Immunogenicity, Reactogenicity, and Immune Memory after Primary Vaccination with a Novel *Haemophilus influenzae-Neisseria meningitidis* Serogroup C Conjugate Vaccine

Heinz-J. Schmitt, Gudrun Maechler, Pirmin Habermehl, Markus Knuf, Roland Saenger, Norman Begg, and Dominique Boutriau

*Johannes-Gutenberg-Universita¨t, Langenbeckstr. 1, 55101 Mainz, Germany, and GlaxoSmithKline Biologicals, Rixensart, Belgium*

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We evaluated two formulations of a new combined *Haemophilus influenzae* type b (Hib)-meningococcal serogroup C (MenC)-tetanus toxoid (TT) conjugate vaccine and two formulations of a new MenC-TT vaccine (trials 711202/001 and 711202/008; clinical trial register numbers NCT00135486 and NCT00135564 [www.ClinicalTrials.gov]). A total of 520 healthy infants were randomized to receive primary vaccination (at 2, 3, and 4 months) with either MenC-TT plus diphtheria-tetanus-acellular pertussis (DTPa)–hepatitis B virus (HBV)–inactivated poliovirus (IPV)/Hib, Hib-MenC-TT plus DTPa-HBV-IPV, or MenC-CRM197 plus DTPa-HBV-IPV/Hib (control). At 12 to 15 months, subjects received a polysaccharide challenge with meningococcal polysaccharide C plus a DTPa-HBV-IPV/Hib booster. Immune responses were assessed 1 month after dose 2, 1 month after dose 3, and prior to and 1 month after the booster. After primary vaccination, there was no difference between groups in seroprotection rates as measured by titers of serum bactericidal antibody (SBA) to MenC (≥1:8) or concentrations of antipolyribosyl ribitol phosphate (PRP) antibody (≥0.15 μg/ml). Prior to the booster, there was no difference between groups in SBA seroprotection rates, whereas anti-PRP seroprotection rates were significantly higher after priming with Hib-MenC-TT. Booster doses induced large increases in SBA and anti-PRP antibodies in primed groups, indicating successful priming with induction of immune memory. Reactogenicity and safety were similar in all groups during the primary and booster phases. A novel Hib-MenC-TT conjugate vaccine induced MenC and Hib responses comparable to those induced by licensed monovalent vaccines. A Hib-MenC-TT conjugate vaccine provides vaccination against two major pathogens in a single injection and is a suitable candidate for use in primary or booster vaccination schedules.

Historically, *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis*, and *Streptococcus pneumoniae* have been responsible for the vast majority of occurrences of bacterial meningitis in children <5 years old. The introduction of Hib conjugate vaccines during the 1990s fundamentally altered the epidemiology of Hib disease. The availability of effective conjugate vaccines against *S. pneumoniae*, Hib, and *N. meningitidis* serogroup C raises the possibility that invasive disease due to these three organisms may be virtually eliminated in young children.

Before the introduction of meningococcal serogroup C (MenC) conjugate vaccination, serogroups B and C were the two most common causes of meningococcal disease in developed countries, including Europe, Australia, and the Americas (1, 4, 12, 17). The burden of endemic disease is highest in infants and children <5 years old (1, 12). In Europe during 1999 to 2000, 16% of cases occurred in children <1 year old (38.6/100,000), and 43% in children <5 years old (14.4/100,000) (12). Increasing case numbers due to serogroup C have been observed in the United Kingdom, Spain, Belgium, Ireland, The Netherlands, and Greece (7, 11, 24). In 1999, a mass vaccination campaign with the first MenC conjugate vaccines was launched in the United Kingdom, aiming to protect the whole population below the age of 25 years. The vaccine was also introduced into the routine infant immunization schedule. An overall reduction in disease of 81% was observed (10).

A novel Hib-MenC-tetanus toxoid (TT) vaccine (containing 5 μg of Hib antigen and 5 μg of MenC antigen) was recently licensed in the United Kingdom (Menitorix; GlaxoSmithKline [GSK] Biologicals). This study evaluated the immunogenicity and safety of the new Hib-MenC-TT vaccine, as well as those of a MenC-TT vaccine, when administered for primary vaccination at the ages of 2, 3, and 4 months. Persistence and immune memory were also assessed.

(The results of these studies have been presented in part at the 4th World Congress of the World Society for Pediatric Infectious Diseases, 1 to 4 September 2005, Warsaw, Poland.)

