Antibody to serotype 8 rotavirus in Ecuadorian and German children

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SUMMARY

Only 2 out of 71 German patients infected with rotavirus (3%) and 8 out of 147 German control patients (5%) showed serum antibody to the new serotype 8 rotavirus. Such antibody was detected in the sera of 232 of 870 Ecuadorian children (27%). Twelve Ecuadorian sera showed neutralizing activity only against serotype 8 and not to the other serotypes (1–4) tested, indicating that human serotype 8 rotavirus circulates in South America.

Rotavirus is a major cause of infectious gastroenteritis in children. Six serotypes of rotavirus have been identified in children: 1, 2, 3, 4, 8 and 9 [1]. The nucleotide sequence of the gene encoding the serotype-specific capsid glycoprotein, VP7, of all six human serotypes has been determined [2, 3]. Gene sequencing defined nine regions of VP7 which are variable across rotavirus serotypes established by cross-neutralization tests using animal hyperimmune sera. It is however still controversial whether antibody responses in children distinguish these six serotypes. Homotypic [4] and heterotypic [5–7] antibody responses have been reported after natural rotavirus infection. Up to now analysis has been restricted to serotypes 1, 2, 3 and 4 whose epidemiological importance has been established. In the present report we extend this analysis to serotype 8 rotaviruses, represented by rotavirus strain 69M, which was isolated from an Indonesian child with gastroenteritis [8] and strain 678, which was isolated from a British calf with diarrhoea [9].

A population-based serum collection from Ecuadorian children obtained during a representative nutritional and health survey in Ecuador [10] and a hospital-based serum collection from the University Children's Hospital at Bochum, West Germany (but excluding gastroenteritis patients) were screened for the presence of neutralizing antibody to serotype 8 rotavirus at a 1:90 serum dilution in the neutralization test described by Gerna and colleagues [11]. When the number of infected cells was reduced by 90% in comparison with virus-infected control cells the serum sample was said to be neutralizing. When the number of infected cells was only reduced by 50%, the serum sample was said to be partially neutralizing. Both serum collections and the prevalence of neutralizing antibody to serotype 1–4 human rotavirus have been described previously [12, 13].
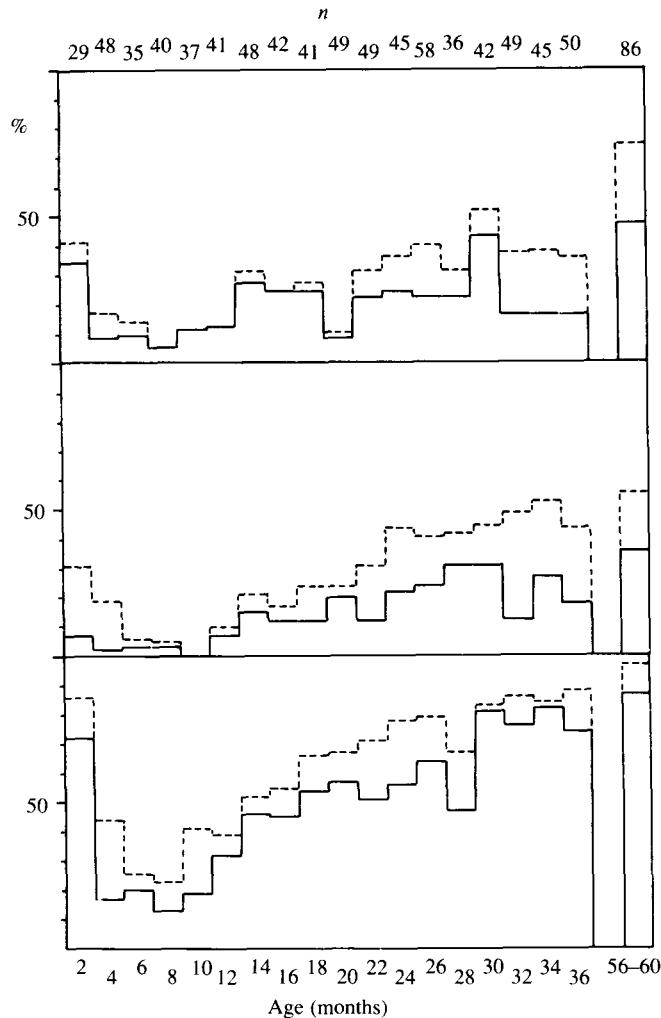
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Fig. 1. Prevalence of neutralizing antibody to serotype 8 human rotavirus 69M (upper panel), serotype 8 bovine rotavirus 678 (middle panel) and, for comparison, serotype 4 human rotavirus Hochi (lower panel) in different age groups of Ecuadorian children. Age groups are indicated by the upper age limit in months: 2 means children < 2 months of age, 4 means children > 2 and < 4 months of age etc. n gives the number of children in each two-month age interval. Neutralization criterion was 50% (‑‑‑) and 90% (——) reduction in the numbers of infected cells.

