Immune Response to Rotavirus Polypeptides after Vaccination with Heterologous Rotavirus Vaccines (RIT 4237, RRV-1)

By LENNART SVENSSON,1,2* HOOSHMAND SHESHBERADARAN,3 TIMO VESIKARI,3 ERLING NORRBY2 AND GÖRAN WADELL4

1Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, 2Department of Virology, Karolinska Institute, School of Medicine, S-105 21 Stockholm, Sweden, 3Department of Clinical Sciences, University of Tampere, SF-335 20 Tampere, Finland and 4Department of Virology, University of Umeå, S-901 85 Umeå, Sweden

(Accepted 30 March 1987)

SUMMARY

The antibody response to individual rotavirus polypeptides after vaccination with live attenuated heterologous rotavirus vaccines was studied with a radioimmunoprecipitation assay (RIPA). Following vaccination with bovine rotavirus strain RIT 4237 and rhesus monkey rotavirus strain RRV-1, an antibody response was demonstrated mainly against the inner capsid polypeptides VP2 and VP6 and to a lesser extent against VP3/VP4. There was no qualitative difference between the vaccines in the antibody response; both vaccines induced antibodies against the same polypeptides. Quantitatively the antibody response to RRV-1 was somewhat stronger. All pre-vaccination sera contained by RIPA low levels of rotavirus antibodies. The effect of these antibodies on the 'take' of the vaccines is discussed.

Heterologous rotaviruses and human–animal rotavirus reassortants have been proposed as candidate vaccines against human rotavirus diarrhoea (Clark et al., 1986; Kapikian et al., 1985a; Midthun et al., 1985; Vesikari et al., 1983). Two animal rotaviruses are currently under evaluation in clinical trials (Anderson et al., 1986; Kapikian et al., 1985a, b; Vesikari et al., 1983, 1984, 1986a). One of these is strain RIT 4237, an attenuated bovine rotavirus which has been shown to induce cross-protection to rotavirus diarrhoea in young infants in Finland (Vesikari et al., 1984, 1985). The RIT 4237 virus shares group antigens with human rotavirus, but is serotypically distinct (Hoshino et al., 1984). The other major vaccine candidate is a rhesus rotavirus, RRV-1, developed by Kapikian et al. (1985a). The RRV-1 virus also shares group antigens with human rotavirus but may have an immunological advantage over the RIT 4237 virus by being immunologically very closely related, if not identical, to human rotavirus serotype 3 (Hoshino et al., 1984). The RRV-1 virus is less attenuated and more immunogenic than RIT 4237 and when used at high doses it causes, in contrast to RIT 4237, significant febrile reactions in children (Vesikari et al., 1986a). So far, there is little information on the protective efficacy of the RRV-1 vaccine, but it may be assumed that, like the bovine rotavirus, the rhesus rotavirus will also induce cross-protection against human rotavirus. Little is known about the mechanism of protection against human rotavirus diarrhoea evoked by immunization with heterologous rotaviruses. In particular, the role of neutralizing antibodies is unclear. In spite of the fact that the RIT 4237 vaccine induces neutralizing antibodies only against the homologous bovine virus and not against human serotypes (Vesikari et al., 1983) it nevertheless conveys protection against human rotavirus diarrhoea. Of the two surface polypeptides (VP3 and VP7) involved in virus neutralization (Bastardo et al., 1981; Offit et al., 1985; Taniguchi et al., 1985), VP3 has been found to induce neutralizing antibodies against heterotypic rotavirus (Offit et al., 1985;
However, the role of VP3 in eliciting protection against rotavirus-induced diarrhoea is not yet known, nor is it known to what extent VP3 and VP7 induce an immune response following vaccination.

Recently, by use of a radioimmunoprecipitation assay (RIPA), we characterized the antibody response to different rotavirus proteins following a natural infection (Svensson et al., 1987). In the present study we have analysed the immune response to individual rotavirus polypeptides following vaccination with the heterologous rotavirus vaccines RIT 4237 and RRV-1.

The passage history and preparation of the live attenuated bovine rotavirus vaccine RIT 4237 and the rhesus rotavirus vaccine RRV-1 have previously been reported (Delem et al., 1984; Kapikian et al., 1985a).

