Evaluation of RIX4414, A Live, Attenuated Rotavirus Vaccine, in a Randomized, Double-Blind, Placebo-Controlled Phase 2 Trial Involving 2464 Singaporean Infants

Kong Boo Phua,[1] Seng Hock Quak,[2] Bee Wah Lee,[2] Shanta Christina Emmanuel,[3] Paul Goh,[4] Htay Htay Han,[5] Beatrice De Vos,[6] and Hans Ludwig Bock[5]

[1]Department of Medicine, KK Women's and Children's Hospital, [2]Pediatric Department, National University of Singapore, [3]National Healthcare Group Polyclinics, [4]SingHealth Polyclinics, and [5]GlaxoSmithKline Biologicals, Singapore; [6]GlaxoSmithKline Biologicals, Rixensart, Belgium

Background. At present, no rotavirus vaccine is commercially available for use worldwide. Hence, a live, attenuated monovalent vaccine was developed with human strain RIX4414 (G1P1A P[8] specificity). Vaccination trials involving infants are ongoing in developed and developing countries.

Methods. This study was a randomized, double-blind, placebo-controlled trial conducted at pediatric hospitals and polyclinics in Singapore for the evaluation of the immunogenicity, reactogenicity, and efficacy of 2 oral doses of RIX4414. In total, 2464 healthy infants (who were 11–17 weeks old when the first dose was administered, which is in accordance with the local immunization schedule) were enrolled to receive RIX4414 at 3 concentrations of virus ($10^{4.7}$, $10^{5.2}$, or $10^{6.1}$ focus-forming units) or placebo at 1-month intervals, concomitantly with routinely administered infant vaccines.

Results. The RIX4414 vaccine was highly immunogenic, and virtually all vaccine recipients (98%–100%) experienced "vaccine take" (i.e., a combined immunogenicity end point based on seroconversion and/or shedding of RIX4414 in postvaccination stool samples) after receipt of 2 doses at all 3 dosage levels. Depending on the virus concentration, the anti–rotavirus IgA seroconversion rate varied from 76% (95% confidence interval [CI], 68%–83%) to 91% (95% CI, 85%–95%). Two doses of RIX4414 were well tolerated, with no increase in high fever, severe diarrhea, or vomiting after either dose or with increased viral concentration, compared with placebo. There was no observed interference with routine vaccinations of infants when RIX4414 was coadministered. The calculated efficacy of RIX4414 against rotavirus gastroenteritis was 82% ($P = .046$); however, this result was considered to be of limited conclusive value because of the low number of rotavirus gastroenteritis episodes identified during the follow-up period.

Conclusions. The live, attenuated rotavirus vaccine (RIX4414) was well tolerated and highly immunogenic in Singaporean infants. The immunogenicity of routinely administered infant vaccines was not impaired by concomitant administration of RIX4414 vaccine.

Among infants and young children, rotavirus infection is the leading cause of episodes of acute gastroenteritis. It may account for 20% of all diarrhea-related deaths [1]. The global number of deaths among children <5 years old is approximately half a million, of which >80% involve children in developing, low-income countries [2]. Moderate-to-severe rotavirus gastroenteritis is estimated to cause >2 million hospitalizations and 25 million clinic visits among children <5 years old each year worldwide [2]. Although hospitalization rates vary, rotavirus infection is an important cause of hospitalization in different countries in Asia [3–6]. Because ro-

tavirus infection is a major public health problem, as a result of the associated morbidity and mortality, there is a definite need for an effective intervention that can prevent severe rotavirus illness worldwide.

The withdrawal of the first licensed rotavirus vaccine (a rhesus-human reassortant vaccine) because of its reported association with intussusception [7–9] prompted the development of new, safer rotavirus vaccines. Among other vaccines, a human rotavirus candidate vaccine was developed by attenuating the virulent wild-type 89–12 strain (G1P1A P[8] specificity) by multiple passages in cell culture [10]. The rationale for the selection of this human candidate vaccine was based on evidence that natural rotavirus infection protects against subsequent severe illness, regardless of infecting serotypes [11–15]. The 89–12 vaccine has been shown to be efficacious and immunogenic, and its only adverse effect was an increase in the frequency of mild fever among vaccine recipients, compared with placebo recipients [10, 16, 17]. A new rotavirus vaccine (RIX4414; Rotarix; GlaxoSmithKline Biologicals) was subsequently developed from the 89–12 parent strain by cloning and further attenuating it by passaging in Vero cells. When tested in adults (in Belgium), toddlers (in Germany), and infants (in Finland), RIX4414 vaccine was well tolerated and immunogenic [18]. The vaccine was shown to be highly efficacious over the course of 2 rotavirus epidemic seasons in Finland [19]. These studies [18, 19] reported the absence of clinical reactions, as well as a high rate of antibody response, in European infants after administration of 2 oral doses of RIX4414 rotavirus vaccine. The present study was performed in Singapore to assess the immunogenicity, reactogenicity, and efficacy of 2 oral doses of RIX4414 vaccine, administered at different viral concentrations concomitantly with routinely administered infant vaccines, in healthy infants previously uninfected with human rotavirus.

SUBJECTS AND METHODS

Vaccine

The vaccine RIX4414 (Rotarix) was manufactured by GlaxoSmithKline Biologicals. To produce RIX4414, the parent 89–12 vaccine strain was further passaged in Vero cells and cloned [18, 20]. The vaccine was a lyophilized preparation supplied in single-dose vials with calcium carbonate buffer for reconstitution.

Study Design

The present study was conducted according to good clinical practice and in accordance with the Declaration of Helsinki, as amended in Somerset West, Republic of South Africa, in October 1996. The study protocol dated 22 September 2000 and the statement of informed consent were approved by the same Ethics Committee and the Ministry of Health for all centers prior to study initiation. Written, informed consent was obtained from the parents or guardians before enrollment.

