An Adjuvanted, Low-Dose, Pandemic Influenza A (H5N1) Vaccine Candidate Is Safe, Immunogenic, and Induces Cross-Reactive Immune Responses in Healthy Adults

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Background. To protect a naive global population against pandemic influenza, pandemic vaccines should be effective at low antigen doses, because of limited manufacturing capacity.

Methods. In a multicenter, randomized, blind-reader phase 1 trial, groups of 50 healthy young adults received 2 doses, 21 days apart, of influenza A/Vietnam/1194/2004 NIBRG-14 (H5N1) vaccine containing 1.9, 3.8, 7.5 or 15 μg of hemagglutinin with oil-in-water emulsion adjuvant or 7.5 μg of hemagglutinin without adjuvant. Safety was monitored to day 42. Homologous hemagglutination-inhibition (HI) and microneutralization titers were determined after each vaccination. Cross-reactivity against A/Indonesia/05/2005 RG2 was tested after the second vaccination.

Results. No vaccine-related significant or serious adverse events occurred. Injection site reactions, but not systemic reactions, were more frequent with adjuvant than without. Even with only 1.9 μg of hemagglutinin plus adjuvant, 72% of subjects had HI titers ≥1:32 after 2 doses. This proportion was 81%–89% with higher adjuvanted doses but was only 34% without adjuvant. Adjuvanted vaccine induced cross-neutralizing antibodies in 39%–65% of samples, versus 7% without adjuvant.

Conclusions. The emulsion-adjuvanted pandemic influenza vaccine candidate was safe, immunogenic, and induced cross-reactive antibodies. This adjuvanted 1.9-μg candidate is the lowest effective dose tested to date. This could have a major impact on prepandemic vaccination strategies with stockpiled batches of vaccine.

Trial registration. ClinicalTrials.gov identifier: NCT00457509.

Highly pathogenic avian influenza A (H5N1) virus continues to spread, with infected birds identified in many countries in Asia, Europe, the Middle East and in several countries in Africa [1]. As of February 2008, 359 human cases of H5N1 influenza have been confirmed, including 226 deaths (case fatality rate, 63%) [2]. Each new human infection with an avian strain provides the virus with the opportunity to acquire the properties necessary to become a human pandemic strain, such as the ability to transmit easily between humans. This stresses the importance of developing a pandemic influenza vaccine.

For interpandemic influenza, one annual vaccination is sufficient to protect a population that already has some level of immunity through prior infection or vaccination with the same or similar strains. Against influenza A (H5N1) virus, however, humans have no or extremely little preexisting natural immunity. Accordingly, at least 2 doses of an H5N1 vaccine are needed to prime and induce protective immune responses in hu-
mans [3–5]. Furthermore, the quantity of antigen required is substantially higher than the 15 μg of hemagglutinin used per strain in seasonal influenza vaccine. The poor immune response to pandemic vaccine candidates may also be explained by an inherent lack of immunogenicity in humans of the avian H5N1 strains tested.

The global response to an influenza pandemic will require a maximum number of vaccine doses in the shortest possible time after the start of the pandemic. Faced with limited manufacturing capacity, the development of low-dose pandemic influenza vaccines using adjuvants to improve immunogenicity is of utmost importance. Aluminum hydroxide and oil-in-water emulsions have significantly improved the immunogenicity of pandemic vaccine candidates, allowing the quantity of antigen to be reduced [3–7]. Evidence of intrasubtype cross-reactivity of vaccine-induced antibodies have led to the elaboration of pandemic priming strategies, such as the use of vaccine from prepandemic stockpiles at the very start of a pandemic to prime the population [5, 8–12]. Although such a vaccine would not perfectly match the pandemic strain, it would be expected to confer a sufficient baseline level of immunity to facilitate and accelerate subsequent immunization against the pandemic virus itself.

In the present study, we evaluated doses as low as 1.9 μg of a candidate inactivated split-virion clade 1 pandemic influenza vaccine adjuvanted with an oil-in-water adjuvant emulsion for safety, homologous immunogenicity, and cross-reactive immunogenicity.

**METHODS**

In this multicenter, randomized, blind-observer phase 1 trial, the safety and immunogenicity of 4 influenza A/Vietnam/1194/2004 IBRG-14 (H5N1) vaccine candidates containing 1.9, 3.8, 7.5 or 15 μg of hemagglutinin and an oil-in-water adjuvant emulsion were investigated in comparison with 7.5 μg of antigen without adjuvant. The amount of adjuvant in each injected vaccine was identical.

