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Research paper

Relationship between the frequency of influenza vaccination and cell-mediated immunity

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ABSTRACT

Despite established guidelines for population-level assessments of immunity after vaccination, standard methods for individual-level analyses have not been established, limiting the ability to optimize vaccination strategies within a particular season. In this study, we evaluated changes in cell-mediated immunity (CMI) with respect to the number of influenza vaccine doses. In particular, the influenza vaccine was administered to 21 adults during the 2015–2016 season. IFN-γ production induced by the influenza vaccine antigens [A (H1N1), A (H3N2), B (Yamagata lineage), and B (Victoria lineage)] increased after the first dose of vaccination in 11, 10, 10, and 11 subjects, respectively. In 5 of 10 (H1N1), 4 of 10 (H3N2), 3 of 9 (Yamagata lineage), and 3 of 8 (Victoria lineage) subjects who did not exhibit an increase in IFN-γ production after the first dose, CMI was enhanced after the second dose. The production of IFN-γ increased after the first or second dose of the vaccine in 16, 14, 13, and 14 of the 21 subjects, respectively. The results of this study showed that two doses of the influenza vaccine effectively enhance CMI in subjects with primary vaccine failure.

1. Introduction

During the 2015–2016 season, the outbreak of influenza A subtype H1N1 was more marked than that of influenza A subtype H3N2 in Japan. Both Yamagata and Victoria influenza B lineages also became epidemic (National Institute of Infectious Diseases, 2017). According to the Centers for Disease Control and Prevention, the vaccine effectiveness (VE) for influenza A/B viruses in all age categories during the 2015–2016 season was 47%; the VE for influenza A (H1N1) pdm09 virus was 41% and that for influenza B virus was 55% (Centers for Disease Control and Prevention, 2017). Furthermore, the Canadian Sentinel Practitioner Surveillance Network indicated that the VE for influenza A (H1N1) pdm09 virus ranged from 56% to 64% (Chambers et al., 2016).

In Japan, inactivated influenza vaccine (IIV) manufactured in accordance with a guidance document by the European Medicines Agency (EMEA, 1997) is used for prevention. In the United States, quadrivalent IIV (IIV4) has been adopted since the 2013–2014 season. This vaccine has also been used in Japan since the 2015–2016 season.

The EMA guidance document (EMEA, 1997) involves an immunological assessment based on hemagglutination inhibition (HAI) antibody titers, but a cell-mediated immunity (CMI)-based assessment may also be necessary to evaluate an individual's immunity (Centers for Disease Control and Prevention, 2015). However, a simple method to measure CMI has not been established. We previously developed a method to measure CMI related to the influenza vaccine (Otani et al., 2016), but this method has not been used to evaluate the effects of multiple doses.

According to the Advisory Committee on Immunization Practices recommendation (Grohskopf et al., 2015), the frequency of influenza vaccination during the 2015–2016 season in infants and children aged 6 months to 8 years should be one dose if there is a history of influenza vaccination (≥2 doses) prior to 2015. However, there is no regulation regarding the frequency of vaccination at other ages. Accordingly, in the present study, we investigated the influence of the number of influenza vaccine doses on CMI.

Abbreviations: CMI, cell-mediated immunity; GMT, geometric mean titer; HAI, hemagglutination inhibition; IFN-γ, interferon-γ; IIV, inactivated influenza vaccine; IR, interferon-γ release; NT, neutralizing antibody; one dose, 1st dose of a vaccine; two doses, 2nd dose of a vaccine

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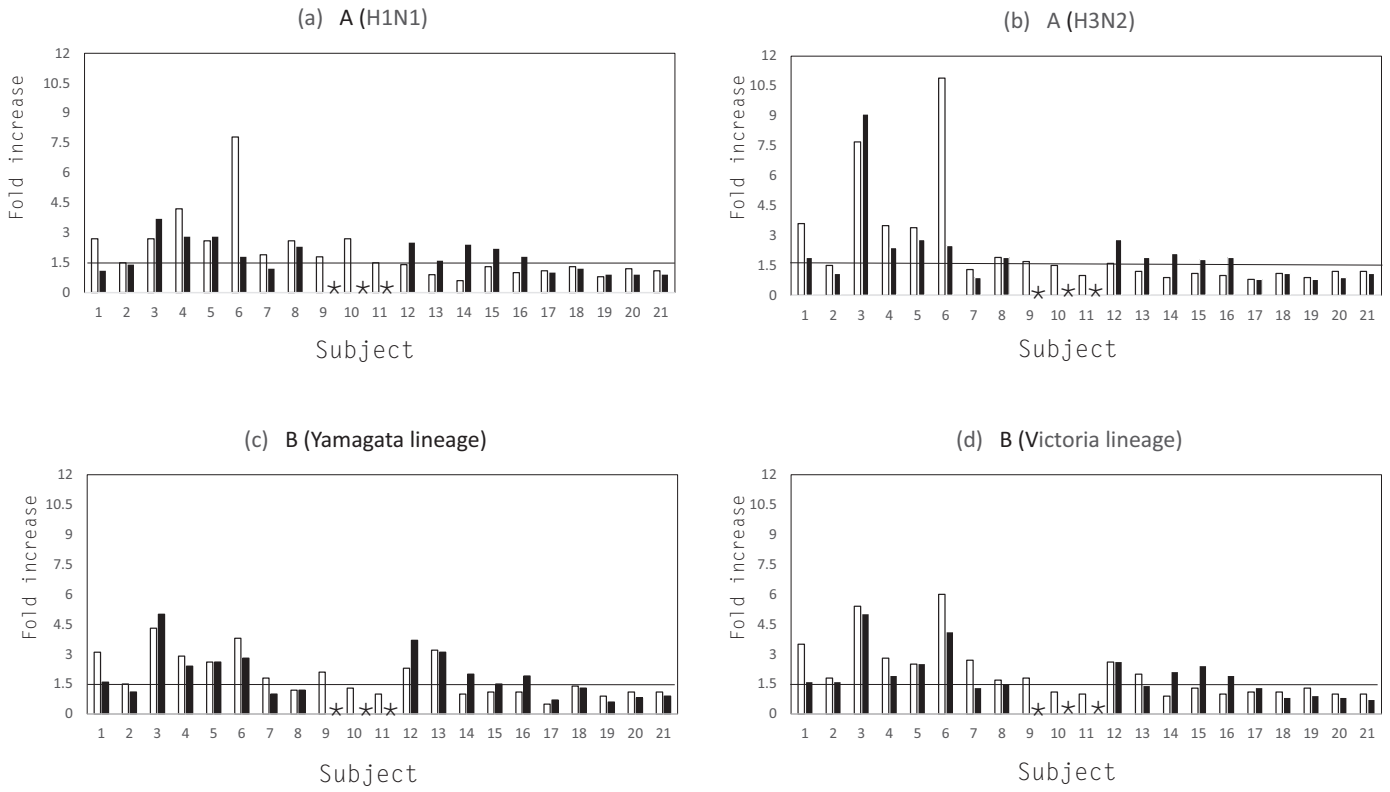