This randomized, double-blind, placebo-controlled study was conducted at 8 centers (pediatric hospitals and polyclinics) in Singapore from January 2001 through April 2003. Infants were randomly assigned (on a 1:1:1:1 basis) to receive 2 oral doses of either RIX4414 vaccine given in 1 of 3 concentrations ($10^{4.7}$, $10^{5.2}$, or $10^{6.1}$ focus-forming units [ffu]) or placebo at 11–17 weeks of age (for the first dose) and 1 month later. The first 2 doses of routine infant vaccinations—diphtheria, tetanus, pertussis, polio (DTPa-IPV [Infanrix-IPV; GlaxoSmithKline Biologicals]) and *Haemophilus influenza* type b (Hib [Hiberix; GlaxoSmithKline Biologicals])—were administered concomitantly with RIX4414 or placebo, according to the local immunization schedule for DTPa-IPV/Hib at ages 3, 4, and 5 months. Hepatitis B vaccine (Engerix; GlaxoSmithKline Biologicals) was administered at birth, at 1 month of age, and at either 5 or 6 months of age, in accordance with the local immunization schedule.

An objective of the trial was to evaluate the efficacy of 2 doses of the vaccine in the prevention of any cases of rotavirus gastroenteritis during the period starting from 2 weeks after administration of the second dose until infants reached age ~18 months. The immunogenicity of the vaccine was evaluated in terms of the serum anti–rotavirus IgA antibody seroconversion rate. "Vaccine take," which is defined as a combined immunogenicity end point based on seroconversion and/or shedding of RIX4414 in postvaccination stool samples, was also determined. The reactogenicity of RIX4414 and any effect of the vaccine on the immune response of coadministered routine infant vaccinations were also determined.

Participants

Infants 11–17 weeks old were eligible for inclusion in the study if they were free of obvious health problems and had been born at full term. Infants were excluded if they had a clinically significant history of gastrointestinal disease or malformation, had received vaccines or treatment prohibited by the protocol, were immunocompromised, had a history of allergic reaction to any vaccine component, or had contact with an immunosuppressed individual or a pregnant woman in their household. Infants with a history of gastrointestinal disease were excluded to avoid any potential risk to them.

Assessments

Immunogenicity. Serum samples were obtained for evaluation of anti–rotavirus IgA antibody levels on the day that the first dose was administered (immediately before vaccination for all subjects), on the day that the second dose was administered (only for a subset of subjects), and at 1 and 2 months after the administration of the second dose (only for a subset of subjects). Serum samples for the evaluation of the immune response to routine infant vaccinations were obtained 1 month

after administration of the third dose of these vaccines. Stool samples (obtained from 50 subjects/group [i.e., the stool sample subset]) were obtained on the day of each vaccination, as well as on days 7 and 15 after each vaccination, for assessment of shedding of the vaccine virus strain.

Reactogenicity. Solicited general symptoms (cough or runny nose, diarrhea, irritability, loss of appetite, fever, and vomiting) were reported by the parents or guardians on diary cards during a 15-day follow-up period after each vaccine dose was administered, and the symptoms were graded according to severity. Any unsolicited symptom occurring up to 42 days after administration of each study vaccine was recorded, and serious adverse events were reported during the entire study period.

Efficacy. Active surveillance for episodes of acute gastroenteritis was performed from 2 weeks after the administration of the second dose until infants were ~18 months old. When an episode of gastroenteritis was suspected, parents and guardians were instructed to record body temperature, the number of episodes of vomiting, the number of looser-than-normal stools, and whether they sought medical intervention or medication, and were asked to obtain at least 2 stool samples on 2 different days within 7 days of the onset of symptoms. The severity of each episode of gastroenteritis was graded by use of a 20-point scoring system described by Ruuska and Vesikari [21]. Rotavirus gastroenteritis was confirmed if at least 1 of the 2 stool specimens was found to be positive for rotavirus by ELISA. Rotavirus isolates were G-typed by use of reverse-transcriptase polymerase chain reaction (RT-PCR).

Laboratory Methods

All blood samples were centrifuged and separated. Serum samples were stored at −20°C until they were shipped to the sponsor.

Anti–rotavirus IgA antibodies were measured by ELISA [22] at the laboratory of Richard L. Ward (Children's Hospital Medical Center, Cincinnati, OH). "Seroconversion" was defined by an anti–rotavirus IgA antibody concentration of ⩾20 U/mL, for infants who were initially (i.e., before administration of the first vaccine dose) seronegative for anti–rotavirus IgA antibodies (i.e., a concentration of <20 U/mL) and/or who had a stool sample that was negative for rotavirus antigen. Concentrations of antibodies to diphtheria, tetanus, hepatitis B surface antigen, Hib polysaccharide polyribosyl ribitol phosphate, and IgG antibodies to pertussis toxin, filamentous hemagglutinin, and pertactin were determined by use of ELISA. Concentrations of antibodies to polio virus types 1, 2, and 3 were measured by use of a virus microneutralization test.

Stool samples obtained for analysis of vaccine shedding were analyzed at the laboratory of Richard L. Ward by use of ELISA to detect rotavirus antigen [16, 17]. Any detection of RIX4414 antigen in stool samples was taken as evidence of a vaccine response (i.e., vaccine take), as long as the subject was initially negative for rotavirus. The genetic sequence of strains resulting in any shedding in placebo recipients was determined by sequencing of the RT-PCR product on an ABI Prism BigDye Terminator Cycle Sequencing Ready Action Kit (Applied Biosystems).

All stool samples obtained during episodes of gastroenteritis were tested by ELISA at the laboratory of Richard L. Ward. ELISA-positive specimens were tested by RT-PCR [23, 24] at GlaxoSmithKline Biologicals (Rixensart, Belgium) to determine the G type. If any G1 rotavirus was detected, vaccine virus was differentiated from wild-type rotavirus by sequence analysis.

Statistical Analysis

The sample size calculation assumed a 12% annual attack rate for rotavirus gastroenteritis in nonvaccinated infants and a vaccine efficacy of 70%. On the basis of these assumptions, the planned sample size of 2460 infants was estimated to provide >90% power to evaluate efficacy against rotavirus gastroenteritis.

The "intention-to-treat population" was defined as all subjects who received at least 1 dose of the vaccine or placebo. The "according-to-protocol (ATP) cohort" for immunogenicity was defined as subjects in the intention to treat population who did not violate any of the eligibility criteria, who complied with all protocol procedures, and for whom immunogenicity end point data were available before vaccination and at least for 1 time point after vaccination.

