# Enhanced Immunogenicity of Seasonal Influenza Vaccines in Young Children Using MF59 Adjuvant

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**Background:** Children have high morbidity and hospitalization rates from seasonal influenza. Meta-analyses suggest that conventional inactivated influenza vaccines are of low efficacy in young children, making vaccines that induce greater and broader immune protection in this vulnerable population a medical priority. Adjuvanted influenza vaccines may offer a solution.

**Subjects and Methods:** Unprimed healthy children (6 to <36 months) were enrolled in an observer-blinded study and randomly assigned to receive 2 doses of MF59-adjuvanted vaccine (Sub/MF59, n = 130) or nonadjuvanted split vaccine (split, n = 139); subgroups of these (n = 43 and 46, respectively) received a booster dose 1 year later. Safety and clinical tolerability were assessed after each dose. Hemagglutination inhibition antibody titers were measured against influenza A and B strains included in the formulation of the vaccines and against mismatched strains.

**Results:** Clinical tolerability and safety were generally comparable between vaccine groups, though some transient, mild solicited reactions were more frequent in the Sub/MF59 group. Postvaccination hemagglutination inhibition antibody titers to all 3 vaccine strains were significantly higher with Sub/MF59 than with split vaccine (all comparisons $P < 0.001$) after each of the 3 vaccine doses. In addition, Sub/MF59 induced significantly higher cross-reactivity against A/H3N2 and A/H1N1 mismatched strains.

**Conclusion:** MF59-adjuvanted influenza vaccine was well tolerated in healthy young children after each of 3 doses and induced greater, longer-lasting, and broader immune responses than a nonadjuvanted split vaccine. The enhanced immunogenicity of the adjuvanted vaccine was most evident in very young children and for the B vaccine strain.

**Key Words:** children, MF59, influenza, vaccine, cross-immunogenicity

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Recent studies on the medical[1–3] and socioeconomic[4] impact of influenza have increasingly highlighted the burden of influenza disease in young children and the unmet need for effective vaccines for this age group. Conventional trivalent inactivated vaccines have a long-track record in the pediatric population, but they do not appear to effectively induce protective antibodies in young unprimed children,[5,6] resulting in only modest efficacy. Vaccine efficacy may be even further compromised in years when there is a mismatch between the viral variants included in the vaccine and the strains actually in circulation.[7]

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The combination of the inherently lower immunogenicity of conventional seasonal influenza vaccines in children and potential strain mismatch creates a challenge for development of more efficacious influenza vaccines, especially in vulnerable subjects such as very young children.

One potential strategy to improve immunogenicity is to use adjuvanted vaccines. MF59 is an oil-in-water emulsion containing naturally occurring squalene oil, and a biodegradable and biocompatible adjuvant. MF59 is thought to act by recruiting and activating antigen-presenting cells at the injection site, thus increasing their capacity to capture, transport, and process the coadministered antigens.[8]

MF59 was first approved for human use in 1997 as an influenza subunit vaccine adjuvant for the elderly. MF59-adjuvanted influenza subunit vaccine has been shown to induce greater immunoresponses than nonadjuvanted comparators, especially those with low prevaccination antibody titers. It also has a good safety profile in vulnerable populations such as the elderly.[9–11] Furthermore, in these populations MF59-adjuvanted vaccine conferred broader immunogenicity than conventional vaccines against drifted strains, and especially against mismatched A/H3N2 influenza antigens.[12–14]

The MF59 adjuvant has been administered previously to toddlers and infants in candidate vaccines against cytomegalovirusand human immunodeficiency virus type 1. These MF59-adjuvanted vaccines were well tolerated and immunogenic in young children.[15,16]

We assessed the immunogenicity, clinical tolerability, and safety of primary and booster doses of an MF59-adjuvanted inactivated influenza subunit vaccine in comparison with a conventional nonadjuvanted split vaccine in healthy children. We also assessed the ability of the vaccines to elicit an immune response against drifted influenza viruses.

## METHODS

### Study Design

The primary observer-blind, randomized study was performed in Finland between November 2006 and August 2007 in healthy children aged 6 to <36 months, not previously vaccinated against influenza. Parents of children who completed the first proof of concept study were invited to include their children in an extension trial (between November 2007 and June 2008) to assess the safety and immunogenicity of a third dose of the same vaccine, administered approximately 1 year later. For both studies, exclusion criteria included known allergy to any vaccine component, known or suspected neurologic reactions following influenza vaccination, any acute infectious or respiratory disease requiring systemic treatment up to 30 days before the study start and laboratory-confirmed influenza disease in the previous 6 months.

Designated unblinded study site staff prepared and administered vaccine according to a computer-generated randomization list and had no further contact with subjects or access to data. The investigator and all other study staff were blinded throughout the study. The study protocols conformed to the ethical guidelines of

the Declaration of Helsinki and Good Clinical Practice and were approved by the Ethics Committee of the University Hospital District of Tampere. Informed consent was obtained from each child's parents or legal guardians before study start.