**Participants.** Healthy 18–40 year-old volunteers were recruited at 3 centers in Belgium. The main exclusion criteria were ongoing febrile illness; immunodeficiency or hepatitis B or C seropositivity; recent receipt (previous 3 months) of blood, blood-derived products, or immunosuppressive treatment; ongoing long-term systemic corticosteroid therapy; any vaccination during the previous 4 weeks or planned in the following month; influenza vaccination during the previous 6 months; or pregnancy. The trial protocol and all relevant documents were approved by the ethics committees of each trial center and the national health authorities. Volunteers gave written informed consent before inclusion. The study was conducted in accordance with all relevant regulations and good clinical practice guidelines.

**Vaccine.** Investigational vaccine candidates were monovalent, inactivated, split-virion vaccine produced by Sanofi Pasteur using the influenza A/Vietnam/1194/2004 NIBRG14 (H5N1) reassortant reference strain (UK National Institute for Biological Standards and Control). This is one of the reference viruses considered suitable for prototype pandemic influenza vaccines [13]. This virus was propagated in embryonated hens’ eggs, using the licensed manufacturing process for the interpandemic vaccine Vaxigrip, as described elsewhere [14], and adapted to the particular avian strain according to biosafety guidelines for the production and quality control of human influenza pandemic vaccines [15].

Before being mixed with the vaccine, the adjuvant, produced by Sanofi Pasteur, was a 5% squalene-in-water emulsion stabilized by 2 nonionic surfactants. This emulsion was prepared to have a very fine (mean particle diameter <100 nm) and monodispersed emulsion with a narrow particle size distribution.

Vaccine doses were prepared just before injection by mixing vaccine from multidose vials and adjuvant from monodose vials according to a reconstitution protocol. This protocol was devised by the sponsor to ensure that the amount of antigen in the final vaccine doses matched the targeted amount and that each adjuvanted vaccine dose contained an identical amount of adjuvant. The final volume per dose was 0.3 mL (nonadjuvanted vaccine), 0.4 mL (adjuvanted 1.9-μg vaccine), or 0.6 mL (the other 3 formulations). The final antigen dose of the injected vaccines was calculated on the basis of the known antigen dose of the multidose vials.

**Procedures.** In a preliminary step, 15 subjects were vaccinated twice, 21 days apart, with the adjuvanted 15-μg vaccine (i.e., the highest dosage vaccine to be injected into subsequent subjects) and were closely monitored for clinical and biological safety at study visits on days 0, 2, 8, 21, 23, and 29. Blood samples obtained at each visit were used to identify abnormalities in liver function parameters, electrolytes and proteins, lipid metabolism parameters, and hematology parameters. Safety data from this preliminary step were reviewed by the sponsor’s safety review board and the ethics committees of all 3 centers to ensure the absence of safety issues before continuing the trial in agreement with the investigators.

A total of 251 subjects were then enrolled and randomly assigned to 1 of 5 groups and vaccinated twice, 21 days apart, with the assigned vaccine. The randomization list, stratified by center, was created by the sponsor’s biostatistics department using the block method (PROC PLAN SAS, version 8.2), to ensure enrollment of a similar number of subjects into each group at any given time. Because the appearance of adjuvanted and nonadjuvanted vaccines differed, to ensure blinding vaccines were prepared and administered (via intramuscular injection into the deltoid) by specific trial personnel who were not involved in the safety assessment.
Subjects were kept under observation for 30 min after each vaccination and were given safety diaries, thermometers, and rulers to record any adverse events. For the first 7 days after each vaccination, diaries included a list of solicited injection site and systemic events. The intensity of nonmeasurable reactions was assessed using a severity scale of mild, moderate, or severe. At the following visit, investigators interviewed the subjects, transcribed events into case report forms, and assessed whether they were vaccine related. By convention, all solicited events were considered vaccine related.

Serum samples for antibody analyses were obtained before and 21 days after each vaccination. These were processed at each trial center within 24 h and shipped frozen to the sponsor’s Global Clinical Immunology Laboratory in Swiftwater, Pennsylvania, for centralized analysis.