The analysis for reactogenicity was performed for the intention-to-treat population. The overall incidence of solicited general symptoms, with exact 95% confidence intervals (CIs), was determined for each study group after administration of each vaccine dose.

The analysis for immunogenicity was performed for the ATP cohort. For calculation of geometric mean concentrations (GMCs), anti–rotavirus IgA antibody titers below the assay cutoff level (<20 U/mL) were given the arbitrary value of half the cutoff level. The GMCs, anti–rotavirus IgA antibody seroconversion rate, vaccine virus shedding, and vaccine take were calculated to evaluate the immune response in each vaccine group. Seroconversion rates, with exact 95% CIs, were calculated for each group. Shedding of the vaccine virus was detected in stool samples by ELISA. Vaccine take, with exact 95% CIs, was calculated for each group.

Analysis of the immunogenicity of routine infant vaccinations for the ATP cohort was performed for the subset of subjects who had received at least 2 doses of DTPa-IPV reconstituted with Hib vaccine and at least 1 dose of hepatitis B vaccine between the day that the first RIX4414 vaccine dose was administered and 2 months after the second RIX4414 vaccine dose was administered. The seropositivity rates, with exact 95% CIs, were determined. For determination of efficacy, the total vaccinated cohort was based on the intention-to-treat cohort

and included all vaccinated subjects for whom efficacy follow-up data were available.

RESULTS

Demographics: Study Population

A total of 2464 subjects (1238 girls and 1226 boys) were enrolled in the study (table 1). Their mean ($\pm$ SD) age was 13.3 $\pm$ 0.90 weeks at the time of administration of the first dose and was 17.9 $\pm$ 1.4 weeks at the time of administration of the second dose. The majority of subjects (93.0%) were Asian, 0.1% were white, and 6.9% were of other ethnic origin. At the time of administration of the first dose, the demographic profile between the groups was similar with respect to age, sex, race, height (mean $\pm$ SD, 60 $\pm$ 2.80 cm), and weight (mean $\pm$ SD, 6.2 $\pm$ 0.70 kg).

Although 2365 subjects completed the study, 99 subjects dropped out (table 1). Five subjects dropped out because of serious adverse events, including 3 deaths. All 5 of these events were assessed by the clinical investigators and were determined to be unrelated to study vaccination. Two infants in the group given $10^{6.1}$ ffu of vaccine (i.e., the $10^{6.1}$-ffu vaccine group) died: 1 infant died at age 2 years, of juvenile chronic myelomonocytic leukemia, which was diagnosed ~12 months after administration of the second vaccine dose, and 1 infant died of accident-induced subarachnoid hemorrhage 3 months after administration of the second vaccine dose. A third infant ($10^{5.2}$-ffu vaccine group) died 19 days after receiving the first vaccine dose. The infant experienced upward rolling of the eyes and tonic movements of short duration; 6 h later, breathing stopped. At the emergency department, resuscitation failed; an autopsy revealed the cause of death as cardiorespiratory failure following acute viral pneumonitis. The other 2 serious adverse events were (1) an *Escherichia coli*–related urinary tract infection ($10^{6.1}$-ffu vaccine group) that developed 18 days after the first vaccine dose and that abated 8 days later and (2) seizure ($10^{5.2}$-ffu vaccine group) that developed 12 days after administration of the first dose and abated after 2 days of hospitalization. In the latter case, 54 days after administration of the second vaccine dose and 19 days after administration of the third dose of DTPa-IPV/Hib, the infant again developed recurrent seizures and had epilepsy diagnosed; episodes were recurring at the end of the study.

The intention-to-treat cohort for evaluation of vaccine reactogenicity and safety was 2464 subjects, and that for evaluation of vaccine efficacy was 2421 subjects. The ATP cohort for assessment of immunogenicity was 640 subjects (table 1).

Reactogenicity

After administration of each dose, irritability and fever were the most commonly reported solicited general symptoms, and diarrhea was the least frequently reported symptom. The incidence of each solicited general symptom was similar between all vaccine and placebo groups, and the incidence of severe symptoms (i.e., those that prevent normal, daily activity) was low in all groups. The data for fever, vomiting, and diarrhea are shown in table 2. The mean duration of fever, vomiting, and diarrhea was similar in the vaccine and placebo groups (data not shown). Vaccination with RIX4414 was associated with a low overall intake of medication, including prophylactic

Table 1. Profile of a phase 2 trial of RIX4414 rotavirus vaccine involving infants in Singapore.

Group of infants	RIX4414 vaccine, by dose			Placebo	Total
	$10^{4.7}$ ffu	$10^{5.2}$ ffu	$10^{6.1}$ ffu		
Received first dose	510	648	653	653	2464
Received second dose	501	639	639	642	2421[a]
Dropped out	17	23	34	25	99
Serious adverse event	0	2	3	0	5
Protocol violation	0	1	2	0	3
Consent withdrawal	8	6	9	10	33
Migration	2	1	7	3	13
Lost to follow-up	6	12	12	12	42
Other	1	1	1	0	3
Completed the trial	493	625	619	628	2365[b]
Cohort					
ITT[c]	510	648	653	653	2464
ATP immunogenicity	155	158	167	160	640
Efficacy	501	639	639	642	2421

NOTE.   Data are no. of infants. ATP, according to protocol; ffu, focus-forming units; ITT, intention to treat.

[a] In total, 98.3% of infants enrolled received the second dose of vaccine.
[b] In total, 96% of infants enrolled completed the trial.
[c] Received at least 1 dose.

Table 2. Percentage of subjects for whom fever, vomiting, or diarrhea was reported during the 15-day follow-up period after administration of each dose of RIX4414 rotavirus vaccine.

