Antibody Persistence after 2-Dose Priming and Booster Response to a Third Dose of an Inactivated, Adjuvanted, Whole-Virion H5N1 Vaccine

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An inactivated, alum-adjuvanted, whole-virion H5N1 vaccine had been evaluated previously. Hemagglutination inhibition (HI) assays showed that the antibody levels declined significantly, with 4.8%–20.8% and 0%–18.8% of participants retaining seroprotection (HI titer ≥1:40) 6 and 12 months after the second dose, respectively. A third dose of the same vaccine given 12 months after the second dose significantly boosted immune responses. Thirty days after the third dose in the 1.25-, 2.5-, 5-, and 10-μg dose groups, 29.4%, 31.3%, 78.6%, and 90.0% of participants had HI titers ≥1:40, respectively. Both the 5-μg and 10-μg doses met European Union criteria. (ClinicalTrials.gov identifier: NCT00660257.)

Human infections with the highly pathogenic avian influenza virus H5N1 were first reported in Hong Kong in 1997 [1]. The virus has become widespread among migratory birds and poultry since 2003, causing a mass of deaths in infected animals [2].

The ability of H5N1 virus to infect human beings via direct contact with infected animals and its rapid dissemination via wild migratory birds confer a high pandemic potential to the virus. As of April 2008, there had been 381 cases of H5N1 infection in humans, 240 of which were fatal, according to the World Health Organization [3]. The virus is regarded as the most probable source for the next human influenza pandemic.

The development of effective H5N1 vaccines is considered a critical priority for preparedness for a potential influenza pandemic. H5N1 vaccines (including split- and whole-virion vaccines and adjuvanted and nonadjuvanted vaccines) have been developed and assessed in several clinical trials [4–8], which showed that an adjuvanted 2-dose vaccine would be needed to elicit a satisfactory immune response in a naïve population. One trial also showed that a split-virion H5N1 vaccine could induce a booster response to a third dose [9]. However, there was no report on antibody persistence after H5N1 vaccine priming. In a previous phase 1 trial, an inactivated, aluminum-adjuvanted, whole-virion H5N1 vaccine manufactured by Sinovac Biotech demonstrated good safety and immunogenicity in adults [7]. Here, we report antibody persistence 1 year after 2-dose priming and the booster response to a third dose.

Methods. The previous phase 1 trial was a randomized, placebo-controlled, double-blind study that included 120 participants aged 18–60 years. They received either placebo or H5N1 vaccine, given intramuscularly at a dose of 1.25, 2.5, 5, or 10 μg in 2 doses separated by 28 days [7]. A total of 118 individuals completed the trial and received 2 planned doses [7]. In the present study, serum samples from 88 participants who had received 2 doses of this vaccine were collected 6 months after the 2-dose priming. Twelve months after the priming, 57 of these 88 vaccine recipients received a third dose of the same vaccine dose they had received, with 24 placebo recipients excluded. Serum samples were collected just before and 15 and 30 days after the third dose. Eligible participants were healthy adults who had received 2-dose priming vaccines 28 days apart and reported no serious adverse events. The main exclusion criteria were the same as in the previous phase 1 trial [7].

The vaccine was an inactivated, whole-virion H5N1 vaccine for which recombinant strain NIBRG-14 (A/Vietnam/1194/2004-A/PR/8/34) was used as seed virus. The NIBRG-14 strain was prepared by the UK National Institute for Biological Standards and Control by reverse genetics and is recommended as a prototype pandemic influenza vaccine strain by the European Union (EU) Committee for Medicinal Products for Human Use (CHMP) [10]. The vaccine was produced in embryonated hens'...
eggs and formulated with aluminum hydroxide, as described elsewhere [7]. There was no preservative in the vaccine, and a 0.5-mL dose contained 1.25, 2.5, 5, or 10 μg of H5N1 antigen (lots 20050604, 20050603, 20050602, and 20050601, respectively).

