Immunogenicity and Reactogenicity of a Monovalent Inactivated 2009 Influenza A Vaccine in Adolescents: With Special Reference to Pre-Existing Antibody

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Objective To evaluate the immunogenicity and reactogenicity of a monovalent 2009 pandemic influenza vaccine in Japanese adolescents.

Study design A total of 111 junior high school and high school students aged 13 to 18 years participated. Subjects received two doses of a monovalent inactivated unadjuvanted 2009 influenza A vaccine. Immunogenicity of the vaccine was evaluated according to the international criteria. We also asked subjects to report adverse reactions.

Results After the first dose of vaccine, the seroprotection rate was 91% (95% CI, 85%-96%), the seroconversion rate was 78% (70%-86%), and the geometric mean titer ratio was 11.9 in all subjects. Antibody titers achieved did not differ significantly after the first and the second doses. With multivariate analysis, an independent negative effect of a prevaccination titer of ≥1:40 on ≥4 fold antibody increase was indicated. No serious adverse reaction was reported.

Conclusion The monovalent pandemic vaccine generally was safe, and a single dose of the vaccine given to adolescents induced sufficient immunity. Pre-existing antibody showed substantial effect on antibody response. The effect of pre-existing titer should be considered when evaluating the immunogenicity of influenza vaccines, especially in studies conducted during pandemic waves. *(J Pediatr 2012;160:632-7)*.

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A novel swine-origin influenza A (H1N1), first identified in the United States in April 2009, rapidly spread to many countries around the world, and the World Health Organization declared a pandemic on June 11, 2009.[1] In Japan, the peak of pandemic influenza activity was observed during October and November 2009. However, it was not until October 2009 that monovalent pandemic H1N1 vaccine was developed and distributed for tiered use. Vaccination was scheduled first for health care workers, and then provided to junior high school and high school students from January 2010, according to the order of priority of the groups.

A number of studies have suggested that a single dose of inactivated pandemic vaccine was enough to induce a protective antibody response in adults.[2-5] In contrast, only few studies have evaluated the immunogenicity in adolescents, despite 45% of 2009 H1N1 infection-associated hospitalizations having occurred in patients <18 years of age.[3,5,6] In this study, we evaluated the immunogenicity and reactogenicity of a monovalent H1N1 pandemic vaccine in Japanese adolescents aged 13 to 18 years.

In assessing the immunogenicity, pre-existing antibody can affect the antibody response.[7,8] However, there is no reference to consideration of pre-existing antibody in the conventionally used licensing criteria. We therefore attempted to evaluate the immunogenicity in adolescents, while considering the effect of pre-existing titer, by using the epidemiologic methodologies of stratification and multivariate analysis.

Methods

This study, including recruitment of subjects, vaccination, and serum collection, was conducted during October and November 2009, at 9 pediatric clinics and 3 hospitals in Osaka, Hyogo, and Fukui prefectures, Japan, involving 111 subjects aged 13 to 18 years (junior high school students and high school students). Participating institutions were enlisted through the network of Osaka Pediatric Association. Exclusion criteria included previously confirmed or suspected infection with 2009 H1N1 virus,

GMT	Geometric mean titer
GMTR	Geometric mean titer ratio
H1N1	Influenza A
HAI	Hemagglutination-inhibition
ORS	Oculo-respiratory syndrome

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acute febrile illness, or signs of severe acute illness at the time of vaccination, history of anaphylaxis caused by vaccine components, known or suspected immunosuppressive disease, recent history of immunosuppressive treatment, and other conditions that made vaccination inappropriate. We were required to enroll study subjects as soon as possible to provide data on immunogenicity in adolescents to the Ministry of Health, Labor and Welfare, Japan, mainly because of the urgency to evaluate the necessity for the second dose of the vaccine.

Vaccine

The vaccine administered was a monovalent inactivated unadjuvanted split-virus 2009 H1N1 vaccine (HP01A in 2009, Biken, Osaka, Japan). The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College, New York, New York), distributed by the Centers for Disease Control and Prevention in the United States. The vaccine was prepared in embryonated chicken eggs with standard methods used for the production of seasonal trivalent inactivated vaccine. The vaccine was supplied as multidose vials containing 0.0008% thimerosal. Vaccine dose was 0.5 mL, containing 15 μg of hemagglutinin antigen, and two doses were administered subcutaneously 3 weeks apart. We instructed participating institutions not to co-administer seasonal trivalent influenza vaccines. The Ministry of Health, Labor and Welfare, Japan, reserved the vaccine for this study on priority.