	Dose of RIX4414						Placebo	
Solicited general symptom	$10^{4.7}$ ffu		$10^{5.2}$ ffu		$10^{6.1}$ ffu			
	Dose 1 (n = 510)	Dose 2 (n = 501)	Dose 1 (n = 648)	Dose 2 (n = 639)	Dose 1 (n = 653)	Dose 2 (n = 639)	Dose 1 (n = 653)	Dose 2 (n = 642)
Fever								
Total[a]	30 (26–34)	32 (28–37)	28 (25–32)	32 (29–36)	25 (22–29)	27 (24–31)	28 (25–32)	29 (26–33)
Severe[b]	0 (0–1)	0 (0–1)	1 (0–2)	1 (0–2)	1 (0–2)	0 (0–1)	0 (0–1)	1 (0–2)
Vomiting								
Total[a]	5 (3–7)	4 (3–6)	5 (3–7)	5 (3–7)	7 (5–9)	4 (2–6)	6 (4–8)	4 (3–6)
Severe[c]	1 (0–2)	1 (0–2)	2 (1–3)	2 (1–3)	1 (1–3)	0 (0–1)	1 (0–2)	1 (0–2)
Diarrhea								
Total[a]	1 (1–3)	2 (1–4)	1 (1–3)	2 (1–4)	3 (2–5)	2 (1–4)	2 (1–3)	1 (0–2)
Severe[d]	0 (0–1)	0 (0–1)	1 (0–1)	0 (0–1)	1 (0–1)	1 (0–2)	0 (0–1)	0 (0–1)

NOTE. Data are percentage of subjects (exact 95% confidence interval). ffu, focus-forming units.
[a] All reports of the specified symptom, irrespective of severity.
[b] Severe fever is defined as temperature >39.5°C (measured rectally) or >39.0°C (measured orally/axillary).
[c] Severe vomiting is defined as ≥3 episodes of vomiting/day.
[d] Severe diarrhea is defined as ≥6 looser-than-normal stools/day.

antipyretics, which was similar to findings for the placebo group (data not shown).

Safety

For the entire study period, 262 serious adverse events were reported for 184 subjects (32 in the $10^{4.7}$-ffu vaccine group, 54 in the $10^{5.2}$-ffu vaccine group, 58 in the $10^{6.1}$-ffu vaccine group, and 40 in the placebo group). Of the 4 serious adverse events assessed as possibly related to vaccination (all subjects had also received concomitantly administered DTPa-IPV/Hib vaccine), there was 1 case of intussusception in a 3-month-old male infant. He developed vomiting, without fever, 6 days after receiving the first dose of RIX4414 ($10^{5.2}$ ffu). The next day, colicky pain and bloody stools accompanied vomiting, and he was hospitalized. Findings of ultrasound examination were indicative of ileocecal intussusception, which was reduced by use of an air enema. The infant recovered without sequelae and was discharged 3 days later. A stool sample obtained from the infant on the day when symptoms began tested positive for rotavirus (as determined by use of RotaClone [Meridian Biosciences]; performed at the study site/local laboratory). Bacterial test results were negative. Convalescent-phase stool samples obtained 1 and 8 days after intussusception were negative for rotavirus (as determined by use of Immuno-card Stat [Meridian Biosciences] and RotaClone, respectively, performed at a central laboratory). Further testing of the latter stool sample by use of RT-PCR (performed 8 days after intussusception and 15 days after vaccination) showed a weak positivity for rotavirus (G1 type), but no sequencing could be performed. Stool samples obtained 4 weeks after the intussusception were RT-PCR neg-

ative for rotavirus and RT-PCR positive for enteroviruses. The subject completed the study.

Another case of intussusception, which was determined to be unrelated to vaccination, was reported in a 14-month-old subject in the placebo group. The subject experienced colicky abdominal pain, vomiting, mild fever (temperature, 37.8°C), chills, and rigors 10 months after administration of the second dose of placebo and of the DTPa-IPV/Hib and hepatitis B vaccines. A stool sample obtained from the subject was negative for both bacteria and rotavirus antigen. An abdominal x-ray film showed dilated bowel loops and a mass in the right fossa consistent with intussusception, which was confirmed by ultrasound examination. Gas reduction failed 3 times. Laparotomy showed intussusception with ileocolic and ileoileal components spread over an area of 36 cm. Resection of the involved loop was performed, and the child recovered 5 days later. The subject completed the study.

Immunogenicity

Seroconversion. No subject was seropositive for anti–rotavirus IgA antibodies before vaccination. For the vaccine groups, the seroconversion rates for anti–rotavirus IgA antibodies ranged from 75%–86% at 1 month after administration of the first dose to 76%–91% at 1 month after administration of the second dose (table 3). For subjects receiving vaccine virus concentrations of ≥$10^{5.2}$ ffu, there was a slight increase in the seroconversion rate at 1 month after administration of the second dose, compared with that noted at 1 month after administration of the first dose (overlapping 95% CIs); seroconversion rates persisted at 2 months after administration of the second dose.

Table 3. **Anti–rotavirus IgA antibody seroconversion (SC) rate and geometric mean concentration (GMC) after administration of each dose of RIX4414 rotavirus vaccine.**

Dose of RIX4414	One month after dose 1			One month after dose 2			Two months after dose 2		
	No. of subjects	SC rate[a] (95% CI)	GMC[b] (95% CI)	No. of subjects	SC rate[a] (95% CI)	GMC[b] (95% CI)	No. of subjects	SC rate[a] (95% CI)	GMC[b] (95% CI)
$10^{4.7}$ ffu	142	75 (67–82)	282 (225–354)	146	76 (68–83)	273 (221–337)	144	72 (64–79)	180 (145–224)
$10^{5.2}$ ffu	147	86 (80–92)	329 (266–406)	145	91 (85–95)	298 (250–355)	150	87 (81–92)	205 (173–243)
$10^{6.1}$ ffu	153	81 (74–87)	328 (270–398)	154	88 (82–93)	249 (206–303)	160	85 (79–90)	172 (141–209)

NOTE. 95% CI, exact 95% confidence interval; ffu, focus-forming units.

[a] Percentage of subjects with titers greater than the assay cutoff level (i.e., ≥20 U/mL).

[b] GMCs were calculated for subjects who were seropositive for anti–rotavirus IgA antibodies at the specified time point and are given as U/mL.

However, distinctly higher rates were found for infants who received vaccine in titers $\geq 10^{5.2}$ ffu (table 3). As shown in table 3, the GMCs for seropositive subjects were similar in all vaccine groups at 1 month after administration of the first dose and at 1 month after administration of the second dose. GMCs decreased slightly at 2 months after administration of the second dose.