The vaccine was administered intramuscularly by a nurse who did not participate in the safety observation and assessment of immunogenicity. Participants remained blinded to the vaccine dose and were kept 30 min after vaccination for observation of adverse events. Adverse events were recorded on diary cards by participants and reviewed by investigators. All adverse events were graded on a standard scale [11].

Serum samples were tested by hemagglutination inhibition (HI) and microneutralization (MN) assays. All samples were coded before titration and were assayed blindly against the homologous NIBRG-14 strain in duplicate. The assays were double-checked by 2 persons. HI assays were performed according to established procedures, with turkey erythrocytes as in previous trials [7, 12–13]. MN assays were conducted as reported elsewhere [14] but with detection of hemagglutination activity in supernatants. MN titers were calculated by the Reed-Muench method. Samples were tested with a starting dilution of 1:10. HI and MN titers <1:10 were recorded as 1:5 (hereafter, titers are reported as reciprocal values).

The statistical analysis was conducted by an independent statistician. The immunologic results were recorded as geometric mean titer (GMT), seroconversion rate, seroprotection rate (for HI only), and proportion of participants with MN titers >40 (for MN only). The seroconversion rate is defined as the percentage of participants who have a prevaccination titer >10 and a postvaccination titer >40 or a prevaccination titer >10 with an increase of at least 4-fold after vaccination. Seroprotection was defined as an HI titer >40. The titers were transformed into a logarithmic scale to calculate GMT.

The trial was approved by the Chinese State Food and Drug Administration and was registered with ClinicalTrials.gov (identifier NCT00660257). All relevant documents were approved by the ethical review committee of the China-Japan Friendship Hospital, and all participants provided written informed consent.

## Results

Six and 12 months after 2-dose priming with H5N1 vaccine, 88 participants (21, 24, 22, and 21 in the 1.25-, 2.5-, 5-, and 10-μg groups) and 57 participants (17, 16, 14, and 10 in the same dose groups), respectively, donated blood samples after the third dose. There were no significant differences in age (for 6 months, \( P = .120 \); for 12 months, \( P = .348 \)) or sex (for 6 months, \( P = .057 \); for 12 months, \( P = .091 \)) among the 4 vaccinated groups at the 2 blood sampling time points.

The results for antibody persistence are shown in tables 1 and 2. Generally, the antibody levels declined substantially 6 and 12 months after vaccination.