Serum Collection and Antibody Titer Measurement

Serum samples were collected before the first dose (S0), 3 weeks after the first dose (S1), and 4 weeks after the second dose (S2). Antibody titer against the vaccine strain was measured with the hemagglutination-inhibition (HAI) assay according to standard methods with chicken erythrocytes.[9,10] Serum samples were treated with receptor destroying enzyme (RDE, Vibrio cholera filtrate, Denka Seiken, Tokyo, Japan) to inactivate nonspecific inhibitors. All samples were assayed at the same time at the laboratory of the Research Foundation for Microbial Diseases of Osaka University.

Survey of Reactogenicity

Subjects and their guardians were asked to record on a questionnaire, after each dose, symptoms of the oculo-respiratory syndrome (ORS) noted within 24 hours, and systemic and local reactions noted within 48 hours. The specified symptoms of the ORS included red eye, facial edema, and respiratory symptoms (cough, wheezing, chest tightness, difficulty breathing, difficulty swallowing, hoarseness, and throat tightness), as in an earlier report.[11] Systemic reactions included fever, fatigue, myalgia or arthralgia, headache, and rash, whereas local reactions included erythema, swelling, induration, itching, and pain. We also asked about medical visits caused by these symptoms.

Statistical Analysis

On the basis of conventional international criteria, these 3 immunogenic end points were selected: seroprotection rate, seroconversion rate, and geometric mean titer ratio (GMTR).[12,13] Seroprotection was defined as an HAI titer of 1:40 or more. Seroconversion was defined as a prevaccination HAI titer <1:10 and a postvaccination HAI titer ≥1:40, or a prevaccination HAI titer ≥1:10 and ≥4-fold antibody increase after vaccination. In addition, we calculated the seroresponse rate, the proportion of subjects who achieved ≥4-fold increase. In this calculation, an HAI titer <1:10 was treated as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results were presented in the original form by obtaining the antilogarithms.

The data was stratified for analysis by sex, school age (ie, junior high school or high school), and prevaccination titer (ie, <1:10, 1:10-1:20, or ≥1:40). The significance of titer increase by vaccination within a category was assessed with the Wilcoxon signed-rank test, and inter-category comparisons of the geometric mean titer (GMT) or GMTR were made with the Wilcoxon rank sum test or the Kruskal-Wallis test. The χ^2 test, Mantel-extension trend test, and McNemar test were also used when appropriate.

The independent effects of potential predictors, such as prevaccination titer and school age, on the antibody response were evaluated with a logistic regression model. The model was constructed with seroresponse as the dependent variable.

Reactogenicity data were expressed as the number and proportion of subjects who had each symptom. Differences in frequencies after the first and the second doses were evaluated with the McNemar test.

All reported P values are two-sided, and a P value <.05 was considered to be statistically significant. All statistical analyses were performed with SAS software version 9.1 (SAS Institute Inc, Cary, North Carolina).

The study was approved by the ethics committee of the Osaka City University Graduate School of Medicine and conducted in accordance with the principles of the Declaration of Helsinki and Japanese regulatory requirements. Written informed consent was obtained from each subject's legal representative.

Results

Immunogenicity

A total of 111 subjects, 63 junior high school students and 48 high school students, consented to participate in the study. Fourteen of these subjects had underlying illness (10 had asthma, 2 were being treated with growth hormone, 1 had diabetes mellitus, and 1 had epilepsy), but all were in a stable medical condition. Of all the subjects, 5 (3 junior high school and 2 high school students) were confirmed to have H1N1 virus infection with the rapid test between the first and the second doses. Additionally, 4 junior high school students were confirmed with H1N1 virus infection between the second dose and the last serum sampling. After excluding these cases, 106 and 102 subjects provided immunogenicity data for analysis after the first and the second dose, respectively.

GMTs are shown in Table I. Before vaccination, 22 of the subjects (21%) had a seroprotective antibody titer (≥1:40). The first dose of vaccine induced significant titer rise in all