Shedding of vaccine virus (stool subset). Shedding of vaccine (RIX4414) virus was detected in a large proportion of vaccinated infants (76%–80% of infants, for all 3 dose levels) on the seventh day after administration of the first dose (figure 1). Shedding of virus decreased steadily to 18%–24% of infants at day 30 (the day that the second dose was administered). At 7 days after the administration of the second dose, 18%–29% of infants were shedding virus, with a gradual decrease to 11%–16% of infants at day 45 (15 days after administration of the second dose). Because no stool samples were obtained later, it was not possible to determine when shedding ended after administration of the second dose. Shedding of vaccine virus was not associated with any increase in the frequency of gastroenteritis-like symptoms in vaccine recipients, compared with that in placebo recipients (data not shown).

Three cases of virus transmission were detected. Three placebo recipients, none of whom had close contact with any vaccine recipients (except, potentially, at the clinic or in the community), were shedding vaccine rotavirus (as determined by RT-PCR) at different predefined time points after vaccination. One subject shed the vaccine virus at day 7 after administration of the first dose but did not experience seroconversion after administration of the first and second doses of placebo. Stool samples subsequently obtained at day 15 after administration of the first dose and on the day that the second dose was administered were negative for rotavirus. The second subject shed the vaccine virus at day 30 after administration of the first placebo dose (the day that the second dose was administered) and was still positive at day 7 after administration of the second dose. This subject experienced seroconversion, whereas stool samples obtained 7 and 15 days after administration of the first dose were negative for rotavirus. The third subject shed the vaccine virus at days 7 and 15 after administration of the second dose and experienced seroconversion after administration of the second dose. Symptoms of gastroenteritis were not reported for any of these subjects. For all cases, the genetic profile of the shed vaccine strain was identical to that of the vaccine strain (RIX4414).

Vaccine take (stool subset). Vaccine take 1 month after administration of the first dose and on combined doses 1 and 2 months after administration of the second dose is shown in table 4. Vaccine take after administration of the first dose was 89%–94%. Vaccine take on combined doses 1 and 2 months after administration of the vaccine was higher (97.8%–100%).
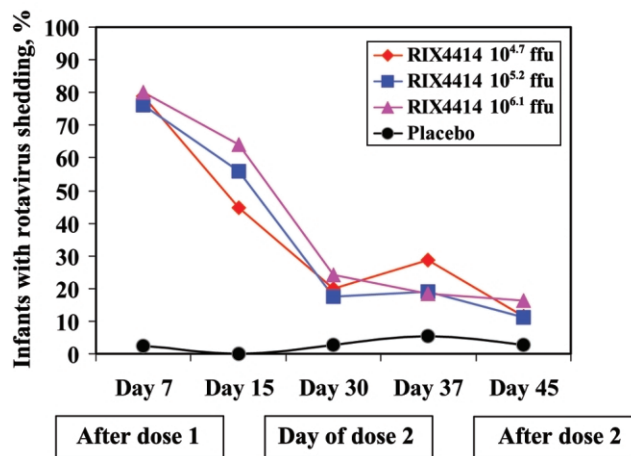

Figure 1. Shedding of vaccine rotavirus after each dose of RIX4414 vaccine.

Seroprotection rates associated with routine infant vaccinations. More than 96% of subjects in each group received 3 doses of the DTPa-IPV/Hib vaccine. More than 91% of subjects in each group received 1 dose of hepatitis B vaccine in the period between administration of the first dose and 2 months after administration of the second dose. The seropositivity rates for all routine infant vaccinations were similar between the vaccine and placebo groups (table 5).

Efficacy

The mean duration of the follow-up period for determination of efficacy was 13 months. The total number of gastroenteritis episodes (regardless of etiology) reported was 387 (276 episodes for 1779 vaccine recipients and 111 episodes for 642 placebo recipients). Most episodes were mild in both the vaccine (68%–72% of episodes) and placebo (75% of episodes) groups (table 6), and only very few cases (10/276 [3.6%] episodes in vaccine recipients and 10/111 [9.0%] episodes in placebo recipients) required hospitalization or an emergency department visit (data not shown). Thus, for 642 placebo recipients (efficacy cohort), the calculated incidence rate of an episode of gastroenteritis for this 13-month period is 111 episodes/642 children (i.e., 0.17 episode/child/13 months).

A smaller number of rotavirus-related gastroenteritis cases than expected were documented during the study. For 41% (160/387) of the reported gastroenteritis episodes, stool samples were not available for determination of the etiology of the gastroenteritis. No results were available for 6% (24/387) of the gastroenteritis episodes because of an insufficient quantity of stool samples collected or because of invalid results. For the 52% (203/387) of stool samples available for detection of rotavirus, only very few cases of rotavirus gastroenteritis ($n = 6$) were identified (2 cases in the $10^{4.7}$ ffu vaccine group and 4

Table 4. Percentage of subjects with vaccine take after administration of dose 1 and on combined doses 1 and 2 months after administration of the second dose of RIX4414 vaccine.

	One month after dose 1		One month after dose 2		Two months after dose 2	
Vaccine or placebo group	No. of subjects[a]	Vaccine take, %[b] (95% CI)	No. of subjects[c]	Vaccine take, %[d] (95% CI)	No. of subjects[e]	Vaccine take, %[f] (95% CI)
RIX4414 vaccine, by dose						
$10^{4.7}$ ffu	35	88.6 (73.3–96.8)	35	100 (90.0–100)	35	100.0 (90.0–100.0)
$10^{5.2}$ ffu	46	91.3 (79.2–97.6)	47	97.9 (88.7–100)	47	97.9 (88.7–100.0)
$10^{6.1}$ ffu	46	93.5 (82.1–98.6)	46	97.8 (88.5–100)	46	97.8 (88.5–100.0)
Placebo	44	4.5 (0.6–15.5)	42	7.1 (1.5–19.5)	43	11.6 (3.9–25.1)

NOTE. Vaccine take after 2 doses is defined as seroconversion occurring after administration of either dose and/or shedding of the vaccine rotavirus in any stool sample obtained from the day that dose 1 was administered until postvaccination blood sampling was performed after administration of the second dose. 95% CI, exact 95% confidence interval; ffu, focus-forming units.