Sera from children vaccinated with the RIT 4237 and the RRV-1 vaccines were obtained from a comparative trial in Finland (Vesikari et al., 1986a). Briefly, children 6 to 8 months old were given a single oral dose (0.5 ml) of the RIT 4237 vaccine lot no. L1109 at 10^{8.3} TCID_{50}/dose into the back of the mouth. The RRV-1 was given orally into the back of the mouth as a single dose, 10^5 to 10^6 p.f.u., in 1 ml. Serum specimens were collected before vaccination and on day 28 after vaccination. Of the 49 paired sera available, twelve serum pairs, six from the recipients of RIT 4237 and six of RRV-1, were selected for analysis in the present study. The selection was made on the basis that each of the 12 children had shown evidence of vaccine ‘take’ by at least one serological test (Vesikari et al., 1986a). Preimmune and convalescent sera from four naturally infected children were kindly provided by Dr I. Uhnoo, Uppsala, Sweden. Preimmune and convalescent sera were collected 1 to 2 days and 16 to 31 days after the onset of illness, respectively. The ages of the four control children with natural rotavirus infection were as follows: child 13, 6 years 3 months; child 14, 20 months; child 15, 8 years 6 months and child 16, 15 months. Two human rotavirus strains, DS-1 (subgroup I, serotype 2) and Wa (subgroup II, serotype 1), previously characterized by Dr R. Wyatt (NIH, Bethesda, Md., U.S.A.), were obtained from Dr B. Tufvesson (Malmö Allmänna Sjukhus, Malmö, Sweden). The human strains as well as simian rotavirus SA-11 (subgroup I, serotype 3) and the RIT 4237 strain (subgroup I, serotype 6) were plaque-purified twice before use.

Radiolabelling of intracellular rotavirus polypeptides was performed essentially as previously described (Svensson et al., 1987). Briefly, at 6 h post-infection, the original medium (Eagle’s MEM, 5 μg/ml trypsin, 8 μg/ml actinomycin D) was replaced by methionine-free Eagle’s MEM supplemented with 5 μg/ml trypsin and 8 μg/ml actinomycin D. Seven hours after infection the medium was replaced by Eagle’s MEM containing one-tenth the normal concentration of methionine, 100 μCi/ml [35S]methionine, 8 μg/ml actinomycin D, 5 μg/ml trypsin. After 3 h of labelling, cells were scraped off and pelleted by centrifugation for 1 h at 13 000 g. The RIPA and SDS–polyacrylamide gel electrophoresis (SDS–PAGE) are described in detail elsewhere (Svensson et al., 1987). The separation SDS gel (14 cm long) consisted of 11 or 9% acrylamide, 0.29% N,N’-methylenebisacrylamide. Trypsin (Type IX) and actinomycin D were purchased from Sigma. The ¹⁴C-methylated protein markers and [³⁵S]methionine were purchased from New England Nuclear.

Typical profiles of 35S-labelled polypeptides of DS-1 virus- and mock-infected cells are shown in Fig. 1. The mol. wt. of the virus-coded proteins were calculated and compared to previously reported data (Offit et al., 1983; Svensson et al., 1987). As reported previously (Svensson et al., 1987) NS1 and VP5 could not be identified in infected cell lysates (Fig. 1). These two proteins could, however, be identified after immunoprecipitation (Fig. 2). As reported previously (Svensson et al., 1987) VP3 and VP4 of the DS-1 strain could not be clearly separated and therefore immunoprecipitates in the mol. wt. region are referred to as VP3/VP4.

All sera were tested by RIPA in dilutions ranging from 1:10 to 1:50 000 against lysates of cells infected by SA-11, DS-1, WA or RIT 4237, and against mock-infected cells. To avoid variability in immunoprecipitation levels, all sera were simultaneously tested on the same batch of radiolabelled cell lysate for each virus strain. The immune response patterns exhibited by the sera were similar regardless of the virus antigen used; therefore only data obtained with the DS-1 strain are presented.
Short communication

Fig. 1. SDS-PAGE of [35S]methionine-labelled rotavirus polypeptides synthesized in the presence of 8 μg/ml actinomycin D. Lane 1, mock-infected cells. Lane 2, 14C-methylated mol. wt. markers, from the top: phosphorylase b (97000), bovine serum albumin (69000), ovalbumin (46000), carbonic anhydrase (30000), lactoglobulin (18000). Lane 3, DS-1 rotavirus-infected cells. Note that VP5 and NS1 with expected mol. wt. of 61000 and 56000, respectively, cannot be recognized.

