Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Sexual diergism in antibody response to whole virus trivalent inactivated influenza vaccine in outbred mice

Irena Živković[a], Biljana Bufan[b], Vladimir Petrušić[a], Rajna Minić[a], Nevena Arsenović-Ranin[b], Raisa Petrović[a], Gordana Leposavić[c,*]

[a] Immunology Research Centre "Branislav Janković", Institute of Virology, Vaccines and Sera "Torlak", 458 Vojvode Stepe, 11221 Belgrade, Serbia
[b] Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, 450 Vojvode Stepe, 11221 Belgrade, Serbia
[c] Department of Physiology, Faculty of Pharmacy, University of Belgrade, 450 Vojvode Stepe, 11221 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 25 August 2015
Accepted 4 September 2015
Available online xxx

Keywords:
Sex differences
Trivalent inactivated influenza vaccine
Antibody response
Th1/Th2 balance

ABSTRACT

An outbred mouse model was used to determine if antibody response to immunization with whole-virus trivalent inactivated influenza vaccine (TIV) differs between the sexes. The antibody response was examined one (serum titer of IgM antibodies), and three and six weeks post-immunization (serum titer of neutralizing and total IgG antibodies and IgG subclass profile). Compared with male in female mice was found (i) the more robust IgM response against all influenza strains included in TIV and (ii) more vigorous neutralizing antibody and total IgG responses against H1N1 influenza virus at both the examined time points post-immunization. The total IgG antibody response against H3N2 and B influenza viruses was comparable between female and male mice three weeks post-immunization, but significantly greater in female mice six weeks post-immunization. The neutralizing antibody response against H3N2 and B influenza viruses did not significantly differ between sexes at both the examined points post-immunization. Finally, three weeks post-immunization subclass profile of IgG specific to the influenza strains included in TIV differed between female and male mice, reflecting the lower titer of IgG1 antibodies in female ones, so that IgG2a (contributing mainly to the total IgG) to IgG1 ratio in mice of this sex was shifted toward the former. In agreement with this shift, compared with male mice, Th1/Th2 balance in female mice was shifted toward Th1, as shown by ELISPOT. Collectively, the results showed influenza virus strain-dependent sexual dimorphism in the magnitude, dynamics and characteristics of antibody response in outbred mice immunized with TIV.

1. Introduction

Sex differences in susceptibility to a range of infections, including viral infections, and their clinical outcome have been well documented. Compared to female, male mice and humans experience higher prevalence and severity of viral diseases [1–3]. On the other hand, females typically mount more pronounced pro-inflammatory innate and adaptive immune responses to viral infections than males [3]. This is shown to be double edged sword leading to a faster virus clearance, but also to some detrimental effects due to development of immune-mediated pathologies, as well [3]. Sexual dimorphism in protective immune response to vaccination against many viral infections, including influenza virus infection, has also been shown [4]. Generally, women generate a more robust antibody response to viral vaccines than men, while the findings related to sex-based differences in cellular immune responses are controversial [4]. However, adverse reactions to vaccines are also significantly more frequent in females than in males [1,4]. The mechanisms mediating sexual dimorphism in antibody responses to infection and vaccination and vaccine adverse side-effects are largely not understood. The lack of this knowledge is a critical barrier preventing optimization of vaccine design, dosage, and usage [5]. Hormonal, genetic and epigenetic factors, and microbiota differences between males and females appear to affect the outcome of infection or vaccination [5].

Although sex differences in immune response, including the immune response to vaccines, are well known, preclinical vaccine studies rarely, if ever, include comparative investigations in

Abbreviations: TIV, trivalent inactivated vaccine; HA, hemagglutinin; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; PBS, phosphate-buffered saline; T-PBS, PBS containing 0.05% Tween 20; OD, optical density; RDE, receptor destroying enzyme; HAI, hemagglutination inhibition; MN, microneutralization; $TCID_{50}$, 50% tissue culture infective dose; MDCK, Madin–Darby canine kidney; ELISPOT, enzyme-linked immunospot; BCIP/NBT, 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium; p.i., post-immunization.

* Corresponding author. Tel.: +381 64 1105075; fax: +381 11 3974 349.
E-mail address: Gordana.Leposavic@pharmacy.bg.ac.rs (G. Leposavić).

http://dx.doi.org/10.1016/j.vaccine.2015.09.006
0264-410X/

female and male animals [6]. Additionally, many of these studies either do not report the sex of experimental animals or combine the responses of males and females [7]. Furthermore, generally, studies investigating sexual dimorphism in immune response mostly include inbred commercial strains. Considering that the influence of X chromosome inactivation on immune responses is lost in commercially available mouse and rat strains bred to homogeneity, the use of these animals in such type of studies is thought to be unsuitable [6].

The current study was aimed to get better insight in sex-related differences in humoral response to influenza vaccine. For this purpose, in adult outbred mice of both sexes the magnitude and dynamics of the total IgM and IgG and functional antibody responses to immunization with trivalent inactivated influenza vaccine (TIV) were examined. Additionally, considering differential contributions of distinct IgG antibody isotypes to protective immunity against influenza infection [8], IgG subclass profile was investigated. This seems to be important considering that, although cellular immune response is clearly important, most vaccines licensed today, including influenza vaccine, depend for their efficacy on serum antibodies [9]. Consequently the antibody response is used as surrogate marker for the vaccine efficacy [9]. Thus, the evaluation of serum levels of virus-specific antibodies offers a convenient and objective means in estimating sexual dimorphism not only in humoral immune response to vaccination, but also in the vaccine efficacy.