MATERIALS AND METHODS

Study design and subjects. An open, randomized multicenter study was conducted at 55 sites in Germany. The study protocol was approved by the ethics review committees of the study centers, and the study was conducted according to the ICH Good Clinical Practice guideline, German drug acts, and the Dec-
In the primary phase, eligible infants were randomized to one of five parallel groups: (i) group MenC, receiving a vaccine containing 10 μg of MenC antigen plus diphtheria-tetanus-acellular pertussis (DTPa)-hepatitis B virus (HBV)-inactivated poliovirus (IPV)/Hib; (ii) group MenCads, receiving a vaccine containing 10 μg of MenC antigen adsorbed onto aluminum plus DTPa-HBV-IPV/Hib; (iii) group Hib10-MenC10, receiving a vaccine containing 10 μg of Hib antigen and 10 μg of MenC antigen plus DTPa-HBV-IPV; (iv) group Hib5-MenC5, receiving a vaccine containing 5 μg of Hib antigen and 5 μg of MenC antigen plus DTPa-HBV-IPV; and (v) the control group, receiving MenC-CRM197 (Meningitec) plus DTPa-HBV-IPV/Hib (Infanrix hexa) (Fig. 1). Primary vaccination was administered at the ages of 2, 3, and 4 months.

All groups except group MenCads were included in the booster phase. Subjects from group MenCads were offered a dose of a licensed MenC conjugate vaccine in the second year of life. An additional group, “group O*”, was added to the booster phase; it consisted of age-matched MenC-naive subjects vaccinated according to the German vaccination schedule (9). At the age of 12 to 15 months, all subjects received one-fifth dose of Men ACWY vaccine (Mencevax, an N. meningitidis polysaccharide C (PSC) conjugated to tetanus toxin), and Mencevax is a trademark of the GSK group of companies.

Healthy infants between the ages of 8 and 16 weeks at the time of first vaccination were eligible for inclusion in the study. Subjects were excluded in case of major congenital defects or serious chronic illness; immunodeficiency; previous/intercurrent vaccination or disease with study vaccine antigens; allergy to any component of the vaccine; use or planned use of other investigational or approved vaccines; allergy to any component of the vaccine; or the immune response to Hib, tetanus toxoid, pertussis, polio, and hepatitis B antigens were measured at month zero and month 3 only.

Serum bactericidal antibodies to MenC (SBA-MenC) were measured by a serum bactericidal test using baby rabbit complement and the C11 strain (9). The cutoff of the test was a dilution of 1:8, which is considered to be protective (2). Different methodologies were used for the SBA-MenC assay during the primary and booster phases: in the primary phase, titers were expressed as continuous values (obtained via curve fitting to derive the exact theoretical twofold-dilution values, while in the booster phase, titers were expressed as equivalent values obtained via curve fitting to derive the exact theoretical dilution giving 50% bactericidal activity, in order to improve the reproducibility of the assay. Therefore, no comparison should be made between the post-primary vaccination and the pre- and post-booster vaccination time points. Antibodies against PSC were measured by an enzyme-linked immunosorbent assay (ELISA) (13). The assay cutoff was 0.3 μg/ml. Antibodies against PRP, diphtheria and tetanus toxoids, pertussis antigens (pertussis toxin [PT], filamentous hemagglutinin [FHA], and pertactin [PRN]), and hepatitis B surface antigen (HBs) were determined by ELISA, as previously described (3). The cutoff values for the assays were 0.15 μg/ml for anti-PRP; 0.1 IU/ml for diphtheria and tetanus toxoid antibodies; 5 ELISA units/ml for PT, FHA, and PRN; and 10 μIU/ml for anti-HBs. For SBA-MenC, PRR, diphtheria, tetanus, and polio, an antibody level greater than or equal to the assay cutoff was considered to be protective. For SBA-MenC, an antibody level greater than or equal to the assay cutoff was considered to be protective. For pertussis, a vaccine response was defined as a postvaccination antibody concentration greater than or equal to the assay cutoff value for initially seronegative infants and as a postvaccination con-
centration greater than or equal to the prevaccination concentration for initially seropositive infants (thereby taking into account the half-life of maternal antibodies).

