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Local and systemic antibody response to rotavirus WC3 vaccine in adult volunteers

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Summary

An evaluation of the safety and immunogenicity of WC3 rotavirus vaccine was evaluated in adult volunteers. Pre- and post-vaccination titers of neutralizing antibody to WC3 and to the four human rotavirus serotypes as well as serum and stool rotavirus IgA levels were measured. Vaccination was safe and did not induce elevation of liver enzymes. None of the 12 volunteers receiving WC3 vaccine shed detectable amounts of virus although antibody rises were detected in 11 of 12 vaccines. Nine developed and increase in WC3 neutralizing antibody, one additional subject had a rise in Wa (human serotype 1) neutralizing antibody while another subject only developed a rise in stool rotavirus IgA. All of the vaccine recipients with a rise in WC3 neutralizing antibody also developed a rise in neutralizing antibody against at least one of the four most common human rotavirus serotypes. A stool IgA rotavirus antibody response was detected in 6 of 9 WC3 recipients with measurable stool antibody. None of the control subjects developed significant rises in any of the antibody titers measured. WC3 rotavirus vaccine appears to be safe and induces systemic and local immune responses in adults suggesting that further evaluation of WC3 should be considered in infants.

Rotavirus vaccine; Immune response; Stool rotavirus IgA

Introduction

Rotavirus infections are a leading cause of morbidity in developed countries and mortality in developing nations (Kapikian and Chanock, 1985). Although infec-

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tions are most common and severe in infancy, reinfection of older children and adults are frequent and can be severe (Kapikian and Chanock, 1985; Hrdy, 1987). The immune mechanisms that protect exposed individuals from infection or illness are not well understood. The presence of local intestinal immunity appears to correlate with protection in animal (Snodgrass and Wells, 1976; Offit and Clark, 1985; Bridger and Brown, 1981) and human studies (Ward et al., 1989). The role of circulating, serotype-specific neutralizing antibody is less clear but has also been correlated to protection from infection as well as symptomatic disease (Ward et al., 1989; Chiba et al., 1986; Kapikian et al., 1983). We, therefore, evaluated the potential of the WC3 strain of bovine rotavirus, a promising vaccine candidate, to induce local rotavirus antibody and serum neutralizing antibody to the 4 most common human rotavirus serotypes.

In previous studies, when WC3 was given to four adult volunteers, none shed detectable levels of virus and only 2 developed a transient increase in serum rotavirus antibody at day 14 (Clark et al., 1986). One of these also had a transient increase in stool rotavirus antibody. When given to infants 5–11 months of age, 95% developed a serum neutralizing antibody response to WC3. The response was slightly less in children 12–23 months of age (80% seroconverted to WC3) (Clark et al., 1986). In another study of infants 3–12 months of age, vaccination induced neutralizing serum antibody to WC3 in 71% of recipients and provided protection against rotavirus diarrhea (Clark et al., 1988).

Previous studies with adult volunteers have shown that subjects infected after oral administration of live human rotaviruses had increases in serum neutralizing antibody to heterotypic human rotavirus strains (Ward et al., 1989; Kapikian et al., 1983; Ward et al., 1986). WC3 has been reported to be serotypically related to Wa and SA-11, serotype 1 and 3 rotaviruses, as shown by a one-way cross-reaction, but appeared to be serotypically unrelated to human rotaviruses belonging to serotypes 2 or 4 (Clark et al., 1986). Only a small portion (8–10%) of children administered the WC3 vaccine who lacked neutralizing antibody to WC3, Wa and SA-11 prior to vaccination developed antibody rises to Wa although approximately 50% had rises to SA-11 (Clark et al., 1988). It was, therefore, of interest to determine whether adults given WC3 would seroconvert to heterotype human rotaviruses, a response of immediate importance for vaccine evaluation.

Materials and Methods

WC3 vaccine

The isolation and characteristics of the WC3 strain of bovine origin rotavirus used in this and other studies have been described in detail (Clark et al., 1986, 1988). The passage history of the WC3 vaccine lot used in this study was identical to that of the vaccine already evaluated in children (Clark et al., 1988) except that the last 3 passages in CV-1 cells were repeated for preparation of the present lot. This same lot was used in an efficacy study of 206 infants aged 2–12 months conducted by

ourselves as well as in infant studies conducted by others in Africa and Israel. The final titer of this vaccine lot was $10^{7.5}$ pfu/ml.

