Prevalence of Serum Neutralizing Antibody to Serotype 9 Rotavirus WI61 in Children from South America and Central Europe

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Neutralizing serum antibody to serotype 9 rotavirus WI61 was detected in 41% of 870 Ecuadorian children and 26% of 140 German children. In both areas an age-related prevalence increase was observed. We identified 11 serum samples from Ecuadorian children which neutralized exclusively serotype 9 rotavirus. Thirteen of 71 (18%) German children hospitalized with serologically defined primary rotavirus gastroenteritis showed a seroconversion to serotype 9 rotavirus; however, in 10 of these 13 patients, the infecting rotavirus could be identified as serotype 1, 3, or 4. Furthermore, all 13 patients showed fourfold increases in titer to at least one further serotype.

Rotaviruses are a major cause of diarrhea in young children (12). The development of an efficient rotavirus vaccine has been complicated by the serological diversity of rotaviruses (12, 14). Six serotypes of group A rotaviruses have been identified in children (6, 13, 19). The epidemiological importance of serotypes 1, 2, 3, and 4 of human rotavirus has been well established (18), while the epidemiological roles of recently described serotypes 8 and 9 have yet to be defined.

It is also a matter of controversy whether antibodies produced after natural rotavirus infection in children distinguish these six serotypes. Homotypic (20) and heterotypic (4, 10, 16) antibody responses have been reported, but to date, analyses have been restricted to antibodies against the four major human rotavirus serotypes. In the present report, we extend the antibody analysis to serotype 9 rotavirus.

Serum samples were obtained from 1,404 Ecuadorian children (0 to 5 years of age) during a nutritional and health survey carried out in Ecuador in 1986 (7). Rotavirus-specific antibodies immunoglobulin G, immunoglobulin M, and immunoglobulin A detected by enzyme-linked immunosorbent assay (ELISA) and neutralizing antibodies to serotypes 1, 2, 3, and 4 have been reported previously (3).

By using the neutralization test described by Gerna et al. (8), serum samples from children aged 0 to 36 and 36 to 60 months were screened for the presence of neutralizing antibody to human serotype 9 rotavirus WI61. A serum sample was counted as positive if a 1:90 serum dilution reduced the number of infected cells by 90% compared with virus-infected control cells. Overall, 360 of 870 serum samples tested (41%) neutralized rotavirus WI61, while 40% of the sera neutralized human rotavirus Wa (serotype 1), 29% neutralized DS-1 (serotype 2), 33% neutralized Ito (serotype 3), 53% neutralized Hochi (serotype 4) (3), and 23% of the sera neutralized the 69M rotavirus (serotype 8) (2).

The prevalence of neutralizing antibody to serotype 9 rotavirus was high in 0- to 2-month-old infants (41%), decreased to 6% in 2- to 4-month-old infants, and thereafter increased gradually with age (Fig. 1A). We identified 11 serum samples which neutralized serotype 9 virus but none of the four major serotypes or serotype 8. On the other hand, 21, 83, 136, and 109 serum samples were identified which neutralized one, two, three, or four of the major serotypes, respectively, in addition to serotype 9 (Fig. 1A).

Figure 1B shows the age-related specificity and neutralizing capacity of the serum samples from Ecuadorian children against the six human rotavirus serotypes. Of the 870 serum samples tested, 33% did not neutralize any of the six serotypes and 14% neutralized only one serotype. The highest prevalence of monotypic sera was detected in 2- to 12-month-old infants. In this age group, 55% of the neutralizing sera neutralized only one serotype. The prevalence of monotypic sera decreased to 22, 15, and 8% of the neutralizing sera in the second, third, and fifth year of life, respectively. Eight percent of the monotypic sera neutralized serotype 1, 11% neutralized serotype 2, 15% neutralized serotype 3, 46% neutralized serotype 4, 9% neutralized serotype 8, and 11% neutralized serotype 9.

Figure 2 shows the age-related prevalence of neutralizing antibody to serotype 9 rotavirus in a hospital-based serum collection from German children. Sera collected for diagnostic purposes in the years 1984 and 1985 at the University Children’s Hospital in Bochum, Federal Republic of Germany, were examined. Gastroenteritis patients were excluded. Isotype-specific ELISA antibody to rotavirus and neutralizing antibodies to serotypes 1, 2, 3, and 4 have been studied previously (5). Overall, 37 of 140 children (26%) between 8 and 48 months of age showed neutralizing antibody to serotype 9 rotavirus, and 50, 20, 41, 50, and 5% of the children had neutralizing antibodies to serotypes 1, 2, 3, 4 (5), and 8 (2) rotavirus, respectively. In addition, serum samples were obtained from 45 women from a neighboring maternity hospital in Bochum, Federal Republic of Germany. Fifty percent of the women showed neutralizing antibody to serotype 9, while 67, 31, 80, 82, and 11% showed antibodies to serotypes 1, 2, 3, 4, and 8 rotavirus, respectively (2, 5).