[a] No. of subjects for whom anti–rotavirus IgA antibody data were available at 1 month after administration of the first dose or for whom vaccine virus was found in stool samples obtained any time between administration of the first dose and 1 month after administration of the first dose.

[b] Percentage of subjects who experienced seroconversion at 1 month after administration of the first dose or for whom vaccine virus was found in stool samples obtained any time between administration of the first dose and 1 month after administration of the first dose.

[c] No. of subjects for whom anti–rotavirus IgA antibody data were available at 1 month after administration of the second dose, who experienced seroconversion at 1 month after administration of the first dose, or for whom vaccine virus was found in stool samples obtained any time between administration of the first dose and 1 month after administration of the second dose.

[d] Percentage of subjects who experienced seroconversion 1 month after administration of either the first or second dose or for whom vaccine virus was found in stool samples obtained any time between administration of the first dose and 1 month after administration of the second dose.

[e] No. of subjects for whom anti–rotavirus IgA antibody data were available at 2 months after administration of the second dose, who experienced seroconversion 1 month after administration of either the first or second dose, or for whom vaccine virus was found in stool samples obtained any time between the administration of the first dose and 1 month after administration of the second dose.

[f] Percentage of subjects who experienced seroconversion 1 month after administration of either the first or second dose or by 2 months after administration of the second dose or for whom vaccine virus was found in stool samples obtained any time between administration of the first dose and 2 months after administration of the second dose.

cases in the placebo group). On the basis of these 6 episodes of rotavirus gastroenteritis, the vaccine efficacy for the pooled vaccine groups was 82% ($P = .046$, by 2-sided Fisher's exact test [level of significance, $\alpha = 0.05$]).

For both episodes of rotavirus gastroenteritis that occurred in vaccine recipients, the severity score was very low (score, 2 and 3, respectively; no medical intervention was reported). In the placebo group, 2 episodes were mild (score, 3 and 6, respectively), 1 was moderate (score, 8), and 1 was severe (score, 13). All episodes required medical interventions, with 1 episode resulting in hospitalization.

DISCUSSION

The present study has demonstrated that 2 doses of the live, attenuated rotavirus vaccine (RIX4414) are well tolerated and immunogenic when administered concomitantly with routine infant vaccinations in Asian infants in Singapore. These results are consistent with those from other studies of the vaccine [18, 19, 25].

The study vaccine was highly immunogenic in Singaporean infants and, in virtually all vaccine recipients, vaccine take was demonstrated. The majority of vaccine recipients experienced vaccine take after receipt of the first dose, with little increase in vaccine take occurring after receipt of the second dose. However, because shedding of rotavirus was detected in >10% of vaccine recipients after administration of the second dose to infants with no evidence of vaccine take after administration

of the first dose, 2 doses are needed to maximize vaccine take. Within the limits of comparison, the vaccine take (in terms of shedding) observed in the present study is higher than that observed in a previous study in Bangladesh that used RotaShield (Wyeth-Ayerst) [26]. It is possible that, in a country such as Singapore, a lower likelihood of exposure to other enteric co-pathogens, as well as a better nutritional and health status, can lead to enhanced immune responses.

Depending on the potency of the vaccine, anti–rotavirus IgA antibody seroconversion rates noted in the present study were comparable to results found in Finland [18] but were higher than those found in a trial conducted in Latin America (Brazil, Mexico, and Venezuela) [27, 28]. This result could be explained by the fact that the age at administration of the first dose was greater in Singapore (3 months) than in Latin America (2 months). Although it is not possible to directly correlate immune response to protection, because serological correlates of protection are lacking, this vaccine can be expected to be effective on the basis of the excellent vaccine take found in the present study.

Although almost all vaccine recipients shed the vaccine virus, the observation that 3 placebo recipients, 2 of whom experienced seroconversion, were shedding the vaccine strain indicated that occasional transmission of the vaccine virus might have occurred during study visits or in the community, although errors in labeling of stool samples cannot be excluded. The possibility of horizontal transmission has been previously observed in association with the use of RotaShield [26].

Table 5. Rates of seropositivity to antigen in routine infant vaccines.

Antibodies	Percentage of subjects with antibody titer above the cutoff value for the assay (95% CI) 1 month after administration of dose 3 of routinely administered infant vaccines, by vaccine or placebo group			
	RIX4414, $10^{4.7}$ ffu	RIX4414, $10^{5.2}$ ffu	RIX4414, $10^{6.1}$ ffu	Placebo
Anti-diphtheria[a]	98 (94–100)	98 (93–100)	97 (92–99)	98 (94–100)
Anti-tetanus[a]	100 (97–100)	100 (97–100)	100 (98–100)	99 (96–100)
Anti-PT[b]	99 (96–100)	99 (96–100)	100 (98–100)	99 (96–100)
Anti-FHA[b]	100 (97–100)	100 (98–100)	100 (98–100)	100 (98–100)
Anti-PRN[b]	100 (97–100)	100 (98–100)	100 (98–100)	100 (98–100)
Anti-HBsAg[c]	93 (87–96)	94 (89–98)	96 (91–99)	94 (89–98)
Anti-PRP[d]	100 (97–100)	100 (97–100)	100 (98–100)	100 (98–100)
Anti-poliovirus type 1[e]	100 (97–100)	100 (97–100)	100 (97–100)	100 (97–100)
Anti-poliovirus type 2[e]	100 (97–100)	100 (97–100)	100 (97–100)	100 (97–100)
Anti-poliovirus type 3[e]	100 (97–100)	100 (97–100)	100 (97–100)	100 (97–100)

NOTE. Cutoff levels for anti-diphtheria, anti-tetanus, anti–hepatitis B surface antigen (HBsAg), anti–polyribosyl ribitol phosphate (PRP), and anti-poliovirus types 1, 2 and 3 are seroprotective. 95% CI, exact confidence interval; ffu, focus-forming units; FHA, filamentous hemagglutinin; PRN, pertactin; PT, pertussis toxin.

