Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Conventional influenza vaccines influence the performance of a universal influenza vaccine in mice

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ARTICLE INFO

Article history:
Received 11 August 2017
Received in revised form 3 November 2017
Accepted 22 November 2017
Available online xxxx

Keywords:
Influenza
Universal vaccine
Mucosal
Recombinant adenovirus

ABSTRACT

Universal influenza vaccines are designed to protect against diverse strains of influenza virus. Preclinical testing of new vaccine candidates is usually done in naïve animals, despite intended use in the human population with its varied immune history including responses to previous vaccinations. As an approach more relevant to human use, we tested a candidate universal influenza vaccine in mice with a history of conventional vaccination. Female BALB/c mice were given two intramuscular doses of inactivated influenza vaccine (IIV) or diphtheria and tetanus toxoids vaccine (DT), one month apart. Another group was given two intranasal doses of live attenuated influenza virus (LAIV). One month after the second dose, mice were given the universal influenza vaccine: recombinant adenoviruses expressing influenza A nucleoprotein (A/NP) and matrix 2 (M2) (A/NP + M2-rAd). Immune responses to universal vaccine antigens A/NP and M2 were assessed by ELISA and interferon-γ ELISPOT. Protection was tested by challenge with mouse-adapted A/FM/1/47 (H1N1) and monitoring for weight loss and survival. Universal vaccine performance was enhanced, inhibited or unaffected by particular prior vaccinations. Mice given Afluria IIV and LAIV had greater antibody and T-cell response to A/NP than mice without prior vaccination, providing examples of enhanced A/NP + M2-rAd performance. Though Fluvirin IIV partially inhibited, the universal vaccine still provided considerable protection unlike conventional vaccination. Fluzone IIV and DT had no effect on A/NP + M2-rAd performance. Thus our results demonstrate that universal vaccine candidate A/NP + M2-rAd was at least partially effective in mice with diverse prior histories. However, the degree of protection and nature of the immune responses may be affected by a history of conventional vaccination and suggests that performance in humans would be influenced by immune history.
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1. Introduction

Influenza remains a significant disease burden. In the United States, estimates attribute about 300,000 hospitalizations and 12,000 deaths to seasonal influenza during the 2015–2016 season [1]. Seasonal influenza epidemics are partially controlled by strain-matched vaccines based on strains selected from those circulating in the population [2,3]. However, current vaccines take months to prepare, leaving the public unprotected from novel influenza viruses that might emerge due to an antigenic shift. Thus, it is important to develop tools for control of influenza that would be readily available in the event of a pandemic.

Universal influenza vaccines aim to protect broadly against widely divergent influenza virus strains. Universal vaccines are designed to induce immune responses to conserved influenza antigens such as nucleoprotein (NP) and matrix (M), or epitopes including the HA stem. DNA, recombinant proteins, peptides or viral vectors are used as expression systems [4–11]. Previously we have developed a recombinant adenoviral (rAd) vaccine that expresses A/NP and matrix 2 (M2). This candidate universal vaccine controls viral replication, protects mice from severe disease [12,13] and reduces transmission [14].

Often new vaccines are tested in naïve animals with no prior vaccination history. Laboratory mice are housed in specific-pathogen free environments, which may influence their immune responses [15]. In contrast, humans are not immunologically naïve, but rather have varied antigen exposure histories which might alter responses to subsequent vaccination [16–18]. In the United States, annual vaccination against seasonal influenza is recommended. Other vaccines, including diphtheria-tetanus toxoids

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https://doi.org/10.1016/j.vaccine.2017.11.065
0264-410X/Published by Elsevier Ltd.

vaccine, are recommended at longer intervals. In this study, we examined whether prior vaccination alters immune responses or protection elicited by the universal influenza vaccine in mice with prior histories of exposure to seasonal influenza vaccines or diphtheria-tetanus toxoids vaccine.

2. Materials and methods

2.1. Animals

Female BALB/cAnNCR (BALB/c) mice were purchased at 6–8 weeks of age from the Division of Cancer Treatment (NCI, Frederick, MD) managed by Charles River Laboratories. All animal protocols were approved by the FDA Animal Care and Use Committee and conducted in animal facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines.

2.2. Conventional vaccines

As detailed in Table 1, we used the following vaccines obtained through the US Army Medical Material Agency: FluMist® Quadrivalent Influenza Vaccine, Live-attenuated, 2015–2016 Formula (MedImmune, Gaithersburg, MD, USA); Afluria® Influenza Vaccine, 2015–2016 Formula (bioCSL Pty Ltd., Parkville, Victoria, Australia); Fluvirin® Influenza Virus, Purified Surface Antigen Vaccine, 2015–2016 Formula (Novartis Vaccines and Diagnostics Ltd., Speke, Liverpool, UK; later purchased by Sequirus); Fluzone® Quadrivalent Influenza Virus Vaccine, 2015–2016 Formula (Sanofi Pasteur Inc., Swiftwater, PA, USA); and Tenivac® Tetanus and Diphtheria Toxoids Adsorbed Vaccine (Sanofi Pasteur Ltd., Toronto, ON, Canada). The following vaccines were obtained through BEI Resources, NIAID, NIH: Fluvirin® Influenza Virus, Purified Surface Antigen Vaccine, 2007–2008, NR-10475; Fluzone® Influenza Virus Vaccine, 2006–2007 Formula, NR-10483 and NR-10482; and Afluria® Influenza Virus Vaccine 2008–2009 Formula, NR-17598.

