed with filter paper and the grids were allowed to dry. The specimens were examined in a Philips EM 300 electron microscope at 60 kV.

Complement fixation. CF antibodies in human sera were determined, with a homogenate of SA 11-infected vervet-kidney cells as antigen, by the technique of Grist, Ross and Bell (1974).

Neutralisation. The human sera were examined for neutralising antibodies against SA 11 virus, in primary vervet-kidney cells and in LLCMK2, CV-1 and Vero cell lines by the standard tube neutralisation test described by Grist *et al.* (1974). The challenge dose of virus was 100 TCD50 and the cultures were examined daily for 28 days for CPE.

RESULTS

SA 11-antibody titres in human sera. Seroconversion was demonstrated in four of six patients by both complement fixation and immunofluorescence (table). One patient failed to develop fluorescent antibody and had only a

TABLE

The detection of immunofluorescent and complement-fixing antibodies in six patients with rotavirus gastroenteritis by the use of simian virus, SA 11, as antigen

Age of patient (months)	Immunofluorescent antibody titres		Complement-fixing antibody titres	
	Acute serum	Convalescent serum	Acute serum	Convalescent serum
9	4	64	8	512
6	4	16	4	128
2	<4	16	4	256
4	<4	64	<4	128
2	<4	<4	4	4
8	32	32	64	32

low titre of complement-fixing antibody, while another had relatively high antibody titres in both the acute and convalescent sera. Among the 10 control patients, eight had fluorescent antibody; two with titres of 8 and 16, respectively, the others with titres of only 4. Similar results were obtained with the CF test; 2 out of 10 control patients had no CF antibody, two had titres of 64, and the remainder titres of 8 and 4, respectively. None of the patients, with or without gastroenteritis, possessed demonstrable neutralising antibodies against SA 11 virus, whether the tests were carried out in vervet-kidney cells or in the various cell lines.

Immunoelectronmicroscopy. (i) *Human sera.* Acute sera from patients with gastroenteritis and sera from control patients without gastroenteritis agglutinated only "rough" virus particles (i.e., devoid of the outer capsid layer), both human and SA 11. On the other hand, convalescent sera from the patients with gastroenteritis agglutinated both "rough" and "smooth" (i.e., outer capsid layer present) particles of the two viruses (figs. 1 and 2). Clumped virus particles, invariably "rough", were occasionally seen in the virus suspensions used for these tests (prepared from stools collected during the first few days of illness), in the absence of either patients' or control sera; strands could be seen between the particles, resembling entibody (fig. 3). Both acute and convalescent sera, as well as the control sera, agglutinated only "rough" EDIM particles. (ii) *Anti-SA 11 antiserum* failed to agglutinate either "rough" or "smooth" human rotavirus particles; this was confirmed in further tests with virus particles from three different patients and antisera prepared in four different rabbits. It agglutinated "rough" and "smooth" SA 11 virus particles, but only "rough" EDIM virus particles. (iii) *Anti-EDIM antiserum* agglutinated "rough" and "smooth" EDIM virus particles but only "rough" SA 11 and human rotavirus particles.

DISCUSSION

Although the taxonomy of the rotaviruses has not yet been fully established, it has been proposed, on the basis of their morphological, biochemical and

biophysical properties, that they be tentatively classified within the family Reoviridae, which at present includes two recognised genera, *Reovirus* and *Orbivirus* (Holmes *et al.*, 1975; Rodger, Schnagl and Holmes, 1975). A distinctive feature of rotaviruses is a thin (about 2 nm) but sharply defined, outer capsid layer (Els and Lecatsas, 1972), which often "strips" spontaneously from the capsid (Schoub *et al.*, 1975). Two forms of virus particles are thus seen (Holmes *et al.*, 1975), double-shelled or "smooth" particles, which possess the outer capsid layer, and single-shelled or "rough" particles which lack the outer capsid layer.