[a] Antibody levels were determined by ELISA (cutoff level, 0.1 IU/mL).
[b] Antibody levels were determined by ELISA (cutoff level, 5 EL.U/mL).
[c] Antibody levels were determined by Australia antigen (AUSAB; Abbott Laboratories; cutoff level, 10 mIU/mL).
[d] Antibody levels were determined by ELISA (cutoff level, 0.15 μg/mL).
[e] Antibody levels were determined by use of a virus microneutralization test (cutoff titer, ≥8).

There was no observed interference when RIX4414 was coadministered with routine infant vaccinations against diphtheria, tetanus, pertussis, inactivated polio, Hib, and hepatitis B. As a result, there should be fewer hurdles in incorporating the vaccine into current immunization schedules.

There were 2 cases of intussusception reported during the present study, 1 of which was deemed to be possibly related to vaccination. Laboratory data could not confirm or dismiss an association with the vaccine, and no conclusion could be reached. Observing 1 case among 2464 infants during the first year of life was in line with the background incidence of intussusception of 66, 41, and 32 cases/100,000 children <1 year old reported for the years 2000, 2001, and 2002, respectively, in Singapore [29].

The observed association of intussusception with the rhesus-based vaccine RotaShield [7, 8] has changed the evaluation of rotavirus vaccines for safety and, probably, the evaluation of other new live viral vaccine candidates for safety as well. Although the sample size evaluated in the present study precludes final conclusions regarding the risk of intussusception, the vaccine was observed to be safe in >2400 Singaporean infants. Natural infection with wild-type rotavirus has not been found to be associated with intussusception, as seen from the lack of peaks in the incidence of intussusception during the winter epidemics of rotavirus disease [4, 30]. Therefore, because RIX4414 is a live, attenuated human rotavirus vaccine, it is expected to be less likely to be associated with intussusception. Whether intussusception was an adverse effect specific to the rhesus-based vaccine can be determined only by conducting large prospective cohort trials with the newer rotavirus vaccine candidates. Results from such

a trial for RIX4414 have demonstrated that the 2 doses of the vaccine did not increase the rate of intussusception, compared with that associated with placebo [31].

The present study was not conclusive regarding efficacy because the number of rotavirus gastroenteritis cases reported was smaller than expected. Aside from logistical problems in the collection of stool samples, the low incidence of gastroenteritis episodes observed during the surveillance period for efficacy (111 episodes in 642 placebo recipients [i.e., 0.17 episode/child/13 months]), compared with the reported incidence of 3.8 episodes/child/year among children <11 months old [2], may have contributed to the low number of rotavirus gastroenteritis episodes detected. Because there have been no recently published epidemiologic reports of rotavirus gastroenteritis in Singapore, the sample size of the present study was determined

Table 6. Severity of gastroenteritis episodes of any etiology reported during the period of evaluation of efficacy.

Severity score[a]	Dose of RIX4414			Placebo[e]
	$10^{4.7}$ffu[b]	$10^{5.2}$ffu[c]	$10^{6.1}$ffu[d]	
1–6 (mild)	67 (68.4)	61 (71.8)	66 (71.0)	83 (74.8)
7–10 (moderate)	29 (29.6)	20 (23.5)	22 (23.7)	18 (16.2)
≥11 (severe)	2 (2.0)	4 (4.7)	5 (5.4)	10 (9.0)

NOTE. Data are no. (%) of episodes. ffu, focus-forming units.
[a] The severity score was calculated by use of the 20-point scoring system described by Ruuska and Vesikari [21].
[b] Ninety-eight gastroenteritis episodes were reported by 74 subjects.
[c] Eighty-five gastroenteritis episodes were reported by 73 subjects.
[d] Ninety-three gastroenteritis episodes were reported by 84 subjects.
[e] One hundred eleven gastroenteritis episodes were reported by 100 subjects.

on the basis of the attack rate observed in other published studies conducted in the United States of America and Finland [16, 32]. However, the calculated efficacy of the vaccine (82%) against any rotavirus gastroenteritis is in the same range as the vaccine efficacy found in studies of RIX4414 conducted in Finland (73%) and Latin America (70%) [19, 27, 28]. In addition, it was observed that rotavirus gastroenteritis episodes in the vaccine group were milder and did not require medical intervention, compared with such episodes in the placebo group. This result is in line with the observed reduction in the overall incidence of gastroenteritis episodes requiring hospitalization or emergency department visits (3.6% for vaccine recipients vs. 9% for placebo recipients) and could reflect a protective effect of the vaccine against the development of severe rotavirus gastroenteritis in this population.

Although there was a low incidence of gastroenteritis episodes observed during the study period, one should not underestimate the disease burden of rotavirus infection in Singapore. On the basis of the recently published preliminary data from the Asian Rotavirus Surveillance Network, rotavirus remains a major cause of severe gastroenteritis among infants and young children (28%–59% of gastroenteritis cases in children requiring hospitalization) [6]. It has been estimated that acute gastroenteritis is the most common gastrointestinal disorder in Singapore children, accounting for 10% of admissions to general pediatric units and 5% of admissions to government hospitals. Rotavirus was found to be the viral agent most commonly responsible for acute gastroenteritis [33]. These data from Singapore and neighboring countries demonstrate the high incidence of rotavirus infection. The finding of a low incidence of rotavirus gastroenteritis during the study period should be considered an exceptional case.

In consideration of the safety and immunogenicity results of the present study and others [31], it appears that the ideal vaccine dosage for this population would be $\geq 10^{5.2}$ ffu. However, statistically significant efficacy data will have to be considered before a final dose selection is made.

These encouraging results from this phase 2 study are a first step in making a safe and effective rotavirus vaccine available in Asia. At present, larger phase 3 efficacy studies are ongoing in Singapore and other countries to confirm the efficacy and, equally important, the safety with respect to intussusception of the RIX4414 rotavirus vaccine.