Assessment of safety. Solicited local adverse reactions (pain, redness, swelling) and solicited general symptoms (fever [defined as a rectal body temperature of ≥38.0°C], irritability/fussiness, drowsiness, loss of appetite) were recorded by completing diary cards for 8 days after each primary vaccination and for 4 days following the booster dose. All other symptoms, serious adverse events (SAEs), and extensive injection site swellings were recorded during the entire study period until 30 days following the last vaccination. Symptom intensity was scored on a 4-point scale. Symptoms of grade 3 intensity were defined as follows: for pain, crying when the limb is moved/spontaneously painful (i.e., pain when the limb is moved and when it is stationary); for redness and swelling, a diameter of >30 mm; for fever, a temperature of >40.0°C; for all other adverse events, prevention of everyday activities and causing the parents/guardians to seek medical advice. The parents/guardians were asked to contact the investigator immediately if the child manifested any signs that were perceived as serious.

Statistical methods. Geometric mean antibody concentrations/titers (GMC/T) with 95% confidence intervals (CIs) were calculated, and antibody concentrations/titers below the assay cutoff were given an arbitrary value of half the cutoff value for the purpose of GMC/T calculation. Seropositivity/seroprotection/vaccine response rates with exact 95% CIs were calculated.

After primary vaccination, exploratory comparisons between groups were performed through computation of standardized asymptotic 95% CIs for the difference in seroprotection/seropositivity/vaccine response rates between each of the study vaccine groups and the control group. Additionally, 95% CIs for the GMC/T ratios between groups were determined using a one-way analysis of variance model on the log_{10} transformation of titers.

After the polysaccharide challenge, an exploratory comparison of the experimental formulations and the MenC-naïve group O was performed in terms of the SBA-MenC and PSC responses through computation of 95% CIs for the GMC/T ratios between groups. Using the same method, the differences between the three experimental groups and the control group in terms of the anti-PRP and anti-tetanus immune responses after the booster dose of DTPa-HBV-IPV/Hib were also computed.

Two vaccine groups were considered significantly different if the standardized asymptotic 95% CI for the difference in seroprotection/seropositivity/vaccine response rates between the two groups did not contain a value equivalent to 0. For GMC/T, groups were considered significantly different if the 95% CI for the GMC/T ratio between groups did not contain a value equivalent to 1. When exploratory analyses were not performed, differences were evaluated by considering the overlap of 95% CIs. The risk of error due to the multiplicity of comparisons was not adjusted.

The incidence and intensity of each solicited adverse event were tabulated with the exact 95% CI. A sample size of 500 subjects to provide 80 evaluable subjects per group for the immunogenicity analysis provided 92% individual power for each of the four comparisons to be done to rule out the null hypothesis: the SBA-MenC seroprotection rate for a MenC group was <10% lower than that for the control group receiving MenC-CRM197 (expected proportions, 95% in group MenC and 98% in the control group; nQuery alpha = 0.025).

RESULTS

A total of 520 healthy infants were enrolled in the primary vaccination phase during 2003 (Fig. 2). A total of 483, 448, and
During the booster phase, no dropouts due to adverse events were observed and none were considered by the investigator to be related to the control group. All three events resolved without sequelae, and poliovirus antibody seropositivity/seroprotection/vaccine response rates (Table 2) at month 3. Antibody GMC/T for all component antigens were of similar orders in all groups, with the exception of diphtheria and tetanus: anti-diphtheria antibody GMCs were significantly higher in the control group, and anti-tetanus GMCs were significantly higher in all four experimental vaccine groups.

There were no statistically significant differences between groups in terms of diphtheria, tetanus, hepatitis B, pertussis, and poliovirus antibody seropositivity/seroprotection/vaccine response rates (Table 2) at month 3. Antibody GMC/T for all component antigens were of similar orders in all groups, with the exception of diphtheria and tetanus: anti-diphtheria antibody GMCs were significantly higher in the control group, and anti-tetanus GMCs were significantly higher in all four experimental vaccine groups.

(ii) Antibody persistence and booster phase. Based on the results of the primary vaccination phase, development of the experimental MenCads vaccine was discontinued, and this group was not evaluated further. Prior to the booster dose, there was no difference between experimental or control groups in the proportion of subjects with SBA-MenC antibody titers of ≥1:8 (Table 3). In contrast to the post-primary vaccination findings, prebooster SBA-MenC GMTs tended to be higher for group Hib5-MenC5 than for the control group and were statistically significantly higher for group MenC. Most subjects were still seropositive for anti-PSC antibody as measured by ELISA, with no difference between groups. Anti-PSC antibody GMCs were significantly lower for groups Hib10-MenC10 and Hib5-MenC5 than for the control group. Prior to the booster doses, anti-PPR antibody seroprotection rates and GMCs were both significantly higher for groups Hib10-MenC10 and Hib5-MenC5 than for the control group. Anti-tetanus seroprotection rates were not significantly different between groups prior to the booster phase.
dose; however, anti-tetanus antibody GMCs continued to be higher for all of the experimental vaccine groups than for the control group (data not shown).

