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Diarrhea in Newborn Cynomolgus Monkeys Infected with Human Rotavirus

Summary: Of six newborn cynomolgus monkeys (*Macaca fascicularis*) naturally delivered and normally nursed five developed diarrhea after oral administration of human rotavirus. Virus excretion was observed in the stool of four animals. This virus was transmitted to four out of six other monkeys causing diarrhea in only one animal.

Zusammenfassung: Diarrhöe bei neugeborenen Cynomolgusaffen nach Infektion mit menschlichem Rotavirus. Sechs neugeborene Cynomolgusaffen (*Macaca fascicularis*) wurden oral mit menschlichem Rotavirus infiziert und natürlich aufgezogen. Diarrhöe wurde bei fünf und Virusausscheidung bei vier der infizierten Tiere beobachtet. Das ausgeschiedene Virus konnte auf vier von weiteren sechs Tieren übertragen werden, von denen nur ein Tier erkrankte.

Introduction

Using electron microscopy rotaviruses were detected in stools of infants and young children in various parts of the world, and their etiologic role in infantile gastroenteritis seems to be established (1). Further progress, however, is hampered by the lack of a productive 'in vitro' system for the propagation of the human rotavirus and of an established animal model for the disease. Successful infection of piglets (2), gnotobiotic calves (3), lambs (4), and rhesus monkeys (5) with the human rotavirus was recently described. Only colostrum-deprived monkeys delivered by Caesarean section developed diarrhea after infection. In search of a more feasible animal model using non-human primates, we inoculated juvenile and newborn cynomolgus monkeys (*Macaca fascicularis*) with the human rotavirus.

Virus Inoculum

The virus was isolated by ultracentrifugation of a clarified 10% stool suspension from fecal specimens of four children hospitalized with acute gastroenteritis. The pellet was resuspended in Eagle's Minimum essential medium supplemented with 2% bovine serum albumin and HEPES buffer pH 7.9. The virus suspension was distributed in 5.0 ml samples and stored at —80 °C. This corresponds to a 0.3% stool suspension. The virus was identified by typical morphology in electron microscopy and by counter-immunoelectrophoresis using a hyperimmune calf diarrhea virus serum. The calf diarrhea virus for immunization purposes was obtained by courtesy of Dr. *G.N. Wood,* Compton, Berks., Great Britain. The virus was shown to be serologically related to human rotavirus (6, 7).

Infection of Juvenile Cynomolgus Monkeys

Four juvenile cynomolgus monkeys approximately four to six months old were inoculated with 5.0 ml of human rotavirus suspension by stomach tube. Four similar animals constituted the control group. Stool samples were collected before inoculation and during the ten following days, and were examined by electron microscopy for the presence of rotavirus. The method has been described in detail (8). Serum samples were collected before and 14 days after inoculation and they were assayed for complement-fixing antibodies using the calf diarrhea virus as described previously (9). Three out of four inoculated animals had diarrhea on Days 1, 3, and 7 respectively. No virus excretion, however, was detected. Most animals had low initial antibody titers which remained essentially unchanged during the observation period. Thus the etiology of the diarrhea remains uncertain.

Infection of Newborn Cynomolgus Monkeys

In the next experiment six newborn naturally delivered and normally nursed animals of the same species were inoculated within 24 hrs of delivery as described previously. One additional animal served as an uninoculated control. Five infected animals developed diarrhea lasting two days on the average (Table 1). Four out of six animals excreted the virus which was identified by typical morphology and by counter-immunoelectrophoresis. Virus excretion lasted 1.8 days on the average. Serum samples were collected from the mothers at the time of inoculation and six weeks after, and from the babies six weeks after inoculation only.

Seroconversion occurred in three mothers during the observation period indicating a possible natural infection from their infected babies (Table 1). Only low antibody titers were observed in the babies.

Virus-containing stool of the animal No. 3257 (see Table1) was used to inoculate six other newborn monkeys. Table 2 shows that virus excretion was observed over a similar

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Table 1: *Diarrhea, virus excretion and antibody profiles of newborn cynomolgus monkeys inoculated with human rotavirus.*

Animal no.	Diarrhea[a]	Virus excretion	Complement fixing antibody titers (reciprocals)		
	(days after infection)		Mother 1st[b]	2nd[c]	Baby[c]
3033	2,3	no	10	40	10
3049	no	no	< 5	40	10
3245	no	5	< 5	10	5
3254	1,2	2,5	n.d.	n.d.	n.d.
3257	1,4	5,7,8	10	20	10
3275	5,6	5	< 5	40	10
Control animal	no	no	n.d.	n.d.	n.d.

[a]) liquid stools
[b]) at the time of inoculation
[c]) 6 weeks after inoculation

period of time at the same rate. Pathologic stools, however, were observed only in one animal. Reduced pathology was also described after one passage of human rotavirus in colostrum-deprived rhesus monkeys (5).

During our study, coronaviruses were observed irregularly in stool samples of almost all animals examined (unpublished results).

Table 2: *Diarrhea and virus excretion of newborn cynomolgus monkeys inoculated with human rotavirus after one passage in a cynomolgus monkey.*

Animal no.	Diarrhea[a] (days after infection)	Virus excretion
3024	no	no
3025	7,8	5
3236	no	4,5
3239	no	5,6
3242	no	5
3297	no	no

[a]) semisolid stools

Conclusion

We conclude that juvenile cynomolgus monkeys are obviously not a suitable animal model for human rotavirus infection. Similarly young animals of related species (*Macaca radiata* and *Macaca mulatta*) did not show clear signs of infection with this virus (5, 10). However, newborn cynomolgus monkeys which were naturally delivered and normally nursed, seem to be a promising animal model for the study of human rotavirus infection. At present we are using this model to examine the protective value of orally administered specific immunoglobulins.

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