**Immunogenicity assays.** Samples were tested for hemagglutination-inhibition (HI) and neutralizing activity against the clade 1 reassortant vaccine seed virus: A/Vietnam/1194/2004 was used in HI assays, and A/Vietnam/1203/2004 was used in neutralization assays. To test for cross-reactivity on day 42, both assays were also performed with the clade 2 influenza A/Indonesia/05/2005 (H5N1) RG2 strain. Assays were performed under blinded conditions.

A previously described HI assay adapted to avian strains was used [16, 17]. Briefly, after eliminating nonspecific inhibitors and anti-species agglutinins, samples were centrifuged and supernatants were submitted to the HI method. From an initial serum dilution of 1:8, ten 2-fold dilutions of serum were prepared and combined with an equal volume of antigen suspension at 4 hemagglutinin units (HAU)/25 μL for 1 h at room temperature. The 4 HAU of antigen used in the assay was determined for each lot of erythrocytes by titrating the antigen (2-fold dilutions) and mixing with equine erythrocytes [17]. After 1 h, hemagglutination was assessed for each dilution, and virus concentration containing 4 HAU was determined. After incubating serum and antibody together for 1 h, 50 μL of 1.0% equine erythrocyte suspension was added, and the reaction was left for another hour before reading. Titers are expressed as the reciprocal of the highest dilution at which hemagglutination was completely inhibited. The titer range of the assay is 1:8–1:8192. Titrations were performed in 2 independent assay runs, and the final titer was the geometric mean. As a serostatus threshold, we considered HI titers ≥1:32 to be similar to titers ≥1:40, which is the seroprotection threshold considered in European and US regulatory guidance; only the initial dilution of the serum in the assay differ: 1:8 vs. 1:10 [18, 19].

Neutralizing antibody activity was analyzed using a micro-neutralization assay based on the methods of the pandemic influenza reference laboratories of the Centers for Disease Control and Prevention and the Health Protection Agency [16, 20]. Heat-inactivated human serum samples were preincubated with a standardized amount of influenza virus before the addition of Madin-Darby canine kidney cells. After overnight incubation, viral nucleoprotein was detected by ELISA in virally infected cells. Serum antibodies to the influenza virus hemagglutinin inhibit the viral infection of cells; therefore, the optical density results of the ELISA are inversely proportional to the serum antibody concentration. Serum was 2-fold serially diluted from a starting dilution of 1:10, and reciprocal dilutions of serum achieving 50% or greater neutralization of virus growth were considered positive. Each sample was tested in duplicate, and the final titer was the mean of the duplicate titers.

**Statistical analysis.** The sample size was chosen in line with European guidelines for annual influenza vaccine trials [18]. No formal statistical hypothesis testing was planned. Analyses were descriptive and were performed by the sponsor’s biostatistics department. Results were summarized using point estimates and 2-sided 95% confidence intervals (95% CI). Reactogenicity was assessed in accordance with European regulatory guidance for interpandemic vaccines (see Results) [18]. In line with European and US regulatory guidance, HI end points were the geometric mean titer (GMT); the proportion of subjects with HI titers ≥32, referred to as the seroprotection rate; the postvaccination-to-prevaccination GMT ratio (GMTR); and the seroconversion rate (the percentage of subjects with a prevaccination titer of <8 and a postvaccination titer of ≥32 or achieving a 4-fold titer increase) [18, 19]. Microneutralization end points were the GMT and the proportion of subjects with 4-fold titer increases. Samples with responses below the limit of detection were assigned a titer of half the detection limit, and titers were transformed into log_{10} titers.

**RESULTS**

In January 2007, 9 men and 6 women aged 18–36 years (median, 23 years) received two 15-μg doses with adjuvant and successfully completed the study to day 42. Interim safety analysis after the completion of this step revealed no safety issues preventing the continuation of the trial. There were no serious adverse events (SAEs) in these subjects, and although out-of-range values were observed for some laboratory parameters, there were no significant or clinically relevant abnormalities or trends in any biological or hematological parameter (data not shown).

**Participants.** In April 2007, 251 subjects were enrolled and randomized (figure 1). Groups were well balanced for demographic characteristics. In each group, the male-to-female ratio was 0.6 or 0.7, the median age was 21 years, and at least 92% were white.

