Short Communication
Analysis of Rotavirus NSP4 Genotypes and Age-Dependent Antibody Response against NSP4 in Shanghai, China

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SUMMARY: This study aimed to determine the genotypes of NSP4 in children with acute rotavirus diarrhea and evaluate serum antibody titers to NSP4 in different age groups in Shanghai, China. A total of 171 stool specimens were collected from hospitalized patients ≤ 5 years of age who had acute rotavirus diarrhea between January 2003 and December 2006. Serum samples were collected from healthy individuals, including 200 for 0–60 months of age and 30 for over 5 years of age. NSP4-B type was the single predominant genotype during 2003–2006 in Shanghai. The titers of NSP4 specific IgG antibody increased with age after birth and peaked during 12–23 months of age, thereafter dropping to a level as low as that in the first 5 months of age. However, high levels of antibody against whole rotavirus were maintained in older children over 5 years of age and in adults. Information on prevalence of NSP4 genotypes in this area of China provides useful data for formulating vaccine policy. Short antibody immune memory compared with that induced mainly by rotavirus structural proteins indicated the protective effect against rotavirus may not persist long term if a single NSP4 protein is applied as the rotavirus vaccine.

Rotavirus is the most common cause of severe dehydrating diarrhea in young children less than 5 years and accounts for approximately 500,000 infant deaths annually (1). The rotavirus nonstructural protein 4 (NSP4) has been found to have an enterotoxin-like activity, originally mapped between amino acid (aa) 114 and 135 (2). Five distinct genetic groups (genotypes), A–E, were classified and genotypes A, B, and C have been detected in humans (3). NSP4 has been shown to induce both humoral and cell-mediated immune responses in humans (4,5). Passive and active immunization against the NSP4 protein have protected mice from diarrhea after both NSP4 and rotavirus challenges (6,7). But it remains unclear whether it is important for NSP4 to be utilized as an immunogen and included in rotavirus vaccine strategies. Information on the age distribution of NSP4 specific antibody in humans is scarce. The aim of the present study was to determine the circulating genotypes of rotavirus NSP4 in children with acute rotavirus diarrhea and also to evaluate the antibody responses to NSP4 in different age groups in Shanghai, China.

A total of 171 stool specimens were collected from hospitalized patients ≤ 5 years of age who had acute rotavirus diarrhea between January 2003 and December 2006. During the same period, 230 serum samples were collected from individuals without acute or chronic gastroenteritis. The 200 children under 5 years of age were arranged into 4 groups: 0–5 months (n = 41), 6–11 months (n = 40), 12–23 months (n = 40); and 24–60 months (n = 79). The other 30 serum samples were obtained from 25 children over 5 years old and 5 adults. This study was approved by the ethics committee of Children’s Hospital, Fudan University. A commercial immunoassay (Wantai, Beijing, China) was applied for detection of group A rotavirus. RNA extracted from stool specimens was subjected to semi-nested RT-PCR as described previously (8). NSP4 genotypes were determined by the size of the second round of PCR product, i.e., 610, 446, and 215 bp representing NSP4 genotypes B, A, and C, respectively.

The nucleotide regions encoding aa 86 to 175 of a simian RRV strain were generated by RT-PCR and subcloned into a vector pET19b (Invitrogen, Carlsbad, Calif., USA). The pET-NSP4 constructs were transfected into BL21 Star (DE3) pLysS cells (Invitrogen) by induction of isopropyl-D-thiogalactopyranoside (IPTG) (Wako, Tokyo, Japan), and the recombinant NSP4 (rNSP4) proteins were purified by affinity chromatography with His-binding resins (Novagen, Madison, Wis., USA). Expressions of purified rNSP4 were characterized by SDS-PAGE and visualized by Coomassie blue staining. A hyperimmune serum to RRV was produced in 7- to 8-week-old BALB/c mice by oral inoculation three times with 10⁶ PFU of RRV. The antigenicity of rNSP4 was characterized by Dot blot analysis with this polyclonal RRV-specific antibody. Purified rNSP4 proteins or supernatants from RRV MA-104 cell culture lysates were diluted in carbonate buffer (pH 9.6) and coated onto 96 microplates (Greiner Bio-One, Ber-

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lin, Germany) overnight at 4°C. After blocking,
twofold diluted serum starting from 1:25, was added
into the wells, and the plates were incubated for 2 h at
room temperature. Goat antihuman IgG conjugated to
horseradish peroxidase (HRP) (1:4,000 dilution; KPL,
Gaithersburg, Md., USA) was added to the plates and a
TMB solution (KPL) was used as a substrate. The IgG
antibody titers were defined as the reciprocal of the
highest dilution of serum with a net OD value (OD with
serum minus OD with diluent buffer) >0.1. Data analy-
sis on serum IgG levels between groups was carried out
with the SPSS 11.5 for Windows.

