Immunogenicity of a Monovalent Pandemic Influenza A H1N1 Virus Vaccine with or without Prior Seasonal Influenza Vaccine Administration

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The immunogenicity of pandemic influenza A H1N1 virus (A/H1pdm) vaccine might be modified by prior seasonal trivalent influenza vaccine (sTIV) administration. We conducted a retrospective analysis of immunogenicity of 243 health care workers (number of sTIV-positive [sTIV+] subjects, 216; number of sTIV− subjects, 27) by hemagglutination inhibition. There was no significant difference in the ratios of antibody titers of ≥40 (41.2% versus 48.1%; P = 0.49) and fold increases in geometric mean titer (3.8 versus 4.5; P = 0.37). sTIV injected 7 to 10 days prior to A/H1pdm vaccine administration did not interfere with the immunogenicity of the latter.

Human infections with pandemic influenza AH1N1 virus (A/H1pdm) were identified in April 2009. The availability of safe and effective vaccines was a critical component of efforts to prevent A/H1pdm infection and expansion of the pandemic (1). In Chiba University Hospital (CUH), seasonal trivalent influenza vaccine (sTIV) administration was conducted 7 to 10 days prior to A/H1pdm vaccine because the precise date of the initial A/H1pdm vaccine supply was unclear owing to limitations in the manufacturer’s production capacity.

There was uncertainty as to whether prior sTIV administration might interfere with the immunogenicity of A/H1pdm vaccine administered a week later. We undertook a clinical trial with health care workers (HCWs) to examine the immunogenicity of A/H1pdm vaccine (3). This study was a retrospective subgroup analysis of a previous study to evaluate the immunogenicity of a monovalent A/H1pdm vaccine administered with or without previous sTIV vaccination in HCWs aged between 20 and 39 years. All subjects provided written informed consent. The study was approved by the CUH Research Ethics Committee (G21035).

**Vaccine.** The A/H1pdm vaccine was monovalent, with a single dose containing 15 μg of hemagglutinin antigen. sTIV contained 15 μg of hemagglutinin antigen of each seed virus per single dose. Both vaccines were inactivated, split-virus, unadjuvanted ones produced by the same procedure and with thimerosal added as a preservative. Both vaccines were administered subcutaneously into the external upper arm in a single dose.

The Japanese Ministry of Health, Labor and Welfare initiated A/H1pdm vaccination of HCWs with top priority on 19 October 2009. Between 26 and 30 October, 409 HCWs without prior A/H1pdm infection were enrolled, and peripheral venous blood samples were collected before and 28 days after vaccination. The vaccination was independent of study participation. Of the 409 subjects, 20 were then excluded because 28-day-postvaccination blood samples were not provided. As a result, immunogenicity analysis was performed on data from 389 subjects. Among these, we selected 243 between 20 and 39 years old for this subgroup analysis.

A/H1pdm (A/California/07/09 [H1N1]) was allowed to proliferate in MDCK cells, and then a viral fraction was obtained and inactivated with formalin. Immunogenicity of the A/H1pdm vaccine was evaluated with a hemagglutination inhibition (HI) antibody assay according to standard methods (7), using the inactivated virus as described previously (3).

Statistical analyses were performed with Dr-SPSS II (SPSS Japan Inc., Tokyo, Japan). The statistical significance of the results of comparisons of data between groups was analyzed by a paired t test and chi-square test as well as a Wilcoxon signed-rank test when appropriate. P values of <0.05 were considered significant. We analyzed three factors as follows: (i) the proportion of subjects with an antibody titer of ≥40, (ii) the proportion of subjects with either seroconversion (prevaccination titer of <10 with postvaccination HI antibody titer of ≥40) or an increase by a factor of 4 or more in antibody titer, and (iii) the fold increase in geometric mean titer (GMT).

At baseline, 6 (3.1%) of 216 subjects with prior sTIV had an antibody titer of ≥40 and 1 (2.8%) of 27 subjects without prior sTIV had an antibody titer of ≥40. There were no significant differences in the proportions of subjects with a baseline antibody titer of >40 or in the baseline GMTs between the groups with and without prior sTIV.