**Safety.** Within 3 days of vaccination, reactions defined in the European regulatory guideline for interpandemic vaccines occurred at comparable rates in all 5 groups. Rates were not higher with adjuvant than without and did not increase with increasing dose of antigen. After the first dose, 20%–36% of subjects in each adjuvanted vaccine group reported at least 1 of these reactions, versus 32% in the group without adjuvant. After the second dose, these values decreased: 15%–22% with adjuvant, 28% without.
The extended set of solicited reactions within 7 days of vaccination was more sensitive in detecting differences in reactogenicity between groups (figure 2). Adjuvanted vaccine caused more solicited injection site reactions than did nonadjuvanted vaccine but not more solicited systemic reactions. Some degree of injection site pain was reported by ≥94% of subjects in each

Figure 1. Trial profile. Adj, adjuvant; AE, adverse event; D0, D21, and D42, days 0, 21, and 42, respectively; SAE, serious adverse event.

Figure 2. Profile of solicited reactions. Shown are the proportion of subjects reporting the occurrence of solicited injection site and systemic reactions on at least 1 day during the 7 days after the first (top) and second (bottom) vaccination. Adj, adjuvant.
Table 1. Antibody responses assessed by hemagglutination-inhibition (HI) and microneutralization (MN) assays after 1 and 2 injections of adjuvanted or nonadjuvanted pandemic A/Vietnam/1194/2004 NIBRG-14 (H5N1) vaccine.

<table>
<thead>
<tr>
<th>Day, parameter</th>
<th>CHMP standard&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.9 µg + adjuvant</td>
<td>3.8 µg + adjuvant</td>
</tr>
<tr>
<td>21 days after first vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMTR&lt;sup&gt;b&lt;/sup&gt; for day 21/day 0</td>
<td>2.54 (1.87–3.26)</td>
<td>2.91 (2.10–4.04)</td>
</tr>
<tr>
<td>MN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMTR&lt;sup&gt;b&lt;/sup&gt; for day 21/day 0</td>
<td>10.2 (7.23–14.48)</td>
<td>12.5 (8.68–17.94)</td>
</tr>
<tr>
<td>21 days after second vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMTR&lt;sup&gt;b&lt;/sup&gt; for day 42/day 0</td>
<td>11.0 (8.54–14.17)</td>
<td>15.5 (11.3–21.4)</td>
</tr>
<tr>
<td>MN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMTR&lt;sup&gt;b&lt;/sup&gt; for day 42/day 0</td>
<td>67.0 (49.4–90.9)</td>
<td>91.3 (66.1–125.9)</td>
</tr>
</tbody>
</table>

**NOTE.** Data in parentheses are 95% confidence intervals. GMT, geometric mean titer.

<sup>a</sup> European Committee for Medicinal Products for Human Use (CHMP) standards for the HI immune response elicited in 18–60-year-olds by interpandemic influenza vaccines. These standards refer to the HI response determined using the standard HI assay on turkey erythrocytes with a detection limit of 1:10 and a seroprotection threshold of 1:40, whereas the results presented summarize the HI response using a modified HI assay on equine erythrocytes with a detection limit of 1:8 and a seroprotection threshold of 1:32.

<sup>b</sup> Proportion of subjects with a titer of ≥32.

<sup>c</sup> Proportion of subjects with a prevaccination titer of < 8 and a postvaccination titer of ≥32 or displaying a 4-fold titer increase.

<sup>d</sup> The GMT ratio (GMTR) is the mean geometric increase between pre- and postvaccination titers.

Adjuvanted vaccine group after the first vaccination, versus 48% without adjuvant. Between 20% and 48% of each adjuvanted vaccine group, but none of the subjects receiving the unadjuvanted vaccine, reported moderate pain after the first injection, defined as pain that interfered with normal behavior or activities. Only 1 subject after each vaccination reported pain as being severe (unable to perform usual activities). Injection site erythema, swelling, and induration, but not ecchymosis, were also more frequent with adjuvant than without. Few subjects reported fever, and none had severe fever. There was no consistent trend for higher reaction rates with higher antigen dose. Reaction rates were lower after the second vaccination than after the first (figure 2). There were no apparent intergroup differences in terms of the duration of either solicited injection site or systemic reactions. Overall (combining all groups and both vaccinations) 86.4% of solicited reactions lasted 1–3 days, 13.0% lasted 4–7 days, and the remaining 0.6% (7 cases of injection site erythema, ecchymosis, or induration) lasted longer. No vaccine-related SAEs occurred, although 1 vasovagal syncope occurring during vaccination in a subject with a history of similar episodes was reported as a SAE related to the trial procedures.