### Year 1: First and Second Dose

In the first study, subjects were randomly assigned in a 1:1 ratio to receive 1 of 2 trivalent inactivated influenza vaccines: a subunit vaccine adjuvanted with MF59 (Sub/MF59, Fluad, Novartis Vaccines) or a licensed nonadjuvanted split vaccine (Vaxigrip, sanofi pasteur). Randomization was stratified according to age (6–11 months, 12–17 months, 18–23 months, 24–29 months, 30–35 months). Two 0.25 mL vaccine doses were given 4 weeks apart, administered intramuscularly in the deltoid region of the nondominant arm, or in the anterolateral aspect of the thigh, if the deltoid mass was insufficient. Both vaccines contained 7.5 $\mu$g per 0.25 mL of each of the 3 influenza antigens recommended by the World Health Organization for the Northern Hemisphere (NH) during the 2006–2007 influenza season: A/New Caledonia/20/99 (H1N1)-like virus, A/Wisconsin/67/2005 (H3N2)-like virus, B/Malaysia/2506/2004-like virus. Both vaccines were provided in 0.5 mL prefilled syringes with a pediatric ring for the administration of half of the adult dose (0.25 mL). The Sub/MF59 influenza vaccine contained 9.75 mg of squalene as adjuvant per 0.5 mL.

### Year 2: Booster Dose

In the extension study, children who had received both vaccine doses received a third dose of the respective vaccine, approximately 1 year after the first dose. For the 2007–2008 NH season, only the recommendation for the A/H1N1-like strain (A/Solomon Islands/3/2006) changed compared with year 1 of this study. In this report, we will use the term "booster dose" to refer to this year 2 vaccination. The booster dose was administered as a single intramuscular dose of 0.25 mL in children aged less than 36 months or 0.5 mL in those aged 36 months or older.

## Study Assessments

### Immunogenicity

In the unprimed children, blood samples were obtained before vaccination (day 0), 4 weeks after the first vaccine dose (day 29), 3 weeks after the second dose (day 50), and at the end of 6 months' follow-up (day 209). Further samples were taken in the extension study prior to (year 2, day 0) and 3 weeks after (year 2, day 21) the booster dose. Immunogenicity was analyzed for the per protocol population, defined as all children who received all the doses of vaccine correctly, provided evaluable serum samples at all scheduled time points, and had no major protocol violations. Hemagglutination inhibition (HI) antibody titers were measured as described previously,[17] against each of the 3 influenza strains in the 2006–2007 and 2007–2008 vaccine formulations, respectively. Results were expressed as geometric mean titers (GMTs) with 95% confidence intervals (CI); geometric mean ratios (GMRs) of post- to prevaccination titer; seroprotection rates, defined as the percentage of subjects achieving an HI titer $\geq$40; and seroconversion rates, defined as the percentage of subjects achieving at least a 4-fold increase in HI titer from a seropositive prevaccination titer ($\geq$10) or a rise from $<$10 to $\geq$40 in those who were seronegative.

In a poststudy immunogenicity analysis, sera collected during the primary trial (2006–2007) were also tested against mismatched A/H3N2 (A/New York/55/2004-like strain) and B (Jiangsu/10/2003-like strain) recommended for 2005–2006 NH vaccination campaign, and against the A/H1N1 strain (A/Solomon Islands/3/2006-like) that emerged during subsequent influenza season (2007–2008), to assess the immune response induced against vaccine mismatched viruses.

### Clinical Tolerability and Safety

Safety was assessed for all subjects who received at least 1 dose of vaccine and had post-baseline safety data. Immediately after each vaccination and for the following 7 days, parents/legal guardians recorded solicited local and systemic reactions on diary cards. Solicited reactions varied according to the age of the subject. As it was conducted 1 year later, the extension study also included older children ($\geq$36 months of age), for whom different criteria were used to assess tolerability in comparison with the earlier initial tolerability assessments. For children $<$36 months of age, systemic reactions were largely based on behaviors (eg, irritability, unusual crying, sleepiness, change in eating habits, vomiting, and diarrhea). Other systemic reactions (eg, chills, malaise, myalgia, arthralgia, headache, and fatigue) were solicited from older children ($\geq$36 months of age) who were better able to express themselves. Similarly, solicited local reactions included ecchymosis, erythema, induration and swelling for all subjects, and either tenderness (children $<$36 months of age) or pain (children $\geq$36 months of age in the extension study) at the injection site. Body temperature, use of analgesic/antipyretic medication and any other adverse events (AEs) were registered for all subjects. All AEs were recorded from study start up to 3 weeks after the last vaccine injection. All serious AEs (SAEs) and AEs necessitating physician consultation, or leading to premature study discontinuation, were recorded throughout the studies.

### Statistical Analysis

Data were analyzed using SAS (SAS Institute; version 9.1 or higher, Cary, NC). The $\chi^2$ test was performed to analyze differences between proportions of subjects. Statistical significance between pre- and postvaccination titers was assessed using a paired Student $t$ test. Different vaccine groups were compared using the Student $t$ test for unpaired data. A $P < 0.05$ was considered statistically significant.

# RESULTS

## Study Population

### Year 1: First and Second Dose

In the primary trial, 281 children were enrolled and randomized and 269 received at least 1 dose of vaccine: 130 in the Sub/MF59 group and 139 in the split group (Figure, Supplemental Digital Content 1, http://links.lww.com/A1127). Of these, 222 subjects completed the initial study, had sufficient blood samples for analysis, and were included in the immunogenicity analysis: 104 in the Sub/MF59 vaccine group and 118 in the split vaccine group. Demographic characteristics and age distributions were well matched between the groups (Table, Supplemental Digital Content 2, http://links.lww.com/A1128).