<table>
<thead>
<tr>
<th>Time point, result</th>
<th>Vaccine dose</th>
<th>1.25 μg</th>
<th>2.5 μg</th>
<th>5 μg</th>
<th>10 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months after 2-dose priming</td>
<td>No. of participants</td>
<td>21</td>
<td>24</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>GMT</td>
<td>6.1 (4.0–9.2)</td>
<td>8.7 (5.6–13.3)</td>
<td>9.7 (6.6–14.2)</td>
<td>12.2 (6.7–22.0)</td>
</tr>
<tr>
<td></td>
<td>Seroconversion</td>
<td>1 (4.8; 0–26)</td>
<td>2 (8.3; 1–29)</td>
<td>3 (13.6; 4–26)</td>
<td>4 (19.0; 6–43)</td>
</tr>
<tr>
<td></td>
<td>Seroprotection</td>
<td>1 (4.8; 0–26)</td>
<td>5 (20.8; 8–43)</td>
<td>3 (13.6; 4–36)</td>
<td>4 (19.0; 6–43)</td>
</tr>
<tr>
<td>12 months after 2-dose priming (just before booster dose)</td>
<td>No. of participants</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>GMT</td>
<td>6.4 (4.1–9.9)</td>
<td>8.4 (5.1–13.8)</td>
<td>5.8 (4.6–7.3)</td>
<td>9.3 (4.1–21.3)</td>
</tr>
<tr>
<td></td>
<td>Seroconversion</td>
<td>1 (5.9; 0–31)</td>
<td>1 (6.3; 0–33)</td>
<td>0 (0; 0–27)</td>
<td>1 (10.0; 1–47)</td>
</tr>
<tr>
<td></td>
<td>Seroprotection</td>
<td>1 (5.9; 0–31)</td>
<td>3 (18.8; 5–47)</td>
<td>0 (0; 0–27)</td>
<td>1 (10.0; 1–47)</td>
</tr>
<tr>
<td>15 days after booster dose</td>
<td>No. of participants</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>GMT</td>
<td>12.8 (7.7–21.1)</td>
<td>29.5 (18.9–46.2)</td>
<td>51.2 (29.3–89.5)</td>
<td>65.0 (36.6–115.5)</td>
</tr>
<tr>
<td></td>
<td>Seroconversion</td>
<td>3 (17.6; 5–48)</td>
<td>5 (31.3; 12–59)</td>
<td>11 (78.6; 49–97)</td>
<td>7 (70.0; 35–95)</td>
</tr>
<tr>
<td></td>
<td>Seroprotection</td>
<td>4 (23.5; 8–51)</td>
<td>7 (43.8; 21–70)</td>
<td>12 (85.7; 56–100)</td>
<td>8 (80.0; 44–100)</td>
</tr>
<tr>
<td>30 days after booster dose</td>
<td>No. of participants</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>GMT</td>
<td>13.9 (7.9–24.4)</td>
<td>18.3 (11.5–29.2)</td>
<td>44.2 (25.7–75.8)</td>
<td>52.8 (34.7–80.2)</td>
</tr>
<tr>
<td></td>
<td>Seroconversion</td>
<td>4 (23.5; 8–51)</td>
<td>3 (18.8; 5–47)</td>
<td>11 (78.6; 49–97)</td>
<td>8 (80.0; 44–100)</td>
</tr>
<tr>
<td></td>
<td>Seroprotection</td>
<td>5 (29.4; 11–57)</td>
<td>5 (31.3; 12–59)</td>
<td>11 (78.6; 49–97)</td>
<td>9 (90.0; 54–100)</td>
</tr>
</tbody>
</table>

**NOTE.** Titers are reported as reciprocal values. Data for geometric mean titers (GMTs) are value (2-sided 95% confidence interval [CI]), and data for seroconversion and seroprotection are no. of participants (%; 2-sided 95% CI).
months after 2-dose priming. Compared with the GMTs in the 4
groups (1.25–10 μg) at 4 weeks after the 2-dose priming (for HI,
13.1–51.5; for MN, 17.7–33.1), the GMTs in the 4 groups at
6 and 12 months after the 2-dose priming declined to 23.7%–
54.7% and 18.1%–52.8% of the 4-week values for HI and to
45.2%–61.6% and 25.8%–67.1% of the 4-week values for MN,
respectively (tables 1 and 2 and Lin et al. [7]). Twelve months
after 2-dose priming, both HI GMTs and MN GMTs were sta-
tistically similar in the 4 groups (for HI, \( P = 0.409 \); for MN,
\( P = 0.058 \)). However, both HI and MN titers remained
\( \geq 40 \) in 1, 4, 3, and 3 participants in the 1.25-, 2.5-, 5-, and 10-
μg groups, respectively, at 6 months after the 2-dose priming and in 1, 3,
and 1 participants in the 1.25-, 2.5-, and 10-μg groups at 12
months. Combining the 4 groups, 14.8% (13/88 participants)
and 8.8% (5/57 participants) had seroprotective HI titers 6 and
12 months after the 2-dose priming, respectively (table 1).

As shown in tables 1 and 2, a third dose remarkably boosted
immune responses in a dose-dependent manner in the 4 groups. Fifteen
days after the third dose, HI GMTs of 12.8 – 65.0 were
achieved in the 4 groups. Both the 5- and 10-μg groups met EU
CHMP criteria for seroconversion rate (≥40%), seroprotection
rate (≥70%), and GMT increase ratio (≥2.5) [10]. Thirty days
after the third dose, HI GMTs of 13.9 – 52.8 were achieved. Both
the 5- and 10-μg groups still met all 3 EU CHMP criteria [10].
After the third dose, HI GMTs, seroconversion rates, and sero-
protection rates were generally higher than those seen after the
second dose (table 1) [7].

