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Research paper

Evaluation of influenza vaccine-immunogenicity in cell-mediated immunity

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ABSTRACT

The immunological effect of influenza vaccines cannot be evaluated accurately using an antibody titer. Therefore, we used a new method that measures cell-mediated immunity to investigate changes in the amount of interferon-gamma (IFN-γ) produced after vaccination in response to the vaccine antigen. The study was conducted during the 2014–2015 influenza season in 23 adults, using a vaccine that contained three types of antigen. The IFN-γ level increased by at least 1.5 times in 65% (15/23) of cases in response to the H1N1 antigen, in 57% (13/23) of cases in response to the H3N2 antigen, and in 57% (13/23) of cases in response to the B antigen. During the study period, 4 subjects developed type A influenza. Our data showed that the IFN-γ level did not increase by 1.5 times in these subjects. We propose that the efficacy of influenza vaccines may be evaluated by measuring changes in the level of IFN-γ produced in response to influenza vaccine.

1. Introduction

Many studies have been conducted to assess the effectiveness of influenza vaccine, which is widely used to prevent infection. Both live attenuated influenza vaccine and inactivated influenza vaccine (IIV) are available, but only IIV is used in Japan. Reportedly, the efficacy of IIV is about 60% in people who are 65 years old or younger [3,11], but it decreases to 50–60% in people who are older than 65 years [3].

In Japan, trivalent IIV (IIV3) was used during the 2014–2015 influenza season, and quadrivalent IIV (IIV4) was used during the 2015–2016 season. IIV3 contains three types of antigen: A/California/7/2009 (X-179A) (H1N1)pdm09, A/New York/39/2012 (X-233A) (H3N2), and B/Massachusetts/2/2012. IIV4 contains four types of antigen: A/California/7/2009 (X-179A) (H1N1)pdm09, A/Switzerland/9715293/2013 (NIB-88) (H3N2), B/Phuket/3073/2013, and B/Texas/2/2013. In Japan, the immunogenicity of the vaccines used is based on evaluation criteria for safety and efficacy established at the time of the yearly choice of strain used to manufacture seasonal inactivated influenza vaccine. These criteria are established by the European Agency for the Evaluation of Medicinal Products [22].

The immunogenicity of most vaccines is evaluated using antibody titers, but this type of test alone is insufficient to determine the effectiveness of influenza vaccine [4]. Recent studies reported that the reaction of cell-mediated immunity (CMI) is related to the development of immunity against influenza infection [9,18]. Therefore, vaccine efficacy can be evaluated by measuring CMI, specifically changes in Granzyme B production, and combining the results of the Granzyme B level and antibody titer [10]. Further investigation of CMI after vaccination is necessary [4].

We developed a simple method to measure CMI and evaluate vaccination-induced CMI by measuring changes in the interferon-gamma (IFN-γ) level. At the same time, humoral immunity was measured using the hemagglutination inhibition (HAI) method to evaluate the relationship between CMI and humoral immunity.

2. Materials and methods

2.1. Study population

Study subjects were 23 healthy adults (aged 36–57 years old). Blood was collected from the subjects immediately before

Abbreviations: CMI, cell-mediated immunity; HAI, hemagglutination inhibition; IFN-γ, interferon-γ; IIV, inactivated influenza vaccine; IR, interferon-γ release.

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influenza vaccine administration, 2 weeks after vaccination, and 8 weeks after vaccination, between September 2014 and March 2015. Each time blood was sampled, an IFN-γ release (IR) assay and HAI test were also performed. This study was approved by the Hyogo College of Medicine Ethics Committee.

2.2. Vaccine and antigens

The influenza vaccine used, IIV3 (BIKEN, Osaka, Japan, Lot HE40A), was used during the 2014–2015 influenza season and was administered subcutaneously as a single 0.5-mL dose. The vaccine antigens in IIV were H1N1, H3N2, and B: A/California/7/2009 (X-179A) (H1N1) pdm09, A/New York/39/2012 (X-233A) (H3N2), and B/Massachusetts/2/2012. Antigens used for IR assay were provided from BIKEN.

2.3. IR assay

The IR assay evaluated the release of IFN-γ in response to influenza antigens. Heparinized whole blood (100 μL) was added to flat-bottomed microtiter plates and incubated with influenza antigen (10 μg HA/mL) diluted with RPMI 1640 medium in a final volume of 200 μL/well. The cocultivations were conducted within 1 h of drawing the blood samples. The culture supernatants (100 μL) were collected 48 h after cultivation, 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. Either phytohemagglutinin (final concentration, 2.5 μg/mL) or medium was added to the blood in place of the influenza antigen to serve as the positive and negative control, respectively. The amounts of IFN-γ released in the negative and positive control wells in all experiments were <4 pg/mL and >100 pg/mL, respectively.