A total of 550 (63%) of 870 sera from Ecuadorian children neutralized at least one of the four serotypes and 476 sera (55%) neutralized human serotype 4 rotavirus [13]. Overall 190 out of 870 Ecuadorian sera tested (22%) neutralized serotype 8 human rotavirus 69M and 149 sera (17%) neutralized serotype 8 bovine rotavirus 678. A total of 232 sera (27%) neutralized at least one of these two serotype 8 rotaviruses: 107 sera neutralized both strains, whereas 83 and 42 sera neutralized only 69M or only 678 rotavirus, respectively. Figure 1 shows the age-related prevalence of neutralizing antibody to human serotype 8 rotavirus 69M (upper panel) in comparison to antibody to bovine serotype 8 rotavirus 678

(middle panel) and to antibody to human serotype 4 rotavirus Hochi (lower panel). Thirty-three per cent of the sera from infants up to 2 months old showed neutralizing antibody to rotavirus 69M; this prevalence decreased to a minimum of 5% in the children 6–8 months old. In older children the prevalence increased slowly to reach 46% in 5 year old children.

It is clear that not all sera neutralized both strains of serotype 8 and this observation raises uncertainties over which was the original infecting strain. A serum sample which neutralizes serotype 8 rotavirus does, however, not necessarily reflect exposure to serotype 8 rotavirus. Cross-reacting antibodies could be induced by exposure to other rotavirus serotypes [6, 7]. To study this point we analysed sera which neutralized serotype 8 human rotavirus 69M for their neutralizing capacity towards the four major human rotavirus serotypes (Table 1).

Eighty-one, 69, 48 and 87% of the 190 sera neutralizing serotype 8 human rotavirus 69M neutralized in addition human rotavirus serotypes 1, 2, 3 and 4 respectively. Notably 41% also neutralized bovine rotavirus NCDV (serotype 6), whereas only 12 sera neutralized serotype 8 rotavirus 69M but none of the four major human rotavirus serotypes. In addition none of these 12 sera neutralized serotype 6 (NCDV) and serotype 10 (V1005, [9]) and only one serum neutralized serotype 9 (WI61) and another serum neutralized serotype 5 (OSU) rotavirus.

Figure 2 shows the age-related prevalence of neutralizing antibody to both serotype 8 rotavirus strains in the hospital-based serum collection from Bochum. Only 8 out of 147 children (5%) between 8 and 48 months of age showed neutralizing antibody to human serotype 8 rotavirus in comparison with 66 children (45%) who showed neutralizing antibody to serotype 4 rotavirus Hochi. Eleven and 82% of 45 maternal sera from Bochum showed neutralizing antibody to rotavirus 69M and Hochi, respectively.

Paired sera were collected from infants who had been admitted during the years 1986 and 1987 to the University Children's Hospital at Bochum with symptoms of acute gastroenteritis or from infants who developed acute gastroenteritis in the hospital. Twofold dilution series starting with a 1:10 serum dilution were used in the neutralization test described by Gerna and his colleagues [11]. Neutralization titres were the mean of three determinations. Acute and convalescent sera from 71 patients hospitalized with a serologically defined primary rotavirus gastroenteritis were studied for seroconversions: 42 children seroconverted to at least one human rotavirus serotype. Interestingly 28 of them seroconverted to two or three serotypes [6]. However only 2 out of the 71 rotavirus patients seroconverted to serotype 8 rotavirus 69M (patient 13: titre increase from 10 to 70; patient 31: titre increase from 10 to 70), but not to serotype 8 rotavirus 678 (all titres < 10). Patient 13 showed also a titre increase to serotype 3 rotavirus (10 to 90) and patient 31 to serotype 1 and 3 rotavirus (10 to 100 and 10 to 90, respectively).

This low prevalence of neutralizing antibody to rotavirus 69M in both German serum collections suggests that German children recognize 69M rotavirus as different from the established rotavirus serotypes. This observation lends further support to the concept that human serotype 8 is distinct as established by animal hyperimmune sera [8, 14], gene sequencing [2, 3] and monoclonal antibodies [15].

