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Impact of antigens, adjuvants and strains on sexually dimorphic antibody response to vaccines in mice

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ABSTRACT

Sexually dimorphic antibody response to vaccines has long been noticed. In addition to sex hormones, other factors such as antigens, adjuvants and strains of mice, as shown by indirect evidence, could also impact the sexual dimorphism. To clarify this, we immunized both gender mice of distinct strains with inactivated FMDV or HBsAg with or without adjuvants, and detected the specific antibody response of the mice. We found that in absence of adjuvants, the recombinant HBsAg but not the inactivated FMDV induced enhanced IgG antibody response in the female BALB/c mice. The o/w emulsion could facilitate the HBsAg to induce the comparable level of IgG antibodies in the male BALB/c mice as that in the females. The o/w emulsion rather than ISA206, a w/o/w emulsion, could assist the inactivated FMDV to induce higher levels of IgM antibodies in the female BALB/c mice. Moreover, the sexually dimorphic antibody response varied among the ICR, BALB/c and the F1 (BALB/c × C57BL/6) mice. Thus the data suggest that antigens, adjuvants and strains all impact the sexually dimorphic antibody response to vaccines and may provide insights for developing gender-based vaccines.

1. Introduction

Infectious diseases are a major cause of morbidity and mortality worldwide. Vaccines have historically been the most effective means to fight and eradicate infectious diseases by activating immune responses, mainly the antibody response [1]. The outcome of the antibody response to vaccination has been associated with gender and this phenomenon is referred as sexual dimorphism. In general, the females tend to develop a stronger antibody response than the males, exemplified by the evidence that the females produced higher level of antibodies when immunized with influenza, measles, hepatitis A, hepatitis B, tetanus [1], brucella [2], smallpox [3], rubella and mumps [4,5] vaccines. However, in certain circumstances, the males were found able to launch elevated antibody response to the influenza [6], measles [7], meningococcal A [8], and meningococcal C [9] vaccines. Naturally, the sexual dimorphism is attributed to sex hormones, which is supported by the data from animal studies and clinical observations, additionally to age and social and psychological factors.

Interestingly, clinical observations hint that antigens formulated in vaccines could also influence the sexual dimorphism of antibody response. Live attenuated rubella [4] and dengue [10] vaccines induced enhanced antibody response in the females, while live attenuated yellow fever [11] and venezuelan equine encephalitis virus vaccines [12] induced elevated antibody response in the males. Inactivated influenza and hepatitis A vaccines [1], or inactivated rabies vaccine [4] induced stronger antibody response in the females or males, respectively. Pneumococcal, meningococcal A [8], and meningococcal C [9] vaccines, with polysaccharide antigens, induced higher level of antibodies in the males. Hepatitis B virus (HBV) vaccine, a subunit vaccine consisting of recombinant hepatitis B virus surface antigen (HBsAg), only elicited weaker antibody response in the males [13].

Besides antigens, adjuvants may also impact the sexually dimorphic antibody response to vaccines, exemplified by indirect evidence obtained from inducing experimental allergic

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encephalomyelitis (EAE) in mice [14]. Complete Freund's adjuvant (CFA) emulsions burying mycobacterium tuberculosis and neuroantigen inside (Int-ENC-CFA) their vesicles induced higher incidence of EAE in female mice than in the males. In contrast, CFA with mycobacterium tuberculosis and neuroantigen on the external surface phase of their vesicles led to annihilation of the sexual dimorphism. Furthermore, in the female SJL/J mice but not the female B10.S/SgMcdJ mice, the higher rate of EAE incidence was induced by the Int-ENC-CFA [14]. Similarly, in inducing anaphylactic shock, the ovalbumin induced higher mortality rate in the female NC, C57BL/6, CFW, DDD and IDT mice, not in the 10 other strains; in inducing cutaneousr passive anaphylaxis and active systemic anaphylaxis, cephalothin (CET) elicited more severe symptoms in the female Hartley guinea pigs than that in the males, while elicited symptoms with equivalent severity in both of the males and females in Strain 2 guinea pigs [15]. These data obtained from the mice and the guinea pigs provide clues that the strains may affect the sexually dimorphic antibody response to vaccines.

Overall, however, the above-mentioned data are limited in clinical observations and indirect evidence from the experiments about the antigen/adjuvant-induced autoimmune disease and allergy, lacking designed experiments to observe how antigens, adjuvants and strains of mice impact the sexually dimorphic antibody response to vaccinations. In this study, we initially to test whether the oil-in-water (o/w) emulsion could enhance antibody response to inactivated foot-and-mouth disease virus (FMDV) vaccine, and noticed that the female ICR mice produced significantly higher levels of specific antibodies than the males. Upon this, we further observed how antigens, adjuvants and strains of mice affected the sexual dimorphism.