The administration of a 10-μg challenge dose of plain PSC elicited a marked booster response in all four groups that had received primary vaccination with a MenC conjugate vaccine (Table 3). The response is in striking contrast to that of MenC-naive subjects in group O; more than 90% of subjects primed with a MenC conjugate vaccine had SBA-MenC titers of ≥1/128 after the polysaccharide challenge, compared with 17.6% in group O. Postchallenge GMTs were significantly higher for groups MenC and Hib5-MenC5 than for the control group.

Similarly, polysaccharide challenge induced increases in anti-PSC antibody levels of at least eightfold for the experimental groups, higher than those for the control group. Notably, 95.7% of unprimed subjects responded to the challenge dose with anti-PSC concentrations of ≥0.3 μg/ml but with lower GMCs.

All subjects received a booster dose of DTPa-HBV-IPV/Hib on the same day as the polysaccharide challenge. One month later, at least 98.8% of subjects had anti-PRP antibody concentrations of ≥0.15 μg/ml and at least 96.5% of subjects had concentrations of ≥1.0 μg/ml. Statistically significantly higher anti-PRP GMCs were observed for the group primed with Hib5-MenC5 than for the control group.

One month after the booster dose, all subjects in all five groups had seroprotective concentrations of anti-tetanus antibodies. Anti-tetanus antibody GMCs continued to be significantly higher for all three experimental vaccine groups than for the control group.

**Safety.** Redness and drowsiness/irritability were the most commonly reported local and general solicited symptoms, respectively, after primary vaccination (Fig. 3 and 4). Symptoms

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**TABLE 3. SBA-MenC, PSC, and PRP antibody persistence (prebooster) and immune response 1 month after polysaccharide challenge and DTPa-HBV-IPV/Hib booster doses (postbooster)**

| Group | Antibody | Assay cutoff | % of subjects responding | GMT (95% CI) | % of subjects responding | GMT (95% CI) | % of subjects responding | GMT (95% CI) | % of subjects responding | GMT (95% CI) |
|-------|----------|--------------|--------------------------|--------------|--------------------------|--------------|--------------------------|--------------|--------------------------|--------------|
| MenC  | SBA-MenC | ≥1/8 (95% CI)* | 88.6 (79.5, 94.7) | 189.1* (124.5, 287.3) | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |
|       |          |              | 100.0 (94.9, 100.0) | 3.85 (3.20, 4.64) | 88.9 (80.0, 94.8) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) | 0.68 (0.50, 0.94) |

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* Asterisks indicate statistically significant differences relative to the control group.

b VR, vaccine response (postvaccination antibody concentration greater than or equal to the assay cutoff value for initially seronegative infants and the prevaccination concentration in initially seronegative infants).
of grade 3 intensity occurred infrequently. Incidences of reported local and general solicited symptoms were within the same range for the different treatment groups. After the booster dose, the incidences of local symptoms of pain, redness, and swelling were higher than those after primary vaccination. Reported general solicited symptoms increased to a lesser degree after the booster dose. No difference in the incidence of local or general symptoms after the booster was observed between groups, including the group that had not previously received primary vaccination with a MenC-containing vaccine (group O).

During primary vaccination, a total of 13 SAEs were re-
ported for 12 subjects (4 in group MenC, 3 in group MenCads, 2 in group Hib10-MenC10, 1 in group Hib5-MenC5, and 2 in the control group): bronchitis, pyelonephritis (3 cases), degenerative brain disease with epilepsy, skull fracture, coordination disturbance, pertussis, apnea, otitis media, thrombocytopenia, pneumonia, and vomiting. None were considered by the investigator to be vaccine related. No deaths were reported. Four SAEs occurred during the booster phase (two in group MenC, one in group Hib10-MenC10, and one in the control group): pseudocroup, viral infection, bronchitis, and febrile convolution.
None were determined by the investigator to be causally related to vaccination.