References

1. De Zoysa, Feachem RG. Interventions for the control of diarrhoeal disease among young children: rotavirus and cholera immunization. Bull World Health Organ 1985; 63:569–83.
2. Parashar UD, Hummelman EG, Breesee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9:565–72.
3. Unicomb LE, Kilgore PE, Faruque SG, et al. Anticipating rotavirus vaccines: hospital-based surveillance for rotavirus diarrhea and estimates of disease burden in Bangladesh. Pediatr Infect Dis J 1997; 16: 947–51.
4. Nelson EAS, Tam JS, Glass RI, Parashar UD, Fok TF. Incidence of rotavirus diarrhea and intussusception in Hong Kong using standardized discharge data. Pediatr Infect Dis J 2002; 21:701–3.
5. Doan LTH, Okitsu S, Nishio O, Pham DT, Nguyen DH, Ushijama H. Epidemiological features of rotavirus infection among hospitalized children with gastroenteritis in Ho Chi Minh City, Vietnam. J Med Virol 2003; 69:588–94.
6. Bresee J, Fang Z-Y, Wang B, et al. First report from the Asian Rotavirus Surveillance Network. Emerg Infect Dis 2004; 10:988–95.
7. Intussusception among recipients of rotavirus vaccine—United States, 1998–1999. MMWR Morb Mortal Wkly Rep 1999; 48:577–81.
8. Withdrawal of rotavirus vaccine recommendation. MMWR Morb Mortal Wkly Rep 1999; 48:1007.
9. Abramson JS, Baker CJ, Fisher MC, et al. Possible association of intussusception with rotavirus vaccination. American Academy of Pediatrics Committee on Infectious Diseases. Pediatrics 1999; 104:575.
10. Bernstein DI, Smith VE, Sherwood JR, et al. Safety and immunogenicity of live, attenuated human rotavirus vaccine 89–12. Vaccine 1998; 16: 381–7.
11. Bernstein DI, Sander DS, Smith VE, Schiff GM, Ward RL. Protection from rotavirus reinfection: 2-year prospective study. J Infect Dis 1991; 164:277–83.
12. Ward RL, Bernstein DI. Protection against rotavirus disease after natural rotavirus infection. US Rotavirus Vaccine Efficacy Group. J Infect Dis 1994; 169:900–4.
13. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infection in infants as protection against subsequent infections. N Engl J Med 1996; 335: 1022–8.
14. Moulton LH, Staat MA, Santosham M, Ward RL. The protective effectiveness of natural rotavirus infection in an American Indian population. J Infect Dis 1998; 178:1562–6.
15. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection: a prospective longitudinal study in young children. N Engl J Med 1983; 309:72–6.
16. Bernstein DI, Sack DA, Rothstein E, et al. Efficacy of live attenuated human rotavirus vaccine 89–12 in infants: a randomised placebo-controlled trial. Lancet 1999; 354:287–90.
17. Bernstein DI, Sack DA, Reisinger K, Rothstein E, Ward RL. Second year follow-up evaluation of live attenuated human rotavirus vaccine 89–12 in healthy infants. J Infect Dis 2002; 186:1487–9.
18. Vesikari T, Karvonen A, Korhonen T, et al. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine. Vaccine 2004; 22: 2836–42.
19. Vesikari T, Karvonen A, Puustinen L, et al. Efficacy of RIX4414 live attenuated human rotavirus vaccine in Finnish infants. Pediatr Infect Dis J 2004; 23:937–43.
20. Colau B, Denamur F, Knott I, Poliszczak A, Thiry G, Vande Velde V. Vaccine. Patent demand WO 01/12797. Filed 15 August 2000.
21. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for severity of diarrheal episodes. Scand J Infect Dis 1990; 22:259–67.
22. Ward RL, Bernstein DI, Shukla R, et al. Effects of antibody to rotavirus on protection of adults challenged with a human rotavirus. J Infect Dis 1989; 159:79–88.
23. Pang X-L, Joensuu J, Hoshino Y, et al. Rotaviruses detected by reverse

transcription polymerase chain reaction in acute gastroenteritis during a trial of rhesus-human reassortant rotavirus tetravalent vaccine: implications of vaccine efficacy analysis. J Clin Virol **1999**; 13:9–16.

24. Gouvea V, Glass RI, Woods P, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acids from stool specimens. J Clin Microbiol **1990**; 28:276–82.

25. Linhares A, Perez-Schael I, Ruiz-Palacios G, Pernambuco E, Jacquet J, De Vos B. Immunogenicity and reactogenicity of an oral human rotavirus (HRV) vaccine in Latin American infants [poster 242]. In: Program and abstracts of the Congress of the World Society for Pediatric Infectious Diseases (Santiago, Chile). Gennep, The Netherlands: World Society for Pediatric Infectious Diseases, **2002**:82.

26. Bresee JS, El Arifeen S, Azim T, et al. Safety and immunogenicity of tetravalent rhesus-based rotavirus vaccine in Bangladesh. Pediatr Infect Dis J **2001**; 20:1136–43.

27. Linhares AC, Ruiz-Palacios G, Guerrero L, et al. Highlights of worldwide development: a Latin American experience [abstract]. In: Program and abstracts of the Vaccines for Enteric Diseases congress (Montego Bay, Jamaica). Surrey, UK: Meetings Management, **2004**.

28. De Vos B, Vesikari T, Linhares AC, et al. A rotavirus vaccine for prophylaxis of infants against rotavirus gastroenteritis. Pediatr Infect Dis J **2004**; 23:S179–82.

29. Phua KB, Tan N, Koh PK, et al. The incidence of intussusception in children under 2 years of age in Singapore [abstract FP-04–6]. In: Program and abstracts of the 11th Asian Congress of Pediatrics (Bangkok). Association of Pediatric Societies of the South East Asian Region, **2003**.

30. Rennels MB, Parashar UD, Holman RC, Le CT, Chang H-G, Glass RI. Lack of an apparent association between intussusception and wild or vaccine rotavirus infection. Pediatr Infect Dis J **1998**; 17:924–5.

31. O'Ryan M, Lanata C, Abate H, et al. A novel rotavirus vaccine RIX4414 is not associated with intussusception [poster G1129a]. In: Program and abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy (Washington, DC). Washington, DC: American Society for Microbiology, **2004**:15.

32. Joensuu J, Koskenniemi E, Pang XL, Vesikari T. Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. Lancet **1997**; 350:1205–7.

33. Quak SH. Gastroenteritis infections in Singapore children. Ann Acad Med Singapore **1991**; 20:265–8.