None of the sera reacted at 1:10 dilution with mock-infected cells. Variation in the number of polypeptides immunoprecipitated with sera from vaccinated and naturally infected children were observed (Fig. 2). It appears that the pre-vaccination sera from all of the vaccinated children contained antibodies against VP2 and VP6. In some cases a very weak precipitation of VP3/VP4 could be seen although the titre levels varied between the sera. The antibody titres were low. Preimmune sera of two of the four naturally infected children (children 15 and 16) also contained rotavirus protein-specific antibodies as shown by RIPA. The most pronounced precipitates were again obtained with VP2 and VP6, but in addition VP3/VP4 were also immunoprecipitated. Two preimmune sera (children 13 and 14) showed no reaction by RIPA with any rotavirus polypeptides.

The immune response following vaccination with either RIT 4237 or RRV-1 appeared to be weaker than that after a natural rotavirus infection. The strongest immune response in the vaccinees was directed against VP6. Titration experiments showed that children who received RRV-1 vaccine had antibody titres between 5000 and 10000 in their convalescent sera against VP6 (the polypeptide inducing the strongest immune response) while the comparable antibody titres for RIT 4237 vaccinees were 500 to 5000. Thus RRV-1 appeared to be slightly more immunogenic than the RIT 4237 vaccine. A comparison of the immune responses to various DS-1 proteins in pre- and post-vaccination sera is shown in Fig. 3. Both vaccines accelerated the antibody response to VP2 and VP6 and to a lesser degree to VP3/VP4. None of the vaccinated
Fig. 2. Immunoprecipitation of [³⁵S]methionine-labelled human rotavirus (DS-1) polypeptides with (a) preimmune and (b) convalescent sera from rotavirus-vaccinated (lanes 1 to 12) or naturally infected children (lanes 13 to 16). All sera were tested at 1:10 dilution. Children 1 to 6 were vaccinated with RRV-1 and 7 to 12 were vaccinated with RIT 4237. Mol. wt. markers (M) were the same as in Fig. 1. Note that lactoglobulin (18000) is not separated, nor are VP7, NS2 or NS3 well separated, due to the use of a lower polyacrylamide concentration (9%).

children developed detectable antibodies against VP1, a core protein, or against VP5, VP7, VP8 or VP9. No reactivity was seen with the three non-structural proteins (NS1 to NS3). In contrast, two naturally infected children (children 13 and 14) seronegative in their preimmune serum, developed antibodies to VP2, VP3/VP4, VP6 and to NS2/NS3.

The present study was designed to analyse by use of a RIPA the immune response to human rotavirus proteins acquired by oral vaccination with the two current heterologous rotavirus vaccine candidates, RIT 4237 and RRV-1. The paired sera were selected on the basis of so called vaccine 'take' from a recently published vaccine trial (Vesikari et al., 1986a). All the preimmune sera tested were found by RIPA to have rotavirus antibodies. These sera were shown in the vaccine trial to be negative by complement fixation and ELISA. If the pre-vaccination antibodies in these children were due to a previous rotavirus infection and were not maternally acquired, the vaccine-induced responses might be regarded as booster reactions. However, the relatively low level immune response seen after vaccination compared to a natural infection
rather suggests that the pre-existing serum antibodies would be of maternal origin. The presence of such antibodies might still interfere with the 'take' of the RIT 4237 and RRV-1 vaccines, as shown previously with other serological tests (Vesikari et al., 1986a, b). The role pre-existing antibodies play in the 'take' of attenuated rotavirus vaccines remains to be elucidated. The RRV-1 vaccine was found to be more immunogenic than the RIT vaccine but there was no qualitative difference in the antibody pattern. Despite the close serological relation to human rotavirus serotype 3 (Hoshino et al., 1984) the RRV-1 vaccine failed to induce detectable antibodies against the outer viral capsid proteins. Although the RIT 4237 vaccine does not appear to induce VP7 antibodies or any significant neutralizing antibody response against human rotaviruses (Vesikari et al., 1983), it protects against rotavirus diarrhoea (Vesikari et al., 1984, 1985). It has been shown previously that neutralizing antibodies against the homologous vaccine virus are elicited by both RRV-1 (Kapikian et al., 1985a; Vesikari et al., 1986a) and RIT 4237 vaccines (Vesikari et al., 1986a). All sera of vaccinees analysed in the present study have previously been studied for neutralizing antibodies against homologous virus: all six RRV-1 recipients had a greater than fourfold antibody titre, whereas the number of RIT 4237 vaccinees with an antibody titre rise greater than fourfold was three of six (Vesikari et al., 1986a; unpublished data).