### Year 2: Booster Dose

Parents of all children who completed the primary study were invited to have their children take part in the extension study 1 year later. After screening, only 89 children were eligible: 43 in the Sub/MF59 group and 46 in the split group (Figure, Supplemental Digital Content 1, http://links.lww.com/A1127). Most of the screening failures resulted from children having already received an influenza vaccine for that season outside of the study, in agreement with the new recommendation issued for the 2007–2008 season in Finland. Of the revaccinated children, 81 subjects were included in the immunogenicity analysis: 41 in the Sub/MF59 vaccine group and 40 in the split vaccine group. The other 8 subjects were excluded from analysis because of a missing blood

sample for at least 1 visit or because they had received the wrong vaccine dose for their age.

## Safety

### Year 1: First and Second Dose

Diary cards for local and systemic reactions and AE reports were collected from all 269 children enrolled in the first study. Solicited local and systemic reactions reported during 1 week after each of the first 2 doses are presented in Table 1. Reactions were typically mild or moderate and transient (subsiding within 2–3 days after vaccination). There were no significant differences in local and systemic reactions between the 2 vaccine groups, with the exception of injection site swelling, where there were more reports of mild or moderate swelling with Sub/MF59 (12%) than with the split vaccine (5%, $P = 0.033$). In general, both local and systemic reactions were reduced after the second vaccine dose compared with the first vaccination.

Twenty-one children in each group had an AE assessed as possibly or probably vaccine-related from the study start to the end of the 6-months follow-up. Two children in each group were withdrawn from the trial because of an AE (1 case of petecchiae and 1 case of urticaria in the Sub/MF59 group; 1 case of asthma and 1 case of upper respiratory infection in the split group). Two SAEs (both cases of pneumonia) were reported during the follow-up period in the Sub/MF59 group. The first case occurred in a 3-year-old girl, pneumonia led to hospitalization, with a rapid improvement of the health conditions after antibiotic treatment; the child was discharged after 3 days. The second case occurred in a 14-month-old boy and it was a case of Respiratory syncytial virus pneumonia. Six SAEs were reported in the split group (2 cases of prolonged bronchitis, 2 cases of gastroenteritis, 1 case of otitis media, and 1 case of asthma). None of these events was judged to be vaccine related.

### Year 2: Booster Dose

After the booster injection, overall solicited reactions were slightly more frequent in the Sub/MF59 group compared with the nonadjuvanted split group (Table, Supplemental Digital Content 3, http://links.lww.com/A1129). Although not statistically significant, there was a trend toward an increased incidence of reactions in children aged ≥36 months, evident for both vaccine groups. The incidence of local and systemic reactions was similar to that observed during the first year and was generally comparable between vaccine groups. In the older age group (≥36 months), 12 of 18 children (67%) receiving the Sub/MF59 vaccine had pain at the injection site, compared with 6 of 23 children (26%, $P < 0.001$) receiving the split vaccine. Only 1 subject in the Sub/MF59 group reported severe pain.

## Immunogenicity

### Year 1: First and Second Dose

Serologic analysis was performed on the 222 subjects who completed the full vaccination schedule in year 1 and had all 4 sera drawn (n = 104, Sub/MF59 group; n = 118, split group). Baseline GMTs were well balanced between vaccine groups (Table 2). In each age and vaccine group, GMTs against H3N2 were significantly higher than those against H1N1, which in turn were significantly higher than those against influenza B. Responses to H3N2 and H1N1 were similar across the age groups, but there was an apparent age dependence in the response to the B strain in the split vaccine, with very low responses in the youngest children. In contrast, the Sub/MF59 group showed similarly high responses across all age ranges tested (Fig. 1).

Overall, postvaccination GMTs and GMRs were significantly higher with Sub/MF59 than with the split vaccine for all 3 vaccine virus strains (all comparisons $P < 0.001$).

In the total study population, both vaccines elicited comparably high seroprotection rates for A/H3N2 (100% Sub/MF59 vs. 99% split), but Sub/MF59 elicited a significantly higher seroprotection rate for A/H1N1 (100% vs. 86% split [$P < 0.001$]) after the recommended 2 doses for this age group (Table 2). After a single dose, significantly greater proportion of the Sub/MF59 group achieved seroprotection (A/H3N2: 91% vs. 49% split [$P < 0.001$] and A/H1N1: 51% vs. 18% split [$P < 0.001$]). Antibody responses to influenza B remained low after a single dose in both vaccine groups; however, after 2 doses, 99% of subjects in the Sub/MF59 group had seroprotective levels of HI antibody compared with only 33% of subjects in the split vaccine group ($P < 0.001$; Table 2). In children 6 to 11 months of age, the difference was even more pronounced, with 100% of children in the Sub/MF59 group achieving seroprotection compared with 12% of those in the split vaccine group ($P < 0.001$; Fig. 2). A similar pattern of results was