Fig. 1. Distribution of cell-mediated immune responses before vaccination and after one or two doses of influenza vaccine. Values are expressed as fold increases relative to those before vaccination. Responses after one dose (□) and two doses (■) in subjects. (a) H1N1, (b) H3N2, (c) Yamagata lineage, and (d) Victoria lineage
*: no data (Subjects 9, 10, and 11 did not receive two doses.)

2. Material and methods

2.1. Study population and vaccine

Twenty-one healthy adults (33–59 years old; 8 males and 13 females) were recruited and registered at Hyogo College of Medicine for this study. Influenza vaccination was performed between September and December 2015. In total, 18 subjects received two doses of the influenza vaccine. Influenza vaccine IIV4 (Lot No. HA150A, The Research Foundation for Microbial Diseases of Osaka University [BIKEN], Osaka, Japan) was used, and 0.5 mL of this vaccine was administered by subcutaneous injection. In the previous study, we investigated immunity at 2 and 8 weeks after vaccination and observed that IFN-γ production was greater at 2 weeks (Otani et al., 2016). Therefore, blood was collected prior to influenza vaccination, 2 to 3 weeks following the first dose, and 2 to 3 weeks following the second dose. IFN-γ release (IR) assays, hemagglutination inhibition (HAI) assays, and neutralizing antibody titer (NT) assays were performed. The interval between the first and second dose was 2–4 weeks according to the manufacturer's instructions (INFLUENZA VACCINE "BIKEN" package insert). The Ethics Review Board of Hyogo College of Medicine approved the study protocol, and blood was collected after obtaining written informed consent from all subjects. All methods were performed in accordance with the relevant guidelines and regulations.

2.2. IR assay

The four vaccine antigens in IIV4 used during the 2015–2016 season were as follows: A/California/7/2009 (X-179A) (H1N1) pdm09 virus, A/Switzerland/9715293/2013 (NIB-88) (H3N2) virus, B/Phuket/3073/2013 (Yamagata lineage), and B/Texas/2/2013 (Victoria lineage). These vaccine antigens were obtained from BIKEN. The IR assay was performed as previously described (Otani et al., 2016). Heparinized whole blood (100 μL) was added to flat-bottomed microtiter plates and incubated with each influenza antigen (HA titer, 10 μg/mL) diluted with RPMI 1640 medium to obtain a final volume of 200 μL/well. The co-cultures were conducted within 1 h of obtaining the blood samples. Supernatants (100 μL) were collected after 48 h of culture and the IFN-γ concentration was quantified using an enzyme-linked immunosorbent assay (IFN-γ Assay Kit; Biosource International, Camarillo, CA, USA) according to the manufacturer's instructions. Phytohemagglutinin (final concentration, 2.5 μg/mL) or medium was added to the blood, rather than the influenza antigen, for the positive and negative controls, respectively. The amounts of IFN-γ released in the negative and positive control wells in all experiments were < 4 and > 100 pg/mL, respectively.

2.3. Evaluation of CMI

A previous study has shown that the IFN-γ level does not increase by > 1.5 times in response to vaccination in influenza-infected subjects (Otani et al., 2016). Based on these previous results, subjects with a ≥ 1.5-fold increase in IFN-γ production were regarded as showing positive reactions.

2.4. Antibody titration

A commercial laboratory (SRL, Inc., Tokyo, Japan) measured the HAI antibody titers against the influenza virus in serum samples. The levels of serum NT against the vaccine viruses were examined using micro-neutralization assays as previously described (Ainai et al., 2012).

2.5. Statistical analyses

To determine the correlation between two sets of immunologic test results, a Pearson product-moment correlation analysis was used. A *p*-