2. Materials and methods

2.1. Reagents

Recombinant HBsAg was kindly provided by Walvax Biotechnology Co. Ltd. (China). Inactivated FMDV was purchased from Baoling Biopharmaceutical Corporation. ISA206 was purchased from Seppic Company (France) and the o/w emulsion was prepared using mineral oil in our lab. HRP-conjugated goat anti-mouse IgG and IgM were from Santa Cruz Biotechnology, Inc.

2.2. Mice

Specific-pathogen-free (SPF) female and male BALB/c, ICR and F1 (BALB/c × C57BL/6) mice, aged 6–8 weeks, were purchased from the Experimental Animal Center, Jilin University (Changchun, China). All mice were maintained at about 22 °C with a 12 h light/dark cycle and had free access to food and water in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and with the approval of the Scientific Investigation Board of Science & Technology of Jilin Province.

2.3. Vaccines formulation

HBV vaccine were prepared by mixing HBsAg (20 μg/ml) with the o/w emulsion at a 1:1 (v/v) ratio and stored at 4 °C. FMDV vaccines were prepared by mixing inactivated FMDV (10 μg/ml) with ISA 206 or the o/w emulsion at a 1:1 (v/v) ratio and stored at 4 °C.

2.4. Animal experiments

Twenty female and seven male (or six female and six male) ICR mice were immunized with inactivated FMDV with or without the o/w emulsion on days 0 and 14, and bled on days 28 and 35, respectively. Six female and six male BALB/c mice (or F1 mice) in each group were immunized with inactivated FMDV with or without adjuvants (the o/w emulsion or ISA206)/HBsAg with or without the o/w emulsion on days 0 and 14, and bled on days 14, 21 and 28, respectively. Blood was drawn from the tail vein, centrifuged at 1250 g for 10 min, and the obtained sera were stored at −20 °C.

2.5. Indirect enzyme-linked immunosorbent assay (ELISA)

Briefly, 96-well plates were coated with recombinant HBsAg protein at 1 μg/well (Walvax Biotechnology Co. Ltd, China) or inactivated FMDV at 1 μg/well (Baoling Biopharmaceutical Corporation, China) in carbonate/bicarbonate buffer (PH 9.6) at 4 °C overnight and blocked using 200 μl of PBS supplemented with 5% dried skimmed milk at 37 °C for 1 h. The test sera were 1:100 diluted with PBST (PBS containing 0.05% Tween 20) and added to the plates (100 μl/well), followed by incubating at 37 °C for 1 h. After being washed with PBST, 100 μl/well of goat anti-mouse IgG/HRP or goat anti-mouse IgM/HRP (1:5000 dilution, Santa) was added. The plates were incubated at 37 °C for 1 h. After being washed with PBST, 50 μl/well of OPD substrate was added to the plates. After 15 min at 37 °C in dark, the enzyme–substrate reaction was stopped by adding 50 μl/well of 1.25 M sulphuric acid and absorbance was read at 492 nm. Antibody levels were expressed by OD_{492nm} value as described [16]. Alternatively, the antibody levels were also expressed as end point titers. For doing this, the test sera and preimmunization sera were serially 2-fold diluted from 1:100 to 1:12800, and then tested by ELISA to obtain the corresponding OD_{492nm} values. The endpoint antibody titers were defined as the reciprocals of the highest dilutions of sera required to yield an OD_{492nm} value three times that seen with an equal dilution of preimmunization sera as previously described [17].

2.6. Statistical analysis

Data analysis was performed using T-tests with SPSS software (version 19.0, SPSS Inc., Chicago, IL). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Phenomenon of the sexually dimorphic antibody response to inactivated FMDV vaccine in ICR mice

The initial purpose of our study is to test whether the o/w emulsion could replace ISA206, a widely used water-in-oil-in-water (w/o/w) emulsion, to formulate vaccines against foot-and-mouth disease (FMD), one of the most serious viral infections in livestock in many Asian and South American countries [18], since ISA206 is too viscous to inject animals and sometimes can induce local and general reactions, like granuloma, abscesses or fever [18]. To examine the efficacy of the o/w emulsion, 20 female ICR mice were immunized with the o/w emulsion formulated inactivated FMDV vaccine twice on days 0 and 14. On days 28 and 35, the mice were bled to collect sera for testing anti-FMDV antibodies by ELISA. By the way, 7 male ICR mice were also immunized with the o/w emulsion formulated inactivated FMDV vaccine to test if the o/w emulsion could also function well in the males (Fig. 1A). Interestingly, we noticed that the o/w emulsion formulated inactivated FMDV vaccine induced higher levels of antibodies in the female ICR mice than those in the males on days 28 and day 35, respectively ($P = 0.0214$ and $P = 0.0361$) (Fig. 1B). Considering that much higher number of the female mice used for immunization may bias the