**TABLE 1.** Summary of Solicited Local and Systemic Reactions After the First and Second Vaccine Doses

| Postimmunization Solicited Reactions | After First Dose | | After Second Dose | | Overall | |
|---|---|---|---|---|---|---|
| | Sub/MF59 n = 130 | Split n = 139 | Sub/MF59 n = 130 | Split n = 139 | Sub/MF59 n = 130 | Split n = 139 |
| Local reactions, number (%) | | | | | | |
| Tenderness | 43 (33) | 36 (26) | 34 (29) | 28 (22) | 58 (45) | 47 (34) |
| Erythema | 32 (25) | 30 (22) | 29 (25) | 22 (17) | 46 (35) | 38 (27) |
| Induration | 10 (8) | 13 (9) | 12 (10) | 11 (9) | 21 (16)† | 20 (14) |
| Swelling | 10 (8)* | 3 (2) | 8 (7) | 5 (4) | 16 (12)† | 7 (5) |
| Ecchymosis | 11 (8) | 13 (9) | 9 (8) | 8 (6) | 18 (14) | 19 (14) |
| Systemic reactions, number (%) | | | | | | |
| Fever ≥38°C | 9 (7) | 6 (4) | 7 (6) | 8 (6) | 16 (12) | 13 (9) |
| Analgesic/antipyretic use | 23 (18) | 17 (12) | 18 (15) | 17 (13) | 34 (26) | 32 (23) |
| Irritability | 41 (32) | 36 (26) | 29 (25) | 24 (19) | 53 (41) | 46 (33) |
| Unusual crying | 15 (12) | 11 (8) | 13 (11) | 11 (9) | 24 (18) | 19 (14) |
| Sleepiness | 24 (18) | 19 (14) | 17 (15) | 13 (10) | 35 (27) | 26 (19) |
| Change in eating habits | 23 (18) | 24 (17) | 14 (12) | 9 (7) | 32 (25) | 30 (22) |
| Vomiting | 4 (3) | 4 (3) | 5 (4) | 5 (4) | 8 (6) | 8 (6) |
| Diarrhea | 11 (8) | 11 (8) | 9 (8) | 8 (6) | 17 (13) | 17 (12) |

*$P = 0.034$.
†$P = 0.033$ vs. split vaccine.

**TABLE 2.** Immunogenicity Endpoints Against A/H3N2, A/H1N1, and B Homologous Influenza Strains (Perprotocol Population)

| | Primary Study | | | | Extension Study | | | |
|---|---|---|---|---|---|---|---|---|
| | Before First Dose | | 3 wk After Second Dose | | Before Booster Dose | | 3 wk After Booster Dose | |
| | Sub/MF59 n = 104 | Split n = 118 | Sub/MF59 n = 104 | Split n = 118 | Sub/MF59 n = 41 | Split n = 40 | Sub/MF59 n = 41 | Split n = 40 |
| A/H3N2 | | | | | | | | |
| GMT (95% CI) | 8.24 (6.25–11) | 8.79 (6.78–11) | 507* (412–623) | 195 (160–237) | 72† (52–100) | 32 (21–49) | 1248* (995–1564) | 391 (294–519) |
| GMR (95% CI) | NA | NA | 61* (50–75) | 22 (18–27) | NA | NA | 17 (12–24) | 12 (8–18) |
| Seroprotection,‡ % (95% CI) | 12 (6–19) | 13 (7–20) | 100 (97–100) | 99 (95–100) | 88* (74–96) | 40 (25–57) | 100 (91–100) | 100 (91–100) |
| A/H1N1 | | | | | | | | |
| GMT (95% CI) | 5.93 (5.01–7.00) | 6.40 (5.47–7.49) | 195* (159–240) | 92 (76–111) | 11 (7.2–18) | 7.3 (4.9–11) | 1027* (749–1409) | 381 (264–549) |
| GMR (95% CI) | NA | NA | 33* (28–38) | 14 (12–17) | NA | NA | 91 (59–140) | 52 (35–79) |
| Seroprotection,‡ % (95% CI) | 5 (2–11) | 7 (3–13) | 100* (97–100) | 86 (79–92) | 15 (6–29) | 5 (1–17) | 100 (91–100) | 100 (91–100) |
| B | | | | | | | | |
| GMT (95% CI) | 5.42 (5.08–5.77) | 5.18 (4.88–5.5) | 105* (88–127) | 20 (17–24) | 9.9* (7.7–13) | 5.1 (4.9–5.3) | 182* (148–223) | 41 (29–59) |
| GMR (95% CI) | NA | NA | 19* (16–23) | 3.95 (3.38–4.62) | NA | NA | 18* (14–24) | 8 (6–12) |
| Seroprotection,‡ % (95% CI) | 3 (1–8) | 1 (0.021–5) | 99* (95–100) | 33 (25–42) | 10 (3–23) | 0 (0–9) | 100* (91–100) | 68 (51–81) |

*$P < 0.001$.
†$P < 0.01$ vs. split vaccine.
‡Seroprotection rate: the percentage of children achieving an HI titer ≥40.
NA indicates not applicable.

seen for seroconversion rates, with statistically significant differences ($P < 0.001$) in favor of Sub/MF59 vaccine achieved against both A strains after first dose, and against A/H1N1 and B strains after second vaccine dose (Fig., Supplemental Digital Content 4, http://links.lww.com/A1130).

All HI antibody titers declined over the follow-up period, but consistently remained significantly higher in the Sub/MF59 group than in the split group. After 6 months, seroprotection rates were 100% and 66% for H3N2, 48% and 20% for H1N1, and 22% and 3% for B strains, for the Sub/MF59 and split vaccines, respectively ($P < 0.001$ for all comparisons). After approximately 1 year, subjects in the Sub/MF59 group maintained HI titers that were higher than those in the split vaccine group for all strains examined (Table 2). The difference was particularly evident for seroprotection rates against A/H3N2 (Sub/MF59 88% vs. split 40%, $P < 0.001$). HI titers against the B strain were low in both vaccine groups (GMT: 9.9 for the Sub/MF59 group and 5.1 for the split vaccine group).