Postvaccination titers of ≥40 were observed in 41.2% (95% confidence interval [CI], 34.6 to 47.8) of the subjects with prior sTIV and in 48.1% (95% CI, 29.3 to 66.9) of those without prior sTIV. The proportions of subjects with a postvaccination anti-
body titer of ≥40 did not differ significantly between the two groups (P = 0.49) (Table 1). Seroconversion or a significant increase in HI occurred in 60.6% (95% CI, 54.1 to 67.1) of subjects with prior sTIV and in 59.3% (95% CI, 40.8 to 77.8) of subjects without prior sTIV, with no significant difference between the two groups (P = 0.89). There was a substantial rise in GMTs after vaccination (for sTIV-positive [sTIV+] subjects, P < 0.001; for sTIV− subjects, P < 0.001), but the differences in the values of GMTs and the factor increases in GMTs were not significant between the two groups (P = 0.64 and P = 0.37, respectively).

This study demonstrated that sTIV injected 7 to 10 days previously did not affect the immunogenicity of A/H1pdm vaccine. Simultaneous administration of sTIV and A/H1pdm vaccine could induce sufficient levels of antibody to both vaccines (8). Ohfuji et al. reported interference with the immune response to the A/H1pdm vaccine by pregnant Japanese women who had recently received sTIV (6). Another study also showed lower GMT levels for A/H1pdm vaccine among seasonally vaccinated groups of infants and children aged 6 months to less than 9 years (5). Our current study, including this subgroup analysis, demonstrated lower immunogenicity among healthy HCWs (3) compared with other studies (2, 4, 9). However, we were able to compare the immunogenicities of A/H1pdm with or without prior sTIV administration because the samples from both groups were tested for the presence of antibodies using the same assay.

Regarding the differing results of the former two studies (5, 6), we speculate that the immunogenicity of infants and pregnant women is possibly modified in comparison with that of healthy HCWs. Since our subjects were healthy, relatively young HCWs, trials need to be conducted in other populations that may have different responses to the vaccine, such as the elderly, children, and those with impaired immunity. In the study of the immunogenicity of Australian infants (5), A/H1pdm vaccination was conducted in August and early September, with seasonal influenza vaccination having been conducted more than 2 months earlier. In the study of pregnant Japanese women (6), lower immunogenicity was demonstrated in subjects receiving sTIV vaccination within 19 days prior to A/H1pdm vaccination. The interval between sTIV and A/H1pdm vaccinations was slightly longer than ours, allowing us to speculate that a shorter interval between the two types of influenza vaccines might prevent an interference effect. Further testing is required to confirm this hypothesis.

This study was a retrospective subgroup analysis, and the two groups with and without sTIV were not assigned to this study. The number of sTIV-vaccinated HCWs was 216, and the number of sTIV-unvaccinated ones was 27. Because of the small sample size, the statistical power to ascertain a difference between the two groups might be insufficient and results need to be interpreted cautiously.

In conclusion, sTIV injected 7 to 10 days prior to a single dose of A/H1pdm vaccine did not interfere with the immunogenicity of the latter, according to HI antibody assays.

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**TABLE 1 Immune responses after A/H1pdm vaccination as measured by hemagglutination inhibition antibody assay**

| Parameter | Prior seasonal vaccination | P value $^a$ |
|-----------|---------------------------|-------------|
| Total no. of subjects | Yes: 216 | No: 27 | 0.47 |
| Age (yr) (mean ± SD) | 30.0 ± 4.7 | 30.0 ± 5.3 |

Before vaccination (baseline)

| No. [% (95% CI)] of subjects with HI antibody titer ≥ 40 | Prior seasonal vaccination | P value $^a$ |
|----------------------------------------------------------|---------------------------|-------------|
| Geometric mean titer (mean ± SD) | Prior seasonal vaccination | P value $^a$ |
| No. [% (95% CI)] of subjects with seroconversion or significant increase in titer $^c$ | Prior seasonal vaccination | P value $^a$ |
| Factor increase in geometric mean titer (mean ± SD) | Prior seasonal vaccination | P value $^a$ |

$^a$ A/H1pdm, pandemic influenza A/H1N1 virus; HI, hemagglutination inhibition; CI, confidence interval. Hemagglutination inhibition antibody titer values of <10 were assigned a value of 5 for the purpose of calculating the geometric mean titer.

$^b$ P-values were analyzed by t test, and the Wilcoxon rank sum test was also used where appropriate.

$^c$ Data represent the proportion of subjects who had either seroconversion (prevaccination titer of <10 with postvaccination HI antibody titer of ≥40) or an increase by a factor of 4 or more in antibody titer.
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