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Hemagglutination by a Human Rotavirus Isolate as Evidence for Transmission of Animal Rotaviruses to Humans

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Human rotavirus strain Ro1845, which was isolated in 1985 from an Israeli child with diarrhea, has a hemagglutinin that is capable of agglutinating erythrocytes from guinea pigs, sheep, chickens, and humans (group O). Hemagglutination was inhibited after incubation with hyperimmune sera or in the presence of glycophorin, the erythrocyte receptor for animal rotaviruses. These results suggest that Ro1845 is an animal rotavirus that infected a human child.

Group A rotaviruses, the major etiologic agent of acute diarrhea of infants and young children as well as young animals of many species (15), can be classified into at least 12 serotypes (3, 11, 19, 28, 29, 31) and two subgroups (9). The genome of rotavirus comprises 11 segments of double-stranded RNA. When these RNA segments are separated by polyacrylamide gel electrophoresis, short and long patterns are apparent, with the short pattern being characterized as an inversion in the migration order of gene segments 10 and 11 (4). With a few exceptions, subgroup I human rotavirus strains have short RNA patterns, whereas most animal rotavirus strains belong to subgroup I and have long RNA patterns. Thus, one may reasonably assume that subgroup I human rotaviruses with long RNA patterns have a high likelihood of being animal rotaviruses (9).

Molecular analyses by RNA-RNA hybridization showed that a few isolates of subgroup I human strains with long RNA patterns were much more closely related genetically to some animal rotavirus strains than to any other human rotavirus strains, providing evidence that rotaviruses cross species barriers under natural conditions (8, 24, 25, 27). Among such strains is the Ro1845 strain, which was isolated in Israel from a child with diarrhea and which has been shown to share a high degree of homology with feline strain Cat97 isolated in Australia as well as canine strain RS15 isolated in Japan (25).

Hemagglutination, an important biological property of rotavirus mediated by an outer capsid spike protein (VP4) which is encoded by gene segment 4 (14), has been demonstrated with a number of animal rotavirus strains, although a few animal strains such as bovine rotavirus UK lack such activity (5, 12–14). In contrast, no human rotavirus strains have ever been shown to hemagglutinate like many animal rotaviruses do, although there are reports describing the hemagglutination of fixed, but not fresh, 1-day-old chicken erythrocytes by human rotavirus virions that were not treated with trypsin (hence, they were noninfectious) (7, 16). It is therefore stressed that, thus far, positive hemagglutination activity among rotaviruses has been demonstrated only with animal strains, although not all animal strains hemagglutinate (14).

Here, we report that strain Ro1845 has hemagglutinin that is capable of agglutinating erythrocytes from various animal species, providing further support for our previous conclusion that Ro1845 may be an animal rotavirus that infected a human child.

The following human and animal rotavirus strains were used in this study: Wa, serotype 1, subgroup II (32); Ro1845, serotype 3, subgroup I (1); feline Cat97, serotype 3, subgroup I (2); canine RS15, serotype 3, subgroup I (22, 26); simian SA11, serotype 3, subgroup I (18); and bovine NCDV, serotype 6, subgroup I (20). These viruses were propagated in MA104 cells in the presence of 0.5 μg of trypsin per ml.

Hemagglutination and hemagglutination inhibition (HI) assays were performed in wells of microtiter plates by using Veronal-buffered saline (VBS; pH 7.0) containing 0.1% (wt/vol) bovine serum albumin and 0.001% (wt/vol) gelatin as a diluent. Human group O, chicken, guinea pig, horse, mouse, and sheep erythrocytes were used at 0.25% concentrations. Infected cell culture supernatant was diluted (two-fold dilution) with 50 μl of VBS and mixed with an equal amount of erythrocyte suspension. Results were read after a 3-h incubation at room temperature, and the titer was expressed as the reciprocal of the highest dilution of antigen showing complete hemagglutination. A virus suspension that contained four hemagglutinating units and a 0.25% suspension of guinea pig erythrocytes were used for HI tests. The test serum was absorbed with packed erythrocytes, mixed with acid-washed kaolin, and inactivated at 56°C for 30 min. The HI titer was expressed as the reciprocal of the highest dilution of antiserum which completely inhibited hemagglutination. Glycophorin type NM, which was purchased from Sigma Chemical Co. (St. Louis, Mo.), was also used in HI assays.