MN assays also showed an impressive dose-dependent
booster immune response after the third dose. Generally, MN
GMTs were higher than HI GMTs. Fifteen and 30 days after the
third dose, HI GMTs of 12.8 – 65.0 were achieved. Both
the 5- and 10-μg groups still met all 3 EU CHMP criteria [10].

After the third dose, HI GMTs, seroconversion rates, and sero-
protection rates were generally higher than those seen after the
second dose (table 1) [7].

Discussion. In this study, we report antibody persistence 6
and 12 months after 2-dose priming of an inactivated, alum-
adjuvanted, whole-virion H5N1 vaccine and the booster re-
sponse to a third dose of the same vaccine. In our previous trial,
the whole-virion H5N1 vaccine showed good immunogenicity
in adults [7]. However, HI and MN titers declined remarkably 6
and 12 months after 2-dose priming. Five of 57 participants had seroprotective HI titers (≥40) 12 months after 2-dose priming, indicating that the antibody titers after 2-dose priming of H5N1 vaccine are not very persistent and that a booster (third) dose is needed to maintain a seroprotective titer.

A third dose of the whole-virion H5N1 vaccine induced strong HI and MN booster responses in primed adults, suggesting that immune memory was retained after 2 priming doses and that an anamnestic response was induced after a booster dose. The previous trial had shown that two 10-µg vaccine doses elicited a response meeting all 3 EU CHMP criteria but that two 5-µg doses did not [7]. The results presented here show that both the 5- and 10-µg doses elicited responses meeting all 3 EU criteria after the third dose. This finding suggests a potential strategy for antigen sparing whereby the immunologically naive population could be primed with 2 lower doses before the outbreak of a pandemic and then boosted by a third dose to achieve seroprotection when a pandemic is beginning. With this strategy, a lower dose of H5N1 vaccine (e.g., 5-µg of whole-virion vaccine) is sufficient to elicit a response that meets all 3 EU criteria after a booster dose. In addition, we found that the third dose was well tolerated regardless of dose level. The incidence of adverse reactions after the third dose was significantly lower than that after the first or second dose, suggesting that the whole-virion H5N1 vaccine is safe when used as a booster dose.

A previous study has shown that a third dose of 7.5–90-µg, nonadjuvanted, split-virion H5N1 vaccine given 6 months after the second dose induced booster responses, with seroprotective HI titers in 21%–64% of participants [9]. Compared with the split-virion H5N1 vaccine, the whole-virion H5N1 vaccine shows better immunogenicity when used as booster dose, inducing higher GMTs, seroconversion rates, and seroconversion rates at a lower dose level. Both the 5- and 10-µg doses of the whole-virion H5N1 vaccine elicited responses meeting all 3 EU criteria after the booster dose. In fact, the whole-virion H5N1 vaccine has also shown better immunogenicity than split-virion vaccines when used for priming doses [5–7]. A third 7.5–30-µg dose of MF59-adjuvanted, surface-antigen H5N3 vaccine given 16 months after the second dose also induced evident anti-H5N1 booster response in primed adults, but the anti-H5N1 booster response was much poorer in adults who received nonadjuvanted H5N3 vaccine [15], indicating that an adjuvant may be necessary to induce a booster booster response.

Thirty days after the third dose, HI GMTs were slightly lower than those seen 15 days after the third dose, which is consistent with results of the previous trial [7]. In contrast, MN GMTs were generally similar between the 2 blood sampling time points. Further study may be necessary to elucidate the kinetics of the immune responses induced by H5N1 vaccines.

In conclusion, we find that the titers of antibody against H5N1 virus decline significantly 1 year after 2-dose priming and that a third dose induces an obvious booster response, with both the 5- and 10-µg doses meeting all 3 EU CHMP criteria. These findings suggest that an immunization regimen including 2 priming doses and a booster dose may promote preparedness for an influenza pandemic with a strategy of antigen sparing.

Acknowledgments

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References