### Year 1: Immune Response to Vaccine Mismatched Strains

For both vaccine groups, prevaccination GMTs and seroprotection rates were higher for the mismatched A/H3N2 virus strain than for mismatched A/H1N1 and B strains. Significantly higher postvaccination GMTs were recorded in the Sub/MF59 group, compared with the split vaccine group for all 3 mismatched strains tested (Table 3). In the Sub/MF59 group, increased postvaccination seroprotection (Fig. 3) and seroconversion (data not shown) rates were attained against both mismatched influenza A strains, but not against the B strain. However, the seroprotection rate against the mismatched B strain was statistically higher in the Sub/MF59 group than the split vaccine group ($P < 0.001$).

### Year 2: Booster Dose

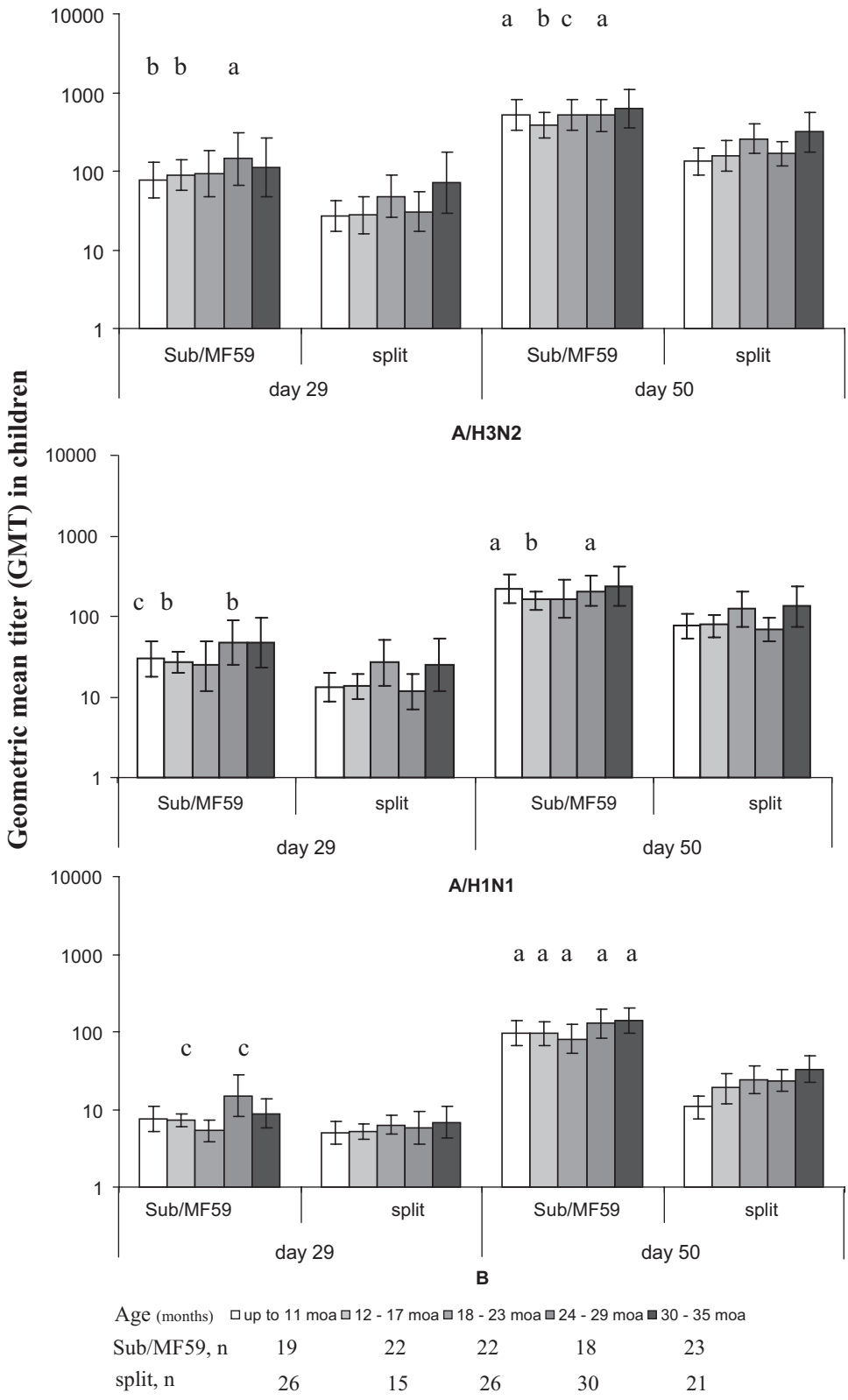
Twenty-one days after the year 2 booster dose, immune responses in the Sub/MF59 group were consistently higher than those observed in the split vaccine group (Table 2). Seroprotection rates of 100% were achieved by both vaccines against both influenza A strains; however, only the Sub/MF59 vaccine resulted in 100% seroprotection against influenza B, compared with 68% seroprotection with the split vaccine ($P < 0.001$). The same trend was observed for seroconversion.