The spectra of various animal erythrocytes that were agglutinated by Ro1845 are shown in parallel with the spectra obtained with some reference animal rotavirus strains (Table 1). Ro1845 agglutinated guinea pig erythrocytes to a high titer, as did simian rotavirus strain SA11 and bovine rotavirus strain NCDV, which have long been known as hemagglutinating strains (5, 13, 14). The target specificity

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TABLE 1. Hemagglutination by rotavirus strains

Erythrocyte	HI titer					
	Wa	Ro1845	SA11	NCDV	Cat97	RS15
Human (group O)	<2	128	8	64	64	512
Sheep	<2	64	<2	8	64	512
Horse	<2	NT[a]	16	NT	NT	NT
Guinea pig	<2	1,024	32	512	2,048	1,024
Mouse	<2	NT	8	NT	NT	NT
Chicken	<2	256	16	<2	128	32
Day-old chicken (fixed)	<2	1,024	64	<2	1,024	256

[a] NT, not tested.

of the hemagglutinin of Ro1845 was identical to that of the hemagglutinin of feline strain Cat97 and canine strain RS15 (Table 1). A slight difference among Ro1845, SA11, and NCDV was noted, however. Both Ro1845 and SA11 agglutinated erythrocytes from chickens, either fresh or fixed, whereas NCDV did not. Furthermore, while both Ro1845 and NCDV agglutinated sheep erythrocytes, SA11 did not. On the other hand, human rotavirus strain Wa did not agglutinate any of the erythrocytes; this result is in good agreement with results of prior studies (7, 16). In addition, preliminary studies showed that human rotavirus strains 1076 (10), K8 (30), and 69M (19), each of which was proposed to possess a distinct VP4 serotype, did not agglutinate human group O erythrocytes (data not shown). Thus, in terms of the property of the hemagglutinin molecule, the Ro1845 strain is much more similar to animal rotavirus strains than to human rotavirus strains.

We then examined whether hemagglutination by Ro1845 was inhibited by various antisera that are known to inhibit hemagglutination by animal rotavirus strains. Hyperimmune sera to strains SA11 and RS15 inhibited agglutination of guinea pig erythrocytes by Ro1845, as they did hemagglutination by homologous strains (Table 2). However, these antisera had significantly lower HI titers to NCDV than to homologous strains. Conversely, anti-NCDV serum had significantly lower HI titers to heterologous strains than to the homologous strain (Table 2).

We further tested the inhibition of hemagglutination by glycophorin. Glycophorin has been demonstrated to be the erythrocyte receptor for animal, but not human, rotaviruses (7, 17) and to inhibit hemagglutination by both virions (7, 17) as well as by expressed VP4 molecules (17). The infected culture supernatant that was adjusted to contain four hemagglutinating units of strain Ro1845 was mixed with various concentrations of glycophorin. To this was added a 0.25% suspension of human group O erythrocytes. We observed that hemagglutination by Ro1845 was inhibited at a concentration of 16 μg of glycophorin per ml. Similarly, hemagglutination by strains NCDV and RS15 was inhibited in the presence of glycophorin at concentrations 16 and 8 μg/ml, respectively. Thus, glycophorin was considered to be the receptor molecule on the erythrocyte for Ro1845, as in the case of SA11, NCDV, and rhesus rotavirus strain RRV (7, 17).

TABLE 2. Comparison of rotavirus strains by HI test

Antigen	Reciprocal of HI titer of hyperimmune serum to rotavirus strain:		
	SA11	NCDV	RS15
SA11	5,120	160	160
NCDV	40	20,480	20
Ro1845	2,560	320	640
Cat97	1,280	320	640
RS15	5,120	320	640

The property of the hemagglutinin of Ro1845 revealed in this study was indistinguishable from the properties of hemagglutinins of animal rotaviruses, particularly those of feline and canine strains, and was clearly different from those of human strains. Thus, the results obtained in this study, when taken together with our previous observations at the molecular level (25), pointed to the conclusion that the origin of strain Ro1845 is an animal rotavirus, presumably of either feline or canine origin. This conclusion was consistent with an implication from retrospective investigations of the then 3-week-old baby from whom Ro1845 was isolated; in that situation, the baby's family raised a dog of less than a half year of age at the time of the baby's diarrheal episode. This situation is reminiscent of the Japanese child who had a clinical history of contact with a cat and from whom AU228 was isolated (27).

Although both AU228 and Ro1845 are considered to be of animal origin, strain AU228 is different from strain Ro1845 in its genogroup and hemagglutination activity; i.e., AU228, a member of the AU-1 genogroup (23), has a high degree of homology with feline strain FRV-1 (27), but not with strain Cat97, and neither AU228 nor AU-1 hemagglutinates (21). Furthermore, Ro1845 and AU228 were shown by hybridization studies not to be closely related to each other (25). Mochizuki et al. (21) have recently shown that there are two distinct genogroups among feline and canine strains and that the members of one genogroup (to which Cat97 belongs) hemagglutinate, whereas the members of the other group (to which FRV-1 belongs) do not possess hemagglutination activity. Thus, Ro1845 may be the first hemagglutinating animal rotavirus strain that has been shown to cross a host species barrier.

Contrary to the prevailing wisdom that animal rotaviruses do not infect humans under natural conditions (15), interspecies transmission of rotaviruses may occur, whenever such chances exist, and can be operative as a mechanism of the evolution of rotavirus. This should be kept in mind when vaccination strategies are developed.

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