Vaccination

Participants in the study were healthy, adult volunteers (14 women, 9 men) with an average age of 31.6 (range 25–45). A general physical examination, urinalysis, complete blood count and a battery of blood chemistries including a pregnancy test, were done prior to enrollment to ensure good health status. Signed consent was obtained from all volunteers. The study and consent form were approved by the Christ Hospital Institutional Review Board. Vaccine or placebo was administered according to a random code supplied by Merieux Institut, France. Immediately prior to vaccination, blood was obtained fro pre-vaccination antibody levels and for determination of SGOT, SGPT, GTT, alkaline phosphatase, LDH and bilirubin levels. A stool specimens was also obtained for rotavirus IgA antibody determination. Subjects were asked to fast for at least 1 h prior to vaccine administration. At the time of vaccination, they were each given 50 ml of 4% $NaHCO_3$ following by 1 ml of vaccine or placebo. Volunteers were asked to keep a diary of any side effects during the first 7 days after vaccination. Stool specimens obtained on days 3, 5 and 7 after vaccination were used to identify subjects who shed virus and a day 14 specimen was used to determine stool rotavirus IgA levels. Stool rotavirus IgA was previously found to peak 14 days after rotavirus inoculation of adults (Bernstein et al., 1989). Blood specimens were obtained on days 7, 14 and 28 to measure liver function enzymes, rotavirus IgA and both liver enzymes and neutralizing antibody, respectively.

Processing of stool specimens

Stool specimens were prepared for detection of WC3 virus and antibody through the following steps. Ten percent suspensions of each specimens were made in Earle's balanced salt solution (EBSS) and shaken at 4°C with glass beads to pulverize particulates. The suspensions were then centrifuged (1000 × *g*, 10 min) to remove most suspended solids and the supernatants were stored at −70°C in 1 ml aliquots.

Detection of WC3 virus

Two methods were used to detect WC3 virus. The first was a plaque assay for infectious viruses. For this, a 1 ml aliquot of processed stool was incubated (37°C, 30 min) with 10 μg of trypsin (1:250; Gibco laboratories, Grand Island, NY) and undiluted as well as diluted specimens (0.2 ml) were added directly to confluent monolayers of MA-104 cells (washed 2 times with EBSS). After a 1 h adsorption period at 37°C, the monolayer was washed once with EBSS, overlayered with medium [Dulbecco's modified minimal essential medium (D-MEM) with antibiotics, 4 μg of trypsin/ml and 25 μg of DEAE-dextran/ml] containing 0.2% agarose, and

incubated for 4 days at 37°C. The soft agarose was poured from plates and the cells were stained with crystal violet to permit plaques to be visualized. Control plates inoculated with known amounts of WC3 virus developed large plaques in this time period under the same conditions. The second procedure used to detect rotavirus in stools was an enzyme-linked immunosorbent assay (ELISA) which has been described in detail in previous publications (Ward et al., 1989; Gilchrist et al., 1988).

Rotavirus antibody determination

Both serum and stool specimens were examined for the presence of rotavirus antibody. Serum neutralizing antibody to WC3 and representatives of each of the four established human rotavirus serotypes were measured by a fluorescent focus reduction assay (FFA). The representative virus were Wa (serotype-1), DS-1 (serotype-2), P (serotype-3) and ST-3 (serotype-4). Stock solutions of viruses were activated by incubation with 20 μg of trypsin/ml for 1 h at room temperature, then diluted to approximately 5000 focus forming units (ffu)/ml with D-MEM. For neutralization, an equal volume of serum (heat inactivated at 56°C, 30 min) serially diluted in the same medium was mixed with virus and incubated (37°C, 1 h). Confluent monolayers of MA-104 cells (4 days old) grown in 96-well microtiter plates were washed twice with D-MEM and 0.1 ml of serum-virus mixture was added to each well. The plates were then centrifuged (1000 × *g*, 1 h, room temperature) to promote virus adsorption to cells. Unadsorbed virus was aspirated from the wells and the cells were washed once with D-MEM before being overlaid with 0.2 ml of D-MEM containing antibiotics and 4 μg of trypsin/ml. After 15 h of incubation at 37°C, medium was aspirated from the wells and cells were fixed with 80% acetone at −20°C. After storage for ≥ 10 min at −20°C, acetone was aspirated and monolayers were allowed to dry completely. Heat inactivated guinea pig serum to Wa virus was diluted 1:800 in phosphate buffered saline (PBS) containing 5% non-fat dry milk and 0.1 ml was added to each well of the microtiter plates. After 1 h at 37°C, the guinea pig serum was aspirated and the wells were washed once with PBS. Fluorescence-conjugated goat anti guinea pig IgG (Cappel Laboratories, Downington, PA; 0.1 ml of a 1:100 dilution in PBS plus milk per well) was added. The plates were incubated (1 h, 37°C) and the conjugated antibody was removed by aspiration. The wells were washed twice with PBS, the cell monolayers were dried and the fluorescence-tagged cells were visualized with a UV microscope. The reciprocal of the serum dilution that reduced recoverable ffu by 60% was considered its titer.