The post-vaccination immune responses were found by the RIPA test to be directed mainly to VP2, VP6 and to a lesser extent to VP3/VP4. The failure of the rotavirus vaccines to induce detectable antibody responses to VP7, the major polypeptide involved in virus neutralization (Offit et al., 1985; Taniguchi et al., 1985), is surprising. Lack of detectable antibody response to the glycosylated VP7 was also seen in the two naturally infected children without pre-existing immunity. Neither child had any VP7-specific antibodies at the time of serum collection (child 13, day 31; child 14, day 16), although both had neutralizing antibodies (low titres) against serotype 2 and 3, respectively (Svensson et al., 1987). However, antibodies to VP3 have previously been shown to induce heterologous protection in vivo (Offit et al., 1985) and such antibodies may also be involved in protection of the vaccinees. The presence of VP3 antibodies in the vaccine sera could not however, be clearly identified in this study, due to comigration of the VP3 and VP4 polypeptides.

Fig. 3. Comparison of immune responses (by RIPA) to various DS-1 polypeptides in pre- and post-vaccination sera and titrations of post-vaccination sera. (a) Child 1; recipient of RRV-1 vaccine; pre 1:50 (lane 1), post 1:100 (lane 2), 1:500 (lane 3), 1:1000 (lane 4), 1:5000 (lane 5), 1:10000 (lane 6). (b) Child 7; recipient of RIT 4237 vaccine; pre 1:50 (lane 1), post 1:100 (lane 2), 1:500 (lane 3), 1:1000 (lane 4), 1:5000 (lane 5), 1:10000 (lane 6).
The RIPA does not detect antibodies of immunoglobulin classes other than IgG and it is possible that, at 28 days post-vaccination, the newly induced neutralizing antibodies are to some extent of the IgM class. The absence of antibodies directed to non-structural proteins probably reflects restricted replication of the vaccine viruses in the enterocytes. The sera from naturally infected children 15 and 16 show that, upon re-infection, the primary immune response is accelerated and expanded, not only to the originally detected polypeptides but also to new polypeptides including non-structural proteins.

It has been suggested that the heterologous candidate rotavirus vaccines could be improved by using animal–human reassortant rotaviruses containing the glycosylated VP7 from human strains. It is doubtful whether such viruses would induce a better antibody response than the original heterologous viruses. In fact, administration of a reassortant bovine rotavirus vaccine containing the gene coding for VP7 of a human strain to adult human volunteers has been disappointing (Clark et al., 1986). However, if reassortant rotaviruses are to be used, not only VP7 but also the role VP3 plays in virulence (Offit et al., 1986) and in induction of heterotypic immunity (Offit et al., 1985; Taniguchi et al., 1985) has to be considered when designing reassortant vaccines.

The induction of rotavirus-specific IgG and IgM antibodies is a valuable marker for evaluation of the 'take' of a vaccine and for analysing the immune response. Intestinal IgA may play an important role in the resistance to rotavirus-induced diarrhoea. Further studies are in progress to clarify to what extent attenuated rotavirus vaccines stimulate rotavirus-specific IgA and to analyse what proteins stimulate this immune response.

We thank Dr Monica Grandien and Dr Albert Z. Kapikian for valuable discussions and Dr Ingrid Uhnoo for the sera from the naturally infected children.

REFERENCES


*(Received 29 December 1986)*