Paired serum samples were collected from 71 children with serologically defined primary rotavirus gastroenteritis who had been admitted during the years 1986 and 1987 to the University Children’s Hospital in Bochum, Federal Republic of Germany. Clinical details, isotype-specific ELISA antibody to rotavirus, and neutralizing antibodies to serotypes 1,

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2, 3, 4, and 8 have been analyzed previously (2, 4). Neutralizing antibody titers to serotype 9 rotavirus were determined by endpoint titration of the sera by using the neutralization test of Gerna et al. (8). A seroconversion was defined as an acute-phase serum titer of <90, a convalescent-phase serum titer of ≥90, and a fourfold or greater titer increase between the two titers. Thirteen of 71 children met our criteria for a seroconversion to serotype 9 rotavirus, whereas 31, 30, and 18 seroconverted to serotypes 1, 3, and 4 of rotavirus, respectively. However, there was no seroconversion to serotype 2 or 8. Overall, 42 of the 71 children seroconverted to at least one serotype. Eleven, 13, 15, and 3 seroconverted to one, two, three, or four different serotypes, respectively. Table 1 shows the serological reactions of children with a fourfold or greater increase in titer to serotype 9 rotavirus. No patients showing such increases exclusively to serotype 9 rotavirus were observed. On the other hand, seven patients showed fourfold increases in titer to serotypes 1, 3, and 9 of rotavirus, and four patients showed fourfold increases in titer to serotypes 1, 3, 4, and 9 of rotavirus. Serotyping of rotavirus strains was done by ELISA as previously reported (9). The infecting serotype was identified as a serotype 1 rotavirus in 7 of the 13 patients with fourfold increases in titer to serotype 9. There is a major controversy in the literature about primary infection inducing only homotypic (20) or homotypic plus heterotypic responses (4, 10, 16). An answer to this question will be important for vaccination strategies. Our data clearly demonstrate primary heterotypic responses to serotypes 3, 4, and 9 but not to serotypes 2 and 8 following serotype 1 infection.

A comparative analysis of the nucleotide sequences has identified nine defined regions on VP7 which are variable across rotavirus serotypes (11). These sequence analyses confirmed earlier cross-neutralization tests which designated 69M and W161 rotaviruses as new rotavirus serotypes. The low prevalence of antibody to serotype 8 rotavirus in sera from German and Ecuadorian children (2) demonstrated that the majority of the children would recognize serotype 8 rotavirus as distinct from the established rotavirus serotypes. However, this is not the case for serotype 9 rotavirus. In Ecuadorian children, antibody to serotype 9 rotavirus is the second most common rotavirus antibody. This confirms reports of a high prevalence of serotype 9 antibody in sera from American children (6).

Preliminary evidence indicates that serotype 9 rotaviruses are not common in the area from which they were isolated (6, 15). Thus, some of the antibodies neutralizing serotype 9 rotavirus may be induced by infection with other serotypes. Our observation that serotype 1-infected rotavirus patients seroconvert to serotype 9 supports this hypothesis. It seems improbable that these children showed an anamnestic response to a previous serotype 9 infection, since serological analysis (Table 1) failed to demonstrate a typical secondary antibody response. It is more likely that at least some rotavirus patients recognize a cross-neutralizing epitope shared between rotavirus serotypes 1, 3, 9, and possibly 4 (4, 10, 16). Rotavirus patients did not recognize this hypothetical cross-reacting epitope on rotavirus serotypes 2 and 8. On the basis of known rotavirus gene sequences (17), this pattern is most easily explained by postulating a VP4-specific antibody response in these children. In fact, immunoblotting techniques and neutralization tests with reassortant rotaviruses demonstrated an antibody increase to VP4 more frequently than to VP7 (1).
Homotypic and heterotypic antibody responses were observed in the Ecuadorian seroprevalence study. Homotypic antibody responses to all six human rotavirus serotypes were detected, although with different frequency. Eleven percent of the monotypic sera neutralized serotype 9 rotavirus. This is a seroepidemiological indication that serotype 9 rotavirus circulated in Ecuador. Definitive proof must await rotavirus serotyping in stool samples by using a serotype 9-specific monoclonal antibody (15).

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