**Immunogenicity.** With the exception of 1 low-positive value measured by each method, antibodies were undetectable before vaccination (data not shown). One vaccination, with or without adjuvant, induced significant HI and neutralizing responses (seroconversion or 4-fold rise) in 22%–40% of subjects. A second vaccination with adjuvant substantially increased the response in all 4 groups, whereas without adjuvant it did not (table 1). With adjuvant, 3.8, 7.5 or 15 µg of hemagglutinin was more immunogenic than 1.9 µg (figure 3), yet even this lowest dose was highly immunogenic, as HI responses satisfied all 3 European immunogenicity criteria (table 1). In contrast, the nonadjuvanted
vaccine met only 1 of the 3 criteria (GMTR). Considering US immunogenicity criteria, the 95% CI of the seroconversion rate was >40% in all adjuvanted vaccine groups, and the 95% CI of the seroprotection rate was >70% with the adjuvanted 7.5- and 15-μg vaccine candidates. Immune responses to adjuvanted vaccine followed a dose-dependent trend from 1.9 to 7.5 μg (the day 42 GMTs in these groups were 44.0, 62.2, and 82.8), but increasing the dose to 15 μg did not appear to further increase immunogenicity (GMT, 84.4) (figure 4).

**Cross-reactive immunogenicity.** Among subjects with detectable antibodies against the vaccine strain on day 42, antibodies were cross-reactive against A/Indonesia/05/2005 in up to 65% of subjects in the adjuvanted vaccine groups but were cross-reactive in very few subjects vaccinated without adjuvant (table 2 and figure 3). The proportion of subjects with cross-reactive responses appeared to increase with increasing dose of vaccine antigen, although 95% CIs overlapped in most cases.

**DISCUSSION**

Effective antigen-sparing strategies for pandemic influenza vaccines are crucial given the limited production capacity and the global demand for vaccine in the event of a pandemic, and calls have been made to determine the most antigen-sparing formulation [21]. Inactivated candidate vaccines that are adjuvanted with oil-in-water emulsion and that have an antigen content of only 7.5 μg or even 3.8 μg have been tested, with the results suggesting that further dose sparing could be achieved [5, 8]. In this first clinical study with a new proprietary oil-in-water emulsion adjuvant added to an inactivated split-virion influenza A (H5N1) vaccine, we further explored dose sparing, starting with a formulation containing only half the antigen content of the lowest dose previously reported [5]. We found that, although higher doses were more immunogenic, even this lowest-dose vaccine (1.9 μg) elicited HI and neutralizing responses after 2 injections that surpassed those of the nonadjuvanted 7.5-μg vaccine and satisfied European immunogenicity criteria [20]. The observed pattern of antigen dose–dependant immune response suggests that this lowest dose approaches the limits of dose sparing that can be achieved with such adjuvanted inactivated pandemic influenza vaccine candidates.

In the study by Leroux-Roels et al. [5], immune responses elicited by adjuvanted vaccine containing 3.8, 7.5, or 15 μg of hemagglutinin were remarkably comparable to those elicited by the same antigen doses studied here: HI seroprotection rates ranged from 84% to 96%, compared with 81%–89% in our trial, and the rate of 4-fold increase in neutralizing titer was 86% for each dose, compared with 88%–96% in our trial. However, a
recent interlaboratory collaborative study of HI and neutralizing assays revealed high levels of variation, highlighting the fact that results from different studies may not be directly comparable and must therefore be interpreted with caution [22]. Although the seroprotection rate is conventionally used to express the immune response to seasonal influenza, it is not yet clear how this translates to protection against H5N1 influenza.

Our data also confirm another important property of these adjuvanted vaccines that may prove invaluable for the implementation of pandemic priming strategies: their ability to induce antibodies against heterologous strains, in this case against a reassortant vaccine candidate strain based on the clade 2 strain A/Indonesia/05/2005. This priming property will be further evaluated in a follow-up study in which the study subjects reported here will return for a booster vaccination.