2. Materials and methods

2.1. Animals

Male and female outbred Swiss mice (8–10-weeks-old) bred under standard housing conditions in the Immunology Research Centre "Branislav Janković" animal facility were used. All experiments were conducted in accordance with the provisions of revised Appendix A of the European Convention ETS 123, and approved by Institutional animal Care and Use Committee (Approval number: 232-06-03742/2012-05).

2.2. Immunization

Mice were injected intramuscularly in each caudal thigh with either 50 μl of seasonal (composition of 2011–2012 season) whole TIV (provided by the Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia) or 50 μl of saline (control animals). The vaccine incorporated A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 (B) virus influenza strains and contained 10 μg (3.33 μg of each virus strain) of the virus surface protein HA.

All animals were monitored for (i) adverse local reaction to immunization, including redness, swelling or the formation of granulomas at the injection site, (ii) changes in external physical appearance, body temperature, body weight and food consumption, which indicate systemic reaction to immunization. There was no significant differences in any of the examined parameters between immunized and control mice.

Mice were bled from retro orbital sinus under ketamine–xylazine anesthesia (ketamine, 80 mg/kg Ketamidor, Richter Pharma AG, Wels, Austria; xylasine, 8 mg/kg Xylased, Bioveta, Ivanovice na Hané, Czech Republic) three and six weeks post-immunization (p.i.). For IgM serum level evaluation mice were bleed one week p.i. The blood was allowed to clot for 1 h at room temperature. Sera were separated by centrifugation at 3000 rpm for 30 min, heat-inactivated at 56 °C for 30 min and stored at −20 °C.

2.3. ELISA

Serum samples were examined for influenza virus strain-specific IgM, IgG, and IgG subclasses by ELISA. Briefly, ELISA plates were coated with 2.5 μg of HA/ml (50 μl per well) of inactivated H1N1 or H3N2 or B influenza virus (incorporated in TIV) diluted in PBS. After overnight incubation at 4 °C, the plates were washed three times with T-PBS and once with PBS and blocked for 1 h at 37 °C with 1% and 2% BSA, for IgG and IgM, respectively. Antigen-coated plates were washed and incubated with 50 μl of serial dilutions of sera samples in T-PBS containing 1% BSA for 1 h at room temperature. After washing, the plates were incubated with horseradish peroxidase-conjugated goat anti-mouse-IgM (1:5000), IgG (1:50,000) or individual IgG isotypes IgG1, IgG2a, IgG2b and IgG3 (1:1000) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) for 1 h at room temperature. The reaction was developed using o-phenylenediamine and H_2O_2 and stopped using 2 M H_2SO_4. OD was measured using Multiscan Ascent (Labsystems) at 490 and 620 nm. Titers were calculated as the reciprocal of the highest dilution of test sera that gives an OD of 3 standard deviations above the control sera.

2.4. HAI assay

Functional antibodies can be measured directly, using MN assay, or indirectly, using HAI assay, which detects antibodies targeting the sialic acid binding sites of the surface HA protein. Prior to being subjected to HAI assay, mice sera were treated with RDE (Denka Seiken Co. Ltd., Tokyo, Japan) to inactivate non-specific hemagglutination inhibitors. Briefly, 3 parts of RDE were added to 1 part of serum and incubated overnight at 37 °C. RDE-treated sera were inactivated by incubation at 56 °C for 30 min and then six parts of saline were added, creating a 1:10 dilution of serum. RDE-treated sera were two-fold serially diluted in V-bottom 96 wells microtiter plates and 25 μl of either H1N1 or H3N2 or B strain incorporated in TIV (adjusted to 4 HA units per 25 μl) was added to each well. After 15 min incubation at room temperature, 50 μl of 0.5% TRBCs were added and mixture incubated for 30 min at room temperature before evaluation of agglutination. HAI titer was recorded as the inverse of the last dilution that inhibited agglutination.

2.5. MN assay

MN assay was performed according to WHO protocol [10]. Firstly, 50% $TCID_{50}$ for each vaccine virus strain was determined. Serially diluted RDE-treated sera were pre-incubated with a 100 x $TCID_{50}$ of live virus prior to the addition of MDCK cells. After overnight incubation, the cells were stained with the crystal violet dye. MN titer was expressed as reciprocal of the highest dilution of serum that gave 50% neutralization of $100 \times TCID_{50}$ of virus in MDCK cells.

2.6. ELISPOT assay

To identify IFN-γ- and IL-4-secreting cells, ELISPOT assay was used. The assays were performed according to the manufacturer's instructions (R&D Systems, Minneapolis, USA). Briefly, 1×10^6 freshly isolated splenocytes from the control and TIV-immunized mice were added to the wells of plates pre-coated with anti-mouse IFN-γ or IL-4, and stimulated with 1 μg/ml of either H1N1 or H3N2 or B inactivated virus incorporated in TIV for 48 h in a 37 °C incubator with 5% CO_2. Biotinylated IFN-γ or IL-4 detection antibodies, streptavidin–alkaline phosphatase conjugate and BCIP/NBT substrate solution were used for detection. IFN-γ- or IL-4-secreting cells were quantified by counting spots under a dissecting

Please cite this article in press as: Živković I, et al. Sexual diergism in antibody response to whole virus trivalent inactivated influenza vaccine in outbred mice. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.09.006