2.3. Recombinant adenoviral (rAd) vaccines

Adenoviruses expressing influenza A nucleoprotein from A/PR/8 (A/NP-rAd), influenza A matrix 2 consensus sequence (M2-rAd), or influenza B nucleoprotein (B/NP-rAd) from B/Ann Arbor/1/86 have been previously described [4,14,19]. B/NP-rAd was used as an antigen-specificity control.

2.4. Vaccination and challenge infection

Female BALB/c mice were given intramuscular inactivated influenza vaccine (IIV, multi- and single-dose formulations) or diphtheria tetanus toxoid (DT) vaccine at 8–10 weeks of age. Each IIV dose contained 3 μg of hemagglutinin (HA) of each vaccine strain in 100 μL. For DT vaccine, the dose was 1 limit of flocculation (Lf) tetanus toxoid and 0.4 Lf diphtheria toxoid in 100 μL. Live attenuated influenza virus (LAIV) was given in an undiluted 50 μL intranasal (i.n.) dose of 7.9×10^5 to 7.9×10^6 fluorescent focus units. Four weeks later, mice were boosted with the same IIV, DT vaccine or LAIV. Four weeks after the boost, mice were given 10^{10} total particles of rAd (5×10^9 A/NP-rAd and 5×10^9 A/M2-rAd) i.n. under isoflurane anesthesia. Antigen-specificity controls received 10^{10} particles of B/NP-rAd for comparison with A/NP + M2-rAd. Four weeks after rAd administration, mice were challenged with 5.6×10^4 $TCID_{50}$ mouse-adapted A/Fort Monmouth/1/47 (H1N1) [A/FM] [20] and monitored for weight loss and survival. Mice with 25% loss of initial body weight were euthanized.

2.5. Immune responses to rAd vaccine

Sera were collected 2 and 3 weeks post immunization with A/NP + M2-rAd. Bronchoalveolar lavage (BAL) fluid was collected 3 weeks post immunization. In sera and BAL, total IgG, IgG1, IgG2a or IgA antibodies were measured by ELISA using plates coated with recombinant NP from strain A/PR/8 or M2 ectodomain peptide 2–24 (M2e) as previously described [6,21].

Three weeks post immunization with rAd, lung and spleen cells from individual mice (n = 2–3/group) were assessed for interferon-γ (IFN-γ) or interleukin-4 (IL-4) production in response to peptides SARS $M_{209-221}$ (SARS), $NP_{147-155}$ (NP147), NP_{55-69} (NP55) and M2e by enzyme-linked immunospot (ELISPOT) as described [12,19].

2.6. Gel electrophoresis and western blotting

Vaccine samples in 4x Sample Buffer and reducing agent (BioRad, Hercules, CA, USA) were heated at 95 °C for 10 min. Chameleon® duo pre-stained protein ladder (LI-COR, Lincoln, NE, USA) was used as a size reference. Precast 4–12% Bis-Tris NuPAGE (Invitrogen, Carlsbad, CA, USA) gels were loaded with NP at different concentrations, rHA, and 19.7 μL of vaccines in buffer and electrophoresed. For staining, gels were fixed with 50% methanol, 7% acetic acid solution, incubated overnight at room temperature (RT) with SYPRO® Ruby (Invitrogen), washed for 30 min with 10% methanol, 7% acetic acid solution, and imaged using ChemiDoc XRS + imaging system (Bio-Rad).

For western blotting, proteins were transferred to nitrocellulose membranes using the iBlot system (Invitrogen) at 30 V for 7 min. Membranes were blocked for 1 h at RT with Odyssey® blocking buffer (LI-COR), incubated overnight at 4 °C with rabbit polyclonal antibody specific to influenza A/NP (GTX125989, GeneTex, Irvine, CA, USA) diluted 1:5000 in blocking buffer, washed, and incubated with donkey anti-rabbit antibody conjugated to IRDye 680 (LI-COR). Blots were visualized using the Odyssey imaging system (LI-COR).

2.7. Statistical analysis

All statistical analyses and area under the curve calculations were performed in Sigma Plot (Systat Software, San Jose, CA,

Table 1
Influenza vaccines.

Brand Name	Year	Vaccine type	Inactivation method
FluMist (quadrivalent, single-dose formulation)	2015/16	Live, attenuated	None
Afluria (trivalent, single-dose formulation)	2015/16	Split-virus	Beta-propiolactone
Fluvirin (trivalent, single-dose formulation)	2015/16	Sub-unit	Beta-propiolactone
Fluzone (quadrivalent, single-dose formulation)	2015/16	Split-virus	Formaldehyde
Fluvirin (trivalent, multi-dose formulation)	2007/08	Sub-unit	Beta-propiolactone
Fluzone (trivalent, multi-dose formulation)	2006/07	Split-virus	Formaldehyde
Fluzone (trivalent, single-dose formulation)	2006/07	Split-virus	Formaldehyde
Afluria (trivalent, single-dose formulation)	2008/09	Split-virus	Beta-propiolactone

Please cite this article in press as: Rowell J et al. Conventional influenza vaccines influence the performance of a universal influenza vaccine in mice. Vaccine (2017), https://doi.org/10.1016/j.vaccine.2017.11.065