2.4. Antibody titration

The antibody titer against influenza virus in serum was measured using an HAI test from a commercial laboratory (SRL, Inc., Tokyo).

3. Results

3.1. Culture duration of the IR assay

To establish the culture time, the reaction between influenza antigen and whole blood was investigated on the basis of IFN-γ production at 24, 48, and 72 h. As shown in Fig. 1, IFN-γ production increased until 48 h, but it decreased after 72 h in some subjects. On the basis of these findings, the incubation time was set at 48 h.

3.2. Influenza vaccine-induced changes in cell-mediated immunity

Of the 23 subjects, 4 developed H3N2 influenza during the study period. In these individuals, the amount of IIV3-induced IFN-γ production against H3N2 antigen was less than 1.5 times the original amount. Therefore, we defined an increase of at least 1.5 times the original amount of IFN-γ production as a positive increase. In the IR assay that we developed, whole blood and antigen were reacted without adjusting the number of cells. Thus, whether IFN-γ production increases after vaccination was investigated, setting the baseline at the level before vaccination.

The positive rates of increase were as follows: H1N1, 65% (15/23); H3N2, 57% (13/23); and B, 57% (13/23). Of the 23 subjects, 11 became positive for all 3 antigens (Fig. 2).

3.3. Influenza vaccine-induced changes in the antibody titer

Using HAI antibody titer ⩾1:40 as positive, the humoral immunity positive rates (using the HAI method) before vaccination were as follows: H1N1, 35% (8/23); H3N2, 100% (23/23); and B, 39% (9/23). At 2 weeks after vaccination, the rates were 48% (11/23), 100% (23/23), and 57% (13/23), respectively. At 8 weeks after vaccination, the rate for H1N1 was 52% (12/23); the titer increased to ⩾1:40 in Sub7 and Sub19, but decreased to <1:40 in Sub23. The rate for H3N2 was 100% (23/23). The rate for B was 39% (9/23); the titer increased to ⩾1:40 at 2 weeks and then decreased to <1:40 at 8 weeks in Sub16, Sub18, Sub22, and Sub23 (Table 1). Regarding geometric mean titers for HAI assay ratios (post-vaccination/pre-vaccination) >2.5 in the 18–60-year age group criteria [22], the geometric mean titer ratios at 2 weeks and 8 weeks after vaccination were as follows: H1N1 (1.5, 2.1), H3N2 (2.1, 2.9), and B (1.5, 1.3), respectively.

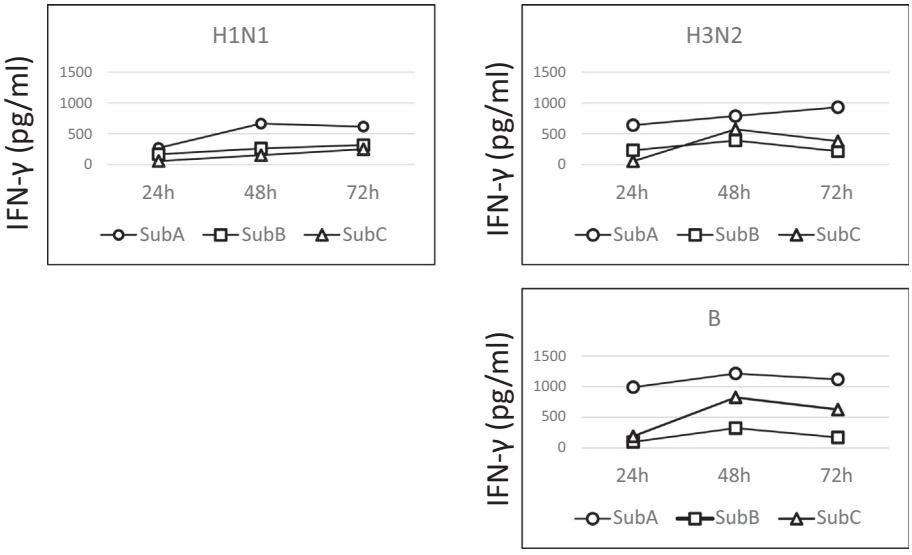

Fig. 1. Interferon-gamma (IFN-γ) production from the whole blood of 3 subjects at specified culture times. Culture supernatant was harvested at 24, 48, and 72 h, and IFN-γ concentration was quantified using an ELISA.