Biologicals xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Sex bias in mouse humoral immune response to influenza vaccine depends on the vaccine type

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ARTICLE INFO

Keywords:
Antibody response
Influenza virus strain
Sex differences
Split virus influenza vaccine
Whole virus influenza vaccine

ABSTRACT

The study explored influence of biological sex on development of humoral immune response to seasonal trivalent whole inactivated virus (WIV) and split virus (SV) influenza vaccines in outbred Swiss mouse model. To this end, mice of both sexes were immunized with WIV (WIV mice) and SV vaccines (SV mice) and examined for specific antibody response. Irrespective of sex, total IgG and neutralizing antibody responses to distinct virus strains were weaker in SV than in WIV mice. In WIV mice of both sexes, irrespective of strain specificity, IgG isotype response was dominated by IgG2a antibodies, while in SV mice nearly equal representation of IgG2a and IgG1 antibodies was found. The analyses of sex differences showed higher titers of H1N1-specific and both H1N1- and H3N2-specific total IgG and neutralizing antibodies in female WIV and SV mice, respectively. Additionally, sexual dimorphism in IgG subclass profile depended on vaccine type. Specifically, compared with males, in females WIV shifted IgG2a/IgG1 antibody ratio towards IgG2a isotype on the account of weaker IgG1 response, whereas in SV mice, irrespective of virus strain, IgG2a and IgG1 isotypes were equally represented in both sexes. These findings indicate the vaccine type-dependent sex bias in antibody response to inactivated influenza vaccines.

1. Introduction

Sex based differences in immune responses, affecting both the innate and adaptive immune responses, likely contribute to differences in the susceptibility to infectious diseases and response to vaccines in males and females [1,2]. Generally, females mount stronger humoral (antibody) vaccine responses, but also experience more adverse reactions following vaccination than males [1,2]. Thus, a "one size fits all" approach in vaccine delivery does not seem to be justifiable [3]. In the same line are data indicating that the magnitude and quality (in terms of IgG subclass profile) of humoral vaccine response depend on quantity of vaccine antigen(s) and type of vaccine, as well as on number of doses administered, and the route of immunization [4–6]. Thus, it may be assumed that sex differences in humoral vaccine response also vary depending on these variables.

Current seasonal trivalent influenza vaccines contain either inactivated influenza antigens or live attenuated influenza viruses derived from two subtypes of influenza type A and one from influenza type B. Inactivated influenza vaccines comprise whole inactivated virus (WIV), split virus (SV), virosomal or subunit antigen, all differing in either structural organization or viral components [7,8]. WIV were the first to be used in widespread annual influenza vaccination campaigns. They are produced by treatment of live influenza virus with β-propiolactone or formaldehyde and retain both the composition and the structure of the native virus. SV are produced in the same way as whole virus vaccines, but influenza virus particles are disrupted using detergents or diethyl ether [7,8]. This type of vaccine still contains all viral proteins, but the original viral particulate organization and viral genomic single-stranded (ss)RNA are mostly lost, losing some of the inherent immunogenicity of the virus [9]. Accordingly, in female inbred naïve mice, immunization with influenza WIV raises stronger and qualitatively different humoral immune response compared with immunization with SV [10,11].

Despite a growing body of evidence that antibody responses to influenza vaccines differ between the sexes, whether diverse types of vaccines influence antibody immune response efficacy differently in males and females has not yet been addressed adequately [3]. This seems to be important considering that, although cellular immune

Abbreviations: HA, hemagglutinin; HAI, hemagglutination inhibition; MDCK, Madin-Darby Canine Kidney; MN, microneutralization; OD, optical density; p.i., post immunization; RDE, receptor destroying enzyme; SV, split virus vaccine; $TCID_{50}$, 50% tissue culture infective dose; T-PBS, PBS containing 0.05% Tween 20; WIV, whole inactivated virus vaccine

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https://doi.org/10.1016/j.biologicals.2018.01.007
Received 4 August 2017; Received in revised form 30 January 2018; Accepted 31 January 2018

response is clearly important, influenza vaccine, as most vaccines licensed today, depends for its efficacy on serum antibodies [12]. Thus, the antibody response is used as surrogate marker for influenza vaccine efficacy [12].

Our previous study showed sexual dimorphism in the antibody response to seasonal trivalent WIV in influenza-vaccination outbred Swiss mouse model [13]. We chose this model to mimicry genetic diversity present in humans. Considering all the aforementioned data, the present study was undertaken to examine putative significance of influence vaccine type (WIV vs SV) for sexual dimorphism in humoral response in Swiss mice. To this end, sera from Swiss mice of both sexes immunized with seasonal trivalent WIV and SV influenza vaccines were examined for the following parameters: (i) total serum titer of IgG specific to distinct influenza strains, (ii) subclass profile of distinct influenza strain-specific IgG, as it may affect immunity to pathogens and (iii) hemagglutination inhibition- and virus neutralization-titers of antibodies specific to each of three influenza strains incorporated into the vaccines, as a parameter routinely used to estimate the efficacy of current influenza vaccines [14].

2. Materials and methods

2.1. Animals and ethics statement

Young adult (10-week-old) outbred Swiss mice, both males and females, bred at the Institute of virology, vaccines and sera “Torlak”, were used in this study. The animals were housed under the standard conditions (temperature and light/dark cycles were maintained at 21 ± 2 °C and 12/12 h), with free access to food pellets and tap water. All experiments were approved by Ethics Committee for the welfare of experimental animals in accordance with the Serbian laws and European regulations on animal welfare (Approval No.232-06-03742/2012-05).