In contrast, the sera from the Ecuadorian children showed a substantially higher prevalence of neutralizing antibody to serotype 8, although its prevalence

Table 1. *Specificity analysis of serotype 8-neutralizing sera in different age groups of Ecuadorian children*

Age in months

	<2	>2<4	>4<6	>6<8	>8<10	>10<12	>12<14	>14<16	>16<18	>18<20	>20<22
No. of sera tested...	29	48	35	40	37	41	48	42	41	49	49
No. of sera neutralizing the indicated serotype (strain)											
Serotype 8 (69M)	10	4	3	2	4	5	13	10	10	4	10
Serotypes 8+1 (69M+Wa)	8	2	2	1	1	2	10	5	8	4	6
Serotypes 8+2 (69M+DS−1)	4	1	0	1	1	2	9	5	8	3	3
Serotypes 8+3 (69M+Ito)	2	1	0	1	2	1	4	0	3	3	10
Serotypes 8+4 (69M+Hochi)	10	1	1	1	3	3	11	8	7	4	7
Serotypes 8+6 (69M+NCDV)	1	0	0	0	0	0	4	2	3	2	4
Serotype 8, but none of serotypes 1, 2, 3 or 4 (only 69M)	0	2	1	1	0	2	1	2	0	0	0

Age in months

|	>22<24	>24<26	>26<28	>28<30	>30<32	>32<34	>34<36	>56<58	>58<60	All ages
No. of sera tested...	45	58	36	42	49	45	50	33	53	870
No. of sera neutralizing the indicated serotype (strain)										
Serotype 8 (69M)	11	13	8	18	8	7	8	13	29	190
Serotypes 8+1 (69M+Wa)	10	12	8	12	7	7	7	13	29	154
Serotypes 8+2 (69M+DS−1)	7	11	8	11	7	7	7	13	23	131
Serotypes 8+3 (69M+Ito)	8	7	4	6	7	5	6	6	15	91
Serotypes 8+4 (69M+Hochi)	10	13	7	16	7	7	7	13	29	165
Serotypes 8+6 (69M+NCDV)	8	6	5	6	5	6	5	9	12	78
Serotype 8, but none of serotypes 1, 2, 3 or 4 (only 69M)	1	0	0	1	0	0	1	0	0	12
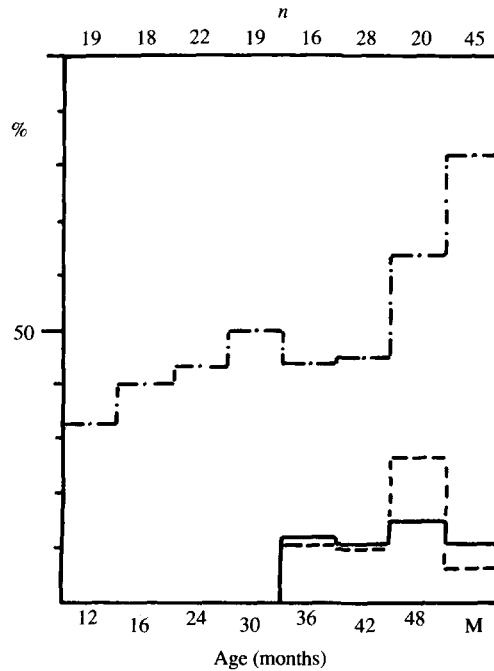
Fig. 2. Prevalence of neutralizing antibody to serotype 8 human rotavirus 69M (———),
serotype 8 bovine rotavirus 678 (– – –) and, for comparison, serotype 4 human rotavirus
Hochi (– · –) in different age groups of 8–48 months old German children. Age groups
are indicated by the upper age limit in months: 12 means children > 8 and < 12
months of age, 16 means children > 12 and < 16 months of age, etc. n gives the
number of children in each age interval. M indicates maternal sera from German
women.

is still lower than to any of the four established human rotavirus serotypes [13].
Except in Indonesia, the epidemiological importance of serotype 8 human
rotavirus has not yet been investigated. In Ecuador we obtained sera which
neutralized only serotype 8 rotavirus among those tested. This is sero-
epidemiological evidence that serotype 8 human rotavirus circulates in South
America. Definitive proof must however await direct serotyping of rotavirus from
stool samples with a serotype 8-specific monoclonal antibody [15]. Most of the sera
which neutralize serotype 8 rotavirus, also neutralize three or four of the
established rotavirus serotypes and 41 % of these sera neutralize also serotype 6
bovine rotavirus NCDV, to which children are not known to be exposed under
natural conditions [16]. Our tests therefore measured more the overall neutral-
ization capacity of the sera than a serotype-specific immune response to serotype
8 rotavirus. The availability of monoclonal antibodies to serotype 8 rotavirus [15]
might allow the development of a competitive binding immunoassay for detection
of a serotype 8-specific immune response [17, 18]. This epitope-blocking immuno-
assay will give a more reliable analysis of serotype 8-specific antibodies in children
than the neutralization test.

(only 69M)

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