Rotavirus IgA in serum and stool was determined by an ELISA procedure using a standard curve as previously described (Ward et al., 1989). Units of rotavirus IgA in stool were standardized to 100 μg of total IgA/ml (Bernstein et al., 1986).

Results

Safety

Two volunteers, one vaccine recipient and one placebo recipient, reported an episode of diarrhea within 24 hours of receiving the vaccine. No other adverse experience were reported by any volunteer during the remainder of the study. All liver function tests for the vaccine recipients were within normal limits at all time points (pre-vaccination, 7 days and 28 days post-vaccination). Two placebo recipients were enrolled with one slightly elevated liver function test value each (alkaline phosphatase or gammaglutamyl transferase). In both cases the values did not rise significantly during the course of the study and remained below two times the upper limit of normal for each test.

Immunogenicity

No viral shedding was detected based on stool specimens collected on days three, five or seven, either by plaque assay or a sensitive ELISA. The limit of detection for the plaque assay was 50 plaque forming units per gram of stool. Nine of the twelve vaccine recipients, however, developed a $\geq$ 3-fold rise in WC3 serum neutralizing antibody (mean fold rise: 5.3) (Table 1). None of the placebo recipients developed a rise (ratio of day 28/day 0 titers ranged from 0.7–1.5). The pre-vaccination GMT of serum neutralizing antibody to WC3 virus was lower in the 9 vaccine recipients who developed an antibody response to WC3 (41.3) compared to the three that did not (99.5, $P = 0.065$, one-tailed Student's t-test.

Of the nine vaccine recipients that developed a rise in WC3 neutralizing antibody, all developed a $\geq$ 3-fold rise against at least one of the four most common human rotavirus serotypes (Table 2). No significant differences were found in the antibody rise to the four human serotypes ($P = 0.30$, analysis of variance), although the geometric mean rise was highest to serotype 4 and least to serotype 3. In addition, one vaccine recipient who did not develop an antibody response to WC3 had a 5-fold increase in Wa antibody following vaccination.

A local (stool) IgA rotavirus antibody response was detected in six of the nine vaccine recipients with measurable antibody levels but in none of the 10 placebo

TABLE 1

WC3 neutralizing antibody response in adult volunteers

	N[a]	No. with $\geq$ 3-fold antibody rise	GMT[b] pre-vaccine	GMT post-vaccine	Geometric mean fold rise
Placebo	11	0	103.5	103.5	1.0
Vaccine	12	9	51.4	273.8	5.3

[a]Number vaccinated.
[b]Geometric mean titer.

TABLE 2

Neutralizing antibody response to the four most common human rotavirus serotypes in the 9 vaccine recipients that developed a rise in WC3 antibody

Serotype[a]	No. with > 3-fold rise	No. with > 2-fold rise	GMT pre-vaccine	GMT post-vaccine	Geometric mean fold rise[b]
1	6	8	742	3259	4.7
2	4	5	123	379	3.1
3	4	7	410	1152	2.8
4	6	8	153	811	5.3

[a]Neutralization was performed using Wa as a representative of serotype-1, DS-1 for serotype 2, P for serotype-3 and ST-3 for serotype 4.
[b]No significant differences were found for the geometric mean fold rises against the four serotypes ($P = 0.30$ by analysis of variance).

recipients with measurable levels (Table 3). The GMT of rotavirus stool IgA increased 6.2-fold in vaccine recipients. Likewise, serum rotavirus IgA titers increased ≥ 3-fold in 8 of the 12 vaccine recipients compared to 0 of 11 placebo recipients. The geometric mean fold rise was 5.3 in vaccine recipients. The 8 subjects with rises in serum rotavirus IgA were included in the 9 subjects that developed serum neutralizing antibody to WC3. One of the 6 vaccine recipients with a rise (14-fold) in stool rotavirus IgA was, however, not included in this group of 9 subjects or the subject who seroconverted to Wa alone. Thus, if all these data are combined, 11 of the 12 vaccine recipients developed an immunological response to rotavirus following WC3 vaccination.