Seroprotection and seroconversion rates in children aged <36 months were generally similar to those in the older age group; lower rates were only seen in this age cohort for the B strain in the split vaccine group (45% vs. 90% for both seroprotection and seroconversion in children aged <36 months and children aged ≥36 months, respectively). In the Sub/MF59 group, GMRs were also similar between the 2 age cohorts (17 and 18 against H3N2, 122 and 63 against H1N1, and 19 and 17 against the B strain in children aged <36 months and children aged ≥36 months, respectively). In contrast, in the split vaccine group, GMRs were lower in the younger age group (8 and 19 against H3N2, 43 and 64 against H1N1, 4 and 16 against the B strain in children aged <36 months and children aged ≥36 months, respectively).

## DISCUSSION

This is the first study of MF59-adjuvanted subunit influenza vaccine in young children. In previous trials, MF59-adjuvanted influenza vaccine has shown a good safety and tolerability profile in other populations.[9,18] In the present study, among 269 children less than 36 months of age, the Sub/MF59 vaccine resulted in slightly higher local reactogenicity, but similar systemic reactogenicity, compared with a conventional influenza vaccine. Both vaccines showed comparable safety profiles following the booster dose administered 1 year after the first dose. Furthermore, the MF59 adjuvant demonstrated that it can significantly increase the immune response in young children against all seasonal influenza subtypes. Sub/MF59 vaccine resulted in higher HI antibody titers compared with the split vaccine across all 3 vaccine virus strains and across all age subgroups in unprimed children aged 6 months to <36 months. The adjuvanted vaccine was also more immunogenic than the split vaccine when given as a booster dose approximately 1 year after priming.

This relatively small-sized study suggest that, considering similar systemic reactogenicity, the substantially increased immunogenicity even in the youngest age groups, and the relating anticipated clinical benefits should outweigh the small increase in generally mild-




| Age (months) | up to 11 moa | 12 - 17 moa | 18 - 23 moa | 24 - 29 moa | 30 - 35 moa |
|---|---|---|---|---|---|
| Sub/MF59, n | 19 | 22 | 22 | 18 | 23 |
| split, n | 26 | 15 | 26 | 30 | 21 |

**FIGURE 1.** GMTs against H3N2, H1N1, and B-strains according to age group in children receiving MF59-adjuvanted vaccine (gray bars) or conventional influenza vaccine (white bars) (per-protocol population) at day 50 (3 weeks after the second dose). Error bars represent the 95% CIs. a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$ versus split vaccine.