The orbiviruses are characterised by a diffuse mantle surrounding the capsid (Lecatsas and Gorman, 1972) which has been shown to be composed of two polypeptides (Verwoerd *et al.*, 1972). The latter authors also observed that infectivity could be virtually abolished by removal of one or both of these polypeptides and then partly restored by allowing the polypeptides to recombine with the capsid. Whether the outer capsid layer of the rotavirus is analogous to the orbivirus outer mantle is conjectural at this stage. The presence of antibodies against the outer capsid layer in convalescent-phase sera, demonstrable by immunoelectronmicroscopy, suggests that this outer structure may well be involved in the infectivity of the virus. In this respect, information on its polypeptide composition, its influence on infectivity of the particle and the activity of antibodies against the purified layer, would be of great interest.

By immunoelectronmicroscopy, we have demonstrated two types of antibody in rotavirus infections. The first is directed against the inner capsid of the virus and displays broad specificity; it probably accounts for the low titres of fluorescent and CF antibody seen in eight of the 10 control patients. Since antibody to rotavirus is widespread amongst animals and human subjects, having been detected in 15 of 23 randomly selected human sera and in 54 of 56 randomly selected pig sera (Bridger *et al.*, 1975), the group antibody would appear to be a relatively persistent antibody. It presumably also develops very early in the course of the illness and may account for the spontaneous agglutination of "rough" virus particles seen in the early stool specimens.

The second antibody is directed against the outer capsid layer of the virus and is narrower in its inter-species specificity than the inner capsid antibody. The human and simian virus outer capsid layers show obvious cross-antigenic relationships, but are both antigenically distinct from the outer layer of the mouse virus. Similar species specificity of the outer capsid layer antigens was seen when human, calf and pig rotaviruses were compared by cross agglutination (Bridger *et al.*, 1975); the calf and pig rotaviruses could be distinguished from the human agent. Preliminary immunofluorescence-blocking experiments have also indicated that the human and SA 11 viruses are more closely related to each other than to the other rotaviruses (unpublished observations).

The antibodies directed against the outer capsid layer, present only in convalescent sera, are presumably the protective antibodies. The persistence of these antibodies and their correlation with host immunity require further investigation.

The antigenic relatedness of the simian to the human rotavirus and its ease of propagation make it very useful agent for the sero-diagnosis of human rotavirus infection. Furthermore, as SA 11 is the only rotavirus that displays cross-antigenicity with the outer capsid layer of the human virus, it may be an ideal candidate for further exploitation for immunoprophylaxis of human gastroenteritis, despite our failure to demonstrate neutralisation of the virus with convalescent human sera *in vitro*.

SUMMARY

The simian rotavirus, SA 11, and the murine rotavirus, EDIM, were investigated for antigenic relatedness to the human rotavirus, by immunoelectron-microscopy. These studies led to the recognition of two types of rotavirus antibody. One agglutinated "rough" virus particles only and was group-reactive; it appears to be widely distributed in various animal species, including human infants. The second antibody agglutinated "smooth" virus particles and was more species-specific, demonstrating only a one-way cross-reaction between the simian and human viruses; it was found only in convalescent-phase human sera and in hyperimmune rabbit sera and is probably protective.

The simian rotavirus is easy to propagate in primary cell culture and in cell lines and should prove useful for serodiagnosis of human gastroenteritis. It may be a candidate for immunoprophylaxis.

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FIG. 1.—“Smooth” particles of SA 11 virus agglutinated by convalescent serum from a patient with gastroenteritis. Arrows indicate antibody strands attached to the outer capsid layer. Electron micrograph (EM). $\times 150\,000$.

FIG. 2.—“Rough” particles of SA 11 virus agglutinated by acute serum from a patient with gastroenteritis. Arrows indicate outer capsid layer free of attached antibody strands; only the inner capsid layer shows attached antibody. EM $\times 200\,000$.

FIG. 3 (insert).—Clumping of “rough” human rotavirus particles in a serum-untreated stool suspension from a patient with acute gastroenteritis. Arrow indicates strands, possibly antibody, attached to the inner capsid layer of virus. EM $\times 200\,000$.