Discussion

In this trial, vaccination of adults with WC3 rotavirus vaccine was safe, did not induce elevation of liver enzymes, and the vaccine strain of virus was not shed at detectable levels. Eleven of the 12 vaccinees, however, did develop immunological

TABLE 3

Local and serum rotavirus IgA responses

	Stool				
	N[a]	No. with 4-fold rise	Pre-vaccination GMT	Post-vaccination GMT	Geometric rise
Placebo	10	0	12.8	15.7	1.2
Vaccine	9	6	3.0	18.5	6.2
	Serum				
	N	No. with 3-fold rise	Pre-vaccination GMT	Post-vaccination GMT	Geometric mean rise
Placebo	11	0	324	342	1.1
Vaccine	12	8	162	861	5.3

[a]Number with measurable stool antibody levels pre- and 14 days post-WC3 inoculation.

evidence of a rotavirus infection. Nine developed an increase in WC3 serum-neutralizing antibody, one additional subject developed a rise in Wa serum neutralizing antibody while another subject only developed a rise in stool rotavirus IgA.

In addition to the response to WC3 all of the subjects with a rise in WC3 neutralizing antibody also developed a rise against at least one of the four most common human rotavirus serotypes. Previous reports indicated that WC3 was most closely related to human serotypes 1 and 3 (Clark et al., 1986) but that infants responded predominantly to serotype 3 if they developed neutralizing antibody to either of these human serotypes (Clark et al., 1986, 1988). Other reports (Losonsky et al., 1986; Vesikari et al., 1983) have noted induction of heterologous neutralizing antibody in only a small percentage of infants following vaccination with RRV (simian) or RIT (bovine) vaccine. In this investigation, WC3 immunized adults responded with a similar rise in antibody to all four human serotypes (range of geometric mean fold rise = 2.8–5.3). Thus, WC3 immunization of adults whose immune systems have been primed by previous rotainfections can induce heterotypic neutralizing antibodies. This implies that WC3 contains cross reactive neutralizing epitopes but that they are not the immunodominant epitopes recognized after primary exposure. A similar heterotypic antibody response has been detected in adult volunteers infected with either the D strain (Kapikian et al., 1983) or the CJN strain of human rotavirus (Ward et al., 1986, 1989). A possible role for serotype-specific serum neutralizing antibody in protection from infection and/or illness has been reported (Ward et al., 1989; Chiba et al., 1986; Kapikian et al., 1983).

Stimulation of a local immune response may be important in providing protection from rotavirus infections. Only limited data is available regarding the intestinal response to rotavirus vaccination in infants, children or adults. Recently, Losonsky et al. (1986) reported detecting a coproantibody response in 11 of 16 children (ages 3–20 months) who received varying doses of RRV rotavirus vaccine including 2 that had no evidence of serum neutralizing antibody to rotavirus. It is not clear how many of these children had been infected with rotavirus prior to their vaccination. In a study by Wright et al. (1987), also using RRV vaccine, a coproantibody response was detected earlier (days 9–12) in vaccinated children with a prior rotavirus infection compared to those undergoing a primary infection with the vaccine (day 21). We have previously reported detecting a coproantibody response in 16 of 19 adults infected with the CJN strain of rotavirus (Bernstein et al., 1986). In this study, coproantibody rises were detected in 6 of 9 WC3 recipients with measurable stool antibody.

WC3 rotavirus vaccine appears to be safe and capable of inducing a systemic and local immune response in adults. Induction of cross neutralizing antibodies in these adults implies that WC3 contains neutralizing epitopes common to all of the 4 established human rotavirus serotypes and infection with WC3 is capable of boosting already existing antibody responses to the human rotavirus strains. Further evaluation of WC3 in unprimed infants appears warranted.

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