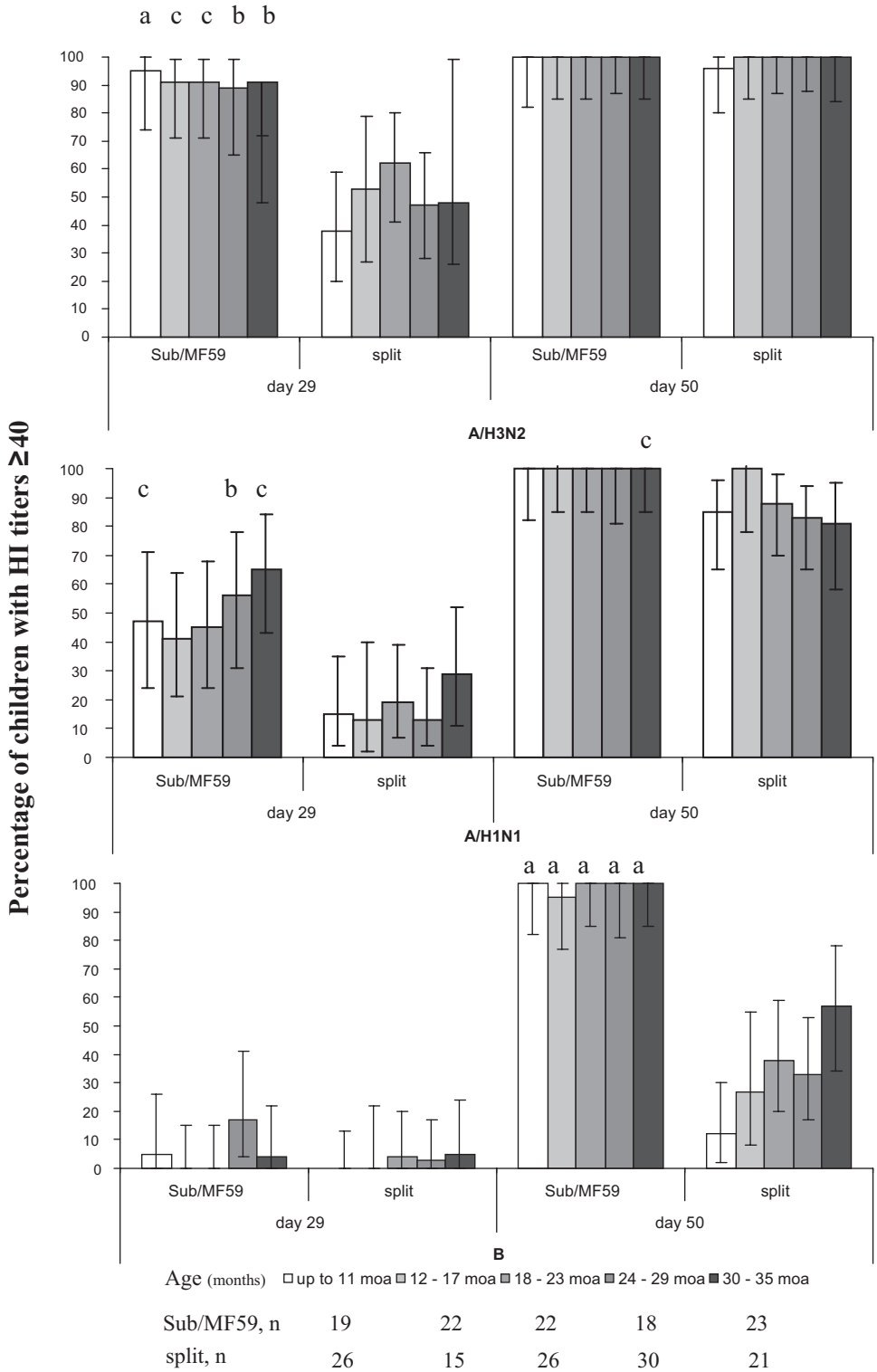



**FIGURE 2.** Seroprotection rates against H3N2, H1N1, and B-strains according to age group in children receiving MF59-adjuvanted vaccine or conventional influenza vaccine (per-protocol population) at day 29 (4 weeks after first dose) and day 50 (3 weeks after the second dose). Seroprotection was defined as the percentage of subjects achieving an HI titer ≥40. Error bars represent the 95% CIs. a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$ versus split vaccine.

**TABLE 3.** Geometric Mean Titers Against A/H3N2, A/H1N1, and B Mismatched Influenza Strains, 21 Days After the Second Dose of Vaccine

| | A/H3N2* | | A/H1N1† | | B‡ | |
|---|---|---|---|---|---|---|
| | Sub/MF59 (n = 104) | Split (n = 118) | Sub/MF59 (n = 104) | Split (n = 118) | Sub/MF59 (n = 104) | Split (n = 118) |
| Prevaccination GMT | 8.08 (6.3–10) | 8.53 (6.69–11) | 5.99 (5.01–7.15) | 6.55 (5.54–7.75) | 5.2 (5–5.42) | 5 (4.82–5.19) |
| Postvaccination GMT | 106§ (80–141) | 41 (31–53) | 55§ (41–72) | 26 (20–34) | 11§ (9.75–12) | 6.07 (5.42–6.8) |
| GMR | 13§ (11–15) | 4.78 (4.1–5.58) | 9.11§ (7.57–11) | 4 (3.36–4.76) | 2.12§ (1.88–2.38) | 1.21 (1.09–1.35) |

*A/New York/55/2004 (H3N2)-like.
†A/Solomon Islands/3/2006 (H1N1)-like.
‡B/Jiangsu/10/2003-like.
§$P < 0.001$ Sub/MF59 vs. split vaccine.

and transient-solicited local reactions with the Sub/MF59 vaccine in this vulnerable population. However, more data on reactogenicity and safety of MF59-adjuvanted influenza vaccine in young children need to be collected.

The United States and some European Union countries (specifically Finland since 2007) have recommended seasonal influenza vaccination for children, especially the very young, based on evidence of the health impact in this age group. Morbidity and hospitalization rates for children less than 5 years of age are similar to those observed in the elderly.[19,20] Criteria are established for the evaluation of influenza vaccines for healthy adults and the elderly,[21] but there are currently no such criteria for children and infants, as data on vaccine efficacy in young children remain limited. For example, 1 study has shown over 80% efficacy against a homologous H3N2 strain.[22] In contrast, a recent Cochrane analysis implies that influenza vaccines have limited field efficacy in young children.[6] Recently, intranasally administered live attenuated trivalent influenza vaccine (LAIV) showed 72.9% to 88.7% efficacy in children compared with placebo.[23,24] In a large field trial, LAIV showed 79.2% and 89.2% greater efficacy than trivalent inactivated vaccine against H3N2 and H1N1, respectively.[25] Since LAIV is not licensed for children below 2 years, and safety issues limit the use of LAIV to children over 2 years of age with no history of wheezing, an effective alternative is still needed for children younger than this age.[25]

In practice, despite the recommendations, young children often receive only 1 injection of influenza vaccine in each season.[26] This study indicates that a single dose of a nonadjuvanted vaccine is not sufficient to induce protective immunity to H3N2, H1N1, or B strains in unprimed children less than 36 months of age. For H3N2, MF59-adjuvanted vaccine yielded high seroprotection rates (91%) even after 1 dose. Therefore, against this most common causative strain of influenza, even a single dose of MF59-adjuvanted influenza vaccine, but not of the nonadjuvanted vaccine, might be expected to provide a good level of protection. Altogether, the protective efficacy of an influenza vaccine would depend on the relative proportions of H3N2, H1N1, and B strains in a given season. With higher proportions of H1N1 and B strains in the season, the advantage of MF59 adjuvanted vaccine over nonadjuvanted influenza vaccines (after 1 and particularly after 2 doses) is likely to be greatest.

Altogether, the protective efficacy of an influenza vaccine in young children with vaccine-induced immunity only may be expected to correlate with the prevalence of seroprotective level of HI antibody titers at the time of the influenza epidemic season. The present study showed that, after a postdose 2, HI antibody levels declined for both vaccines and all influenza strains. However, it is noteworthy to mention that Sub/MF59 vaccine recipients had a particularly durable response against the H3N2 vaccine strain, with 88% (vs. 40% in the split vaccine group) of the subjects retaining seroprotective levels of HI antibodies for approximately 1 year.