**DISCUSSION**

This study has demonstrated the feasibility of a novel combined Hib-MenC conjugate vaccine for the primary vaccination of infants. The reduced-content Hib5-MenC5 vaccine was highly immunogenic and induced immune memory following primary vaccination. High levels of SBA-MenC seroprotection and anti-PSC seropositivity after two doses of the experimental MenC or Hib-MenC vaccines, similar to levels observed after dose 3 in the control group, were observed. A polysaccharide challenge in the second year of life induced very large increases in antibody concentrations and bactericidal titers. Anti-PSC antibody levels were significantly higher in groups that had received primary vaccination with a combined Hib-MenC vaccine, and SBA-MenC titers were significantly higher in the group that had received reduced polysaccharide levels during primary vaccination (Hib5-MenC5). The greater magnitude of the response to challenge for subjects primed with Hib5-MenC5 is consistent with previous observations, where a lower quantity of antigen given during primary vaccination induced a higher immune memory response (15, 20). We observed that the proportions of subjects who remained seroprotected/sero-positive for SBA-MenC and PSC prior to the challenge were not different for the Hib-MenC and control groups, suggesting that the lower SBA-MenC GMT and the lower anti-PSC GMC observed after primary vaccination were of no clinical importance.

To our knowledge, this is the first study where a Hib vaccine containing a reduced quantity of PRP-PRP-TT has been coadministered with an acellular pertussis vaccine and the first in which a Hib-MenC conjugate combination vaccine was evaluated. All groups that received primary vaccination with the experimental MenC or Hib-MenC vaccines demonstrated immune responses that were superior compared to those of the control group in terms of anti-PRP antibody GMCs after primary vaccination, with anti-PRP GMCs similar to those observed after vaccination with licensed monovalent Hib conjugate vaccines (18). The Hib-MenC vaccines showed higher anti-PRP seroprotection rates after two doses and higher seroprotection rates 1 year later, at the time of the booster dose. The effect was most marked for the Hib5-MenC5 vaccine, where the high level of immune response observed after two doses was similar to that after three doses of DTPa-HBV-IPV/Hib.

Our results are consistent with previous observations highlighting the complex interactions between conjugated vaccines and DTPa-based vaccines, whereby concomitant administration of MenC-TT with DTPa and Hib-TT vaccines induced a lower SBA-MenC GMT, whereas the same combination of vaccines induced a higher anti-PRP antibody response, although no impact on seroprotection rates was observed (8). Meningitec (using a CRM197 carrier) was chosen as the control vaccine in this study, because it was the first conjugate vaccine licensed in the United Kingdom and was the most frequently used for infant vaccinations and for the beginning of the vaccination campaigns.

The immune memory response was highest in the group primed with Hib5-MenC5. Two factors may explain the observed response: (i) a lower quantity of antigen during primary vaccination may trigger a higher antibody response postchallenge, as reported previously in references 6 and 15, and (ii) the TT carrier may induce a better memory response. Such an effect was also shown when immune memory was evaluated for United Kingdom toddlers primed with one dose of MenC-TT or MenC-CRM197 conjugate at the age of 12 to 18 months (16).

In light of recent experiences with Hib vaccines, where the importance of a booster dose has been demonstrated, particularly when immunologically challenging immunization schedules are employed (5, 14, 20), there has been debate over the need for a booster dose of MenC vaccine after primary vaccination. Recent data from the United Kingdom indicate that infants vaccinated with MenC conjugates at the ages of 2, 3, and 4 months in the routine infant immunization program were not protected for more than a year after the last dose (23) and that seroprotection rates had dropped to low levels in older children who had received vaccination with a single dose (19). Booster vaccination against Hib and MenC during the second year of life is likely to be needed to reinforce and prolong protection after primary vaccination (19, 20).

The new MenC and Hib-MenC study vaccines were coadministered with licensed DTPa-based combination vaccines without impact on the reactogenicity or safety profile compared to that for the control group. The incidences of reported solicited local and general symptoms were similar for subjects primed with a MenC vaccine and subjects who had not received primary vaccination with a MenC vaccine, suggesting that the three-dose primary vaccination course with additional TT present in the new vaccines had no impact on the reactogenicity of the booster dose. Furthermore, there was no evidence to suggest that the high-persisting anti-tetanus and anti-PRP antibody concentrations prior to the booster dose had any impact on the reactogenicity of the DTPa-HBV-IPV/Hib booster.

This new Hib-MenC combined vaccine with reduced amounts of PRP and PSC provides vaccination against two antigens in one injection and may be used for primary or booster vaccination. It may be safely coadministered with the pentavalent DTPa-HBV-IPV vaccine without affecting the immune response of either vaccine; therefore, it may also provide a valid alternative to DTPa-Hib combinations. A high response to both the MenC and Hib components was seen already after the second dose and confirmed in another study (21), suggesting a potential two-dose primary vaccination regimen with a booster dose in the second year of life. MenC is now recommended as a two-dose vaccine in most countries recommending infant vaccination.

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