2.2. Immunization

Mice were immunized with seasonal trivalent (composition of 2011/2012 season: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 (B) influenza virus) WIV (Institute of Virology, Vaccines and Sera “Torlak”, Belgrade, Serbia; vaccine virus suspension is inactivated by formaldehyde) or SV vaccine (Sanofi Pasteur, Lyon, France; split by Triton X-100, inactivated by formaldehyde). The vaccines were administered intramuscularly in each caudal tight and contained 10 μg of the virus surface protein hemagglutinin (3.33 μg of each virus strain). Both the vaccines were dosed on the amount of hemagglutinin. Mice injected with the saline served as controls. None of immunized mice showed any signs of local reaction to immunization including redness, swelling or the formation of granulomas at the injection site. They were also monitored for indicators of systemic reaction to immunization such as changes in external physical appearance, body temperature, body weight and food consumption. None of these signs was registered in immunized mice when compared with controls.

Serum samples were collected four weeks post immunization (p.i.). This time point was chosen considering the dynamics of humoral immune development and, particularly in appearance of sexual dimorphism in this response [13]. Mice were anesthetized with ketamine-xylazine anesthesia (ketamine, 80 mg/kg Ketamidor, Richter Pharma AG, Wels, Austria; xylasine, 8 mg/kg Xylased, Bioveta, Ivanovice na Hané, Czech Republic), blood was collected from retro orbital sinus and left to clot for 1 h at room temperature. Sera were decomplemented at 56 °C for 30 min and stored at −20 °C.

2.3. ELISA

ELISA was performed to detect influenza virus strain-specific IgG and IgG subclasses (IgG1, IgG2a, IgG2b and IgG3) in serum samples by standard end-point dilution as previously described (13). Briefly, the 96-well plates were coated with 2.5 μg of H1N1 or H3N2 or B influenza virus HA/ml (50 μl per well) diluted in PBS and left at 4 °C overnight. After being washed three times with T-PBS and once with PBS the plates were blocked with 1% BSA for 1 h at 37 °C and then thoroughly washed again. Fifty μl of sera samples serially diluted in T-PBS containing 1% BSA were added to antigen-coated plates and incubated for 1 h at room temperature. Influenza specific serum antibodies were detected using horseradish peroxidase-conjugated goat anti-mouse-IgG (1:50.000) or individual IgG isotypes IgG1, IgG2a, IgG2b and IgG3 (1:1000) (Jackson ImmunoResearch Laboratories Inc., WestGrove, PA, USA). Antibodies were incubated for 1 h at room temperature, reaction was developed using OPD and H_2O_2 and then stopped using 2 M H_2SO_4. Optical density was measured at 490 and 620 nm with Multiscan Ascent (Labsystems). Titers were calculated as the reciprocal of the highest dilution of test sera that gave an absorbance reading value of 3 standard deviations above the control sera at an equivalent dilution.

2.4. Hemagglutination inhibition assay

To inactivate non-specific hemagglutination inhibitors mice sera were treated with receptor-destroying enzyme (RDE, DenkaSeiken Co. Ltd., Tokyo, Japan), prior to hemagglutination inhibition assay (HAI). Three parts RDE were added to one part sera and incubated overnight at 37 °C. RDE-treated sera (samples from WIV or SV vaccine-immunized mice) were two-fold serially diluted in V-bottom 96 wells microtiter plates. Twenty five μl of either H1N1 or H3N2 or B strain incorporated in tested vaccines (adjusted to 4 HA units per 25 μl) were added to each plate and incubated for 15 min at room temperature. Standard dilution of turkey erythrocytes (TRBC) was added. The plates were mixed, covered, and the TRBC were allowed to settle for 30 min at room temperature. The reciprocal of the last dilution which contained non-agglutinated TRBC was considered HAI titer.

2.5. Microneutralization assay

Functional antibodies were measured directly by using micro-neutralization (MN) assay following WHO protocol [15]. Live H1N1 or H3N2 or B virus (100 × 50% tissue culture infective dose, TCID50) was incubated with serially diluted mice serum samples. Virus and serum mixture was transferred to monolayers of Madin-Darby canine kidney (MDCK) cells, and after overnight incubation, the cells were stained with the crystal violet dye. MN titers were expressed as reciprocal of the highest dilution of serum that gave 50% neutralization of 100 × TCID50 of virus in MDCK cells.

2.6. ELISPOT

ELISPOT assays were performed according to the manufacturer's instructions (R&D Systems, Minneapolis, USA) to detect IFN-γ- and IL-4-secreting cells. Cells were isolated from the spleens of WIV or SV vaccinated mice and control animals, two weeks p.i. Cell suspensions (1×10^6) were incubated in anti-mouse IFN-γ or IL-4 pre-coated plates with 1 μg/ml of either H1N1 or H3N2 or B inactivated virus. The plates were incubated for 48 h at 37 °C with 5% CO_2. IFN-γ- and IL-4- secreting cells were detected using biotinylated IFN-γ or IL-4 detection antibodies, streptavidin-alkaline phosphatase conjugate and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) substrate solution. Spots were counted using a dissecting microscope (Olympus VMZ Stereo Microscope, Olympus OpticalCo., Ltd., Tokyo, Japan).

2.7. Statistical analyses

The GraphPad Prism program (GraphPad, San Diego, CA, USA)