Active follow-up of influenza was not conducted in the subjects participating to this study. It is possible that some of the vaccinated children may have been exposed to wild-type influenza during the epidemic season and such exposures may have boosted influenza antibody levels, resulting in an apparent increase in persistence of seroprotective levels of HI antibodies. Should that have happened, the effect might, however, have been greater in the recipients of split vaccine. In fact, these children had lower antibody levels after vaccination and were presumably more susceptible to wild-type influenza. Therefore, exposure to wild-type influenza might only have narrowed the gap in seroprotective levels of HI antibodies between the 2 groups, which, however, remained wide in favor of the Sub/MF59 group.

Previous studies have shown that the immunogenicity of influenza B virus is lower than the immunogenicity of the influenza A strains contained in vaccines.[27,28] In field conditions, the influenza B strain accounts for a substantial proportion of cases regardless of season.[29] In the present study, MF59 improved immunogenicity against the B vaccine virus, whereas the nonadjuvanted vaccine induced only low immune responses. This was particularly evident in the younger age groups (<24 months) where seroprotection in the Sub/MF59 group was 98% compared with 28% in the split vaccine group ($P < 0.001$). Even after the second-year booster dose, seroprotection against the B strain in younger children remained less than 50% in the split vaccine group, compared with 100% in the Sub/MF59 group.

Antigenic drift of influenza viruses requires frequent adaptation of vaccine strains to accommodate this drift. Predicting additional drift in advance of the influenza season remains one of the major challenges to successful vaccination, and the negative impact of vaccine mismatch on vaccine effectiveness and spread in the community is well established.[7,30–32] In a multiyear study evaluating vaccine effectiveness versus the antigenic distance of strain mismatches in the vaccine, a strong correlation was obtained between antigenic distance and vaccine effectiveness.[33] The ability of an influenza vaccine to evoke an immune response against drifted viruses that are different from those included in the formulation would be of major clinical value, especially in very young children who are often naive to influenza virus exposure. In our study, Sub/MF59 induced significantly higher immune responses against mismatched A/H3N2 and A/H1N1 strains than the licensed split vaccine. Higher cross-reactivity was found against the A/H3N2 antigen, confirming previous results in other populations.[12–14] In contrast, no cross-reactivity against the mismatched B strain was detected in either vaccine group, as the mismatched B strain selected for the analysis was of a different lineage (B/Yamagata) than the vaccine strain (B/Victoria). Since the early 1980s, 2 distinct lineages

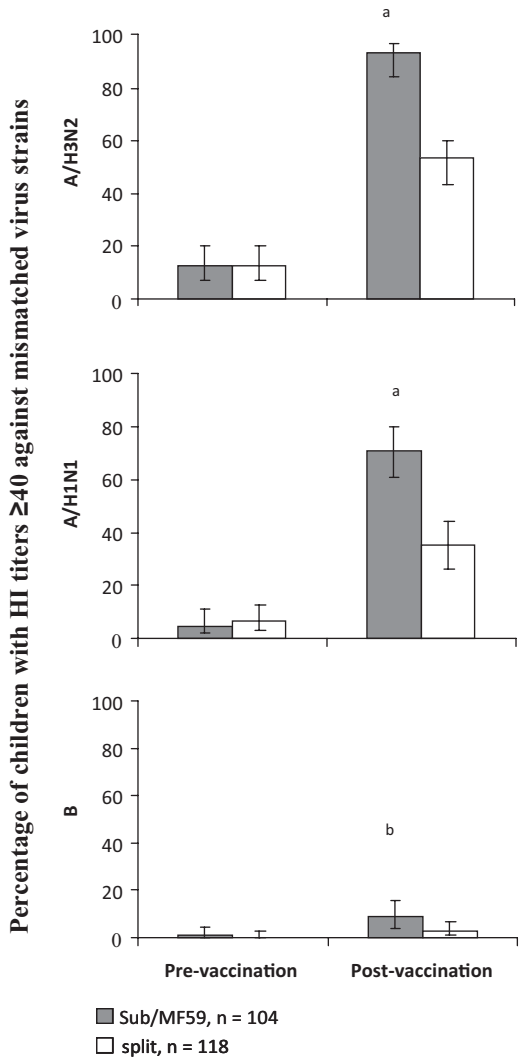



**FIGURE 3.** Seroprotection rates against mismatched H3N2 (A/New York/55/2004-like), H1N1 (A/Soloman Islands/23/2006-like), and B (B/Jiangsu/10/2003-like) strains in 6 to <36 month-old children 3 weeks after receiving the second dose of MF59-adjuvanted vaccine (gray bars, n = 104) or conventional influenza vaccine (white bars, n = 118) (per-protocol population). Seroprotection was defined as the percentage of subjects achieving an HI titer of ≥40. Error bars represent the 95% CIs. a, $P < 0.001$; b, $P < 0.05$ versus split vaccine.

of B influenza strains have cocirculated in humans, with frequent differences in the predominant lineage circulating at a given time in a given geographic region.[34] Since there is no cross-reactivity between the 2 B lineages, public health experts have considered the need to include both B lineages into vaccines to ensure adequate antigenic coverage.[35]

Taken together, the results of this study indicate that MF59-adjuvanted vaccine has acceptable tolerability and greater immunogenicity in young children compared with the conventional split vaccine. Based on these results, expanded studies of MF59-adjuvanted influenza vaccine in young infants, including efficacy trials, are warranted.

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