The exact mechanisms behind the adjuvantation effect of oil-in-water emulsions are unknown, although it is generally accepted that the vehicle properties of the emulsion play an important role and that emulsions recruit antigen-presenting cells to the site of vaccine injection in the muscle. The recruited cells,

**Table 2.** Cross-reactive hemagglutination-inhibition (HI) and neutralization antibody responses against A/Indonesia/05/2005 RG2 among vaccinated subjects with detectable titers against the vaccine strain on day 42

<table>
<thead>
<tr>
<th>Response, parameter</th>
<th>1.9 μg + adjuvant</th>
<th>3.8 μg + adjuvant</th>
<th>7.5 μg + adjuvant</th>
<th>15 μg + adjuvant</th>
<th>7.5 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cross-reactive HI response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects, no.*</td>
<td>48</td>
<td>44</td>
<td>46</td>
<td>48</td>
<td>27</td>
</tr>
<tr>
<td>GMT</td>
<td>6.21 (5.18–7.45)</td>
<td>8.06 (6.47–10.06)</td>
<td>9.09 (6.99–11.83)</td>
<td>10.3 (8.02–13.23)</td>
<td>4.21 (3.91–4.53)</td>
</tr>
<tr>
<td>Subjects with titers ≥8, %</td>
<td>35 (22.2–50.5)</td>
<td>52 (36.7–67.5)</td>
<td>54 (39.0–69.1)</td>
<td>58 (43.2–72.4)</td>
<td>7 (0.9–24.3)</td>
</tr>
<tr>
<td>Subjects with titers ≥32, %</td>
<td>4 (0.5–14.3)</td>
<td>9 (2.5–21.7)</td>
<td>17 (7.8–31.4)</td>
<td>23 (12.0–37.3)</td>
<td>0 (0.0–12.8)</td>
</tr>
<tr>
<td><strong>Cross-reactive neutralizing response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects, no.*</td>
<td>49</td>
<td>46</td>
<td>46</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td>GMT</td>
<td>8.63 (6.91–10.77)</td>
<td>10.6 (8.29–13.53)</td>
<td>11.9 (8.77–16.05)</td>
<td>14.7 (11.3–19.1)</td>
<td>5.87 (4.81–7.15)</td>
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<tr>
<td>Subjects with titers ≥10, %</td>
<td>39 (25.2–53.8)</td>
<td>50 (34.9–65.1)</td>
<td>50 (34.9–65.1)</td>
<td>65 (50.4–78.3)</td>
<td>11 (2.4–29.2)</td>
</tr>
<tr>
<td>Subjects with titers ≥20, %</td>
<td>18 (8.8–32.0)</td>
<td>28 (16.0–43.5)</td>
<td>28 (16.0–43.5)</td>
<td>43 (28.8–57.8)</td>
<td>4 (0.1–19.0)</td>
</tr>
</tbody>
</table>

**NOTE.** Data in parentheses are 95% confidence intervals. GMT, geometric mean titer.

* No. of subjects per group with detectable levels of HI or neutralizing antibodies against clade 1 H5N1 strain.
which take up emulsion and antigen, mature and migrate to the draining lymph nodes for efficient presentation of processed antigen to T lymphocytes [23]. A small droplet size in the emulsion is important for efficient uptake by antigen-presenting cells and for drainage from the injection site through the lymphatic network [24]. The safety and reactogenicity profile of the vaccine candidates proved satisfactory, with no SAEs related to vaccine, no immediate allergic reactions, and no significant abnormalities or trends in biological or hematological parameters. Systemic reactions rates were comparably low in all groups. Although adjuvanted vaccine caused more injection site reactions—particularly injection site pain—the reactions were mainly mild to moderate and in line with previous observations [5, 6, 25, 26]. These results constitute a first step and will need confirmation in further studies in appropriate populations.

In summary, we have shown that this oil-in-water emulsion adjuvant has significant dose-sparing and cross-immunity effects when added to a candidate pandemic influenza vaccine. Even at doses as low as 1.9 µg, the adjuvanted vaccine was able to elicit high levels of antibodies in this H5N1-naive adult population. In the event of an influenza pandemic, this could have a major impact on the number of people who can be protected with each new or stockpiled batch of vaccine.

Acknowledgments

We acknowledge the contributions of Katrien Hoftkens and Fé Droogmans (study nurses at the Centre for Youth Health Care); the participating clinicians, study nurses, and laboratory technicians at the Center for Vaccinology and the Centre de Santé; and, at Sanofi Pasteur, Sanjay Garg and Katherine Fries for work [24]. The safety and reactogenicity profile of the vaccine is important for efficient uptake by antigen-presenting cells and which take up emulsion and antigen, mature and migrate to the lymphatic network [24].

References