We first report the prevalence of circulating NSP4
genotypes causing diarrhea in children in Shanghai,
China. Among the total 171 rotavirus-positive stool
specimens during the year of 2003–2006 (47 in 2003, 42
in 2004, 35 in 2005, and 47 in 2006), NSP4 genotype B
was identified to be the single prevalent type of the
strain circulating in this area. Other similar studies indi-
cated NSP4 genotype B were identified in over 95% of
the detected samples, but NSP4 genotype A was also
present as a minor type. Generally, human rotavirus
VP7 genotypes, G1, G3, G4, and G9 belong to NSP4
genotype B, while G2 viruses are associated with NSP4
genotype A (8–10). Our previous report that G2 strain
was not detected in Shanghai during 2003–2005 possibly
resulted in the absence of NSP4 genotype A during a
similar period (11).

The recombinant peptide corresponding to NSP4
residues 86–175 was expressed in Escherichia coli BL21
Star (DE3) pLysS as a soluble form, and the molecular
mass was 20 kDa (Fig. 1A). The purified rNSP4 was
recognized by polyclonal anti-RRV antibody produced
in mice (Fig. 1B). As the antibody response to NSP4
(86–175 aa) has been confirmed to be heterotypic among
A, B, and C genotypes (12), we use the rNSP4 from
RRV (genotype C) to detect anti-NSP4 antibody direct-
ed to any genotype in sera. Infants less than 5 months
of age had low titers of NSP4 specific IgG antibodies.
The geometric mean titer (GMT) of IgG antibody to
NSP4 protein increased gradually with age after birth
and peaked with titer of 1:400 during 12–23 months of
age (P < 0.001 and P = 0.007 for comparison to 0–5
month group and 6–11 month group, respectively). The
levels of anti-NSP4 IgG antibody then dropped to 1:150
for children aged over 60 months (P = 0.009 for com-
parison to 12–23 month group). By contrast, a high
prevalence of serum IgG antibodies against whole
rotavirus was found in infants less than 5 months. After
a short decline during 6–11 months of age, this serum
response increased and peaked with titer of 1:1,600 dur-
ing 12–23 months of age. High levels of anti-rotavirus
antibody were maintained in older children over 5 years
of age and in adults (Fig. 2).

This pattern of antibody responses to whole rotavirus
in different age groups was similar with that in other
reports (13,14). Infants less than 6 months had remark-
able persistence of maternal antibodies against rotavirus.
After primary infection or reinfection, the antibody
titers increased with age and remained high in older chil-
dren and adults. But little information is available about
serum antibody response to NSP4 changing with age.
Our study demonstrated antibody titers against NSP4
gradually increased with age after birth and peaked dur-
ing 1–2 years of age mostly because children less than 2
years old accounted for 95% of rotavirus diarrhea in
China. However, with rotavirus episodes decreasing this
NSP4 specific antibody dropped to a level as low as that
in infants less than 6 months. These new findings add
important evidence of the insufficient data of antibody
response to NSP4 in humans. Other studies observed
rotavirus natural infections or vaccinations elicited a
significantly lower seroresponse against NSP4 than that
against VP6. Low humoral immune responses against
rotavirus NSP4 may have a short and poor immune mem-
ory compared with that induced by rotavirus structural
proteins (12,15).

In conclusion, information on prevalence of circulat-
ing NSP4 genotypes in Shanghai, China, provide useful
data for formulating vaccine policy, but the short last-
ing antibody response indicated that the protective ef-
fect against rotavirus may not persist long term if a single NSP4 protein is applied as the rotavirus vaccine.

REFERENCES


