Universal Influenza Vaccine Based on Conserved Antigens Induces Broad, Long-Term Immunity and Protection

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Abstract

A universal influenza vaccine based on antigens that change little from year to year could protect against influenza pandemics. NIH has recommended that a universal influenza vaccine provide protection against groups 1 and 2 influenza A viruses for at least a year. However, most animal studies of universal vaccines test protection a short time after vaccination. Unlike hemagglutinin (HA) and neuraminidase (NA), influenza nucleoprotein (NP) and matrix 2 (M2) change slowly and could protect against new virus strains when HA- and NA-matched vaccines are not available. This study extends the interval between vaccination and challenge, to test durability of immunity and broad protection. Vaccines used were replication-deficient adenoviral vectors (rAd) expressing NP of influenza A or B viruses, or M2 of influenza A, given intranasally. Antigen specific antibodies and T-cells persisted for one year. Long-term immunity protected mice against lethal challenge with H1N1, H3N2, or influenza B challenge viruses. Protection was weaker against highly pathogenic H7N9 avian influenza virus but was improved by boosting one year after priming. Thus, candidate universal influenza vaccines based on NP and M2 protect against a wide variety of influenza virus strains for at least a year after a single dose. Such vaccines could be deployed off-the-shelf whenever a novel strain with pandemic potential emerges. While this universal vaccine does not eliminate infection, it could dramatically reduce viral loads, illness, death, and transmission, during an unexpected outbreak or pandemic.

Introduction

Vaccination to respiratory virus infections is a major public health issue, made more difficult by emergence of new viruses and evolution of circulating viruses. Conventional influenza vaccines are intended to induce antibody responses to hemagglutinin (HA) and neuraminidase (NA). Preparedness for influenza outbreaks would be greatly enhanced by vaccines that could provide broad protection against a wide range of influenza viruses. Such a vaccine, termed a universal influenza vaccine, should protect against diverse strains and should also provide long-term protection.

The responses to universal influenza vaccine candidates based on conserved antigens, including nucleoprotein (NP), matrix 2 (M2), and the HA stem are not all neutralizing or sterilizing, but permit mild, transient infection. In animal studies, A/NP and M2 vaccination provides broad short-term protection against influenza A viruses with widely divergent HA and NA, including highly pathogenic avian H5N1 viruses. A single intranasal dose can provide long-term protection.

For adenovirus constructs given mucosally, local T cell memory in the lungs and protection against challenge are known to persist. In this study, we examined durability and breadth of protection and immune responses a full year after a single dose vaccination. This study includes vaccination against influenza A (using A/NP and M2 as target antigens) and influenza B (using B/NP as target antigen). We also investigated responses to new antigens expressed from the same adenoviral vector at this long interval after priming to test feasibility of vector reuse, and responses to boosting with the same antigen.

Materials and Methods

Animals and immunization

6-8 weeks old BALB/c mice were immunized intranasally in 50 µl with 5×10^9 viral particles (v.p.) each of A/NP-rAd and M2-rAd mixed together, 5×10^9 v.p. of A/NP-rAd or M2-rAd separately, 1×10^{10} v.p. of B/NP-rAd, or in some experiments with A/NP-rAd or empty-rAd at 1×10^{10} v.p.

Challenge Viruses

Influenza B/Texas/06/2011 and B/Malaysia/2506/2004;
H1N1: mouse adapted(ma) A/Fort Monmouth/1/47, A/Mexico/4108/2009;
H3N2: ma A/Philippines/2/82 × A/PR/8/34 reassortant (X-79);
H7N9: A/Guangdong/17SF003/2016

Peptides and proteins used for ELISA and (IFN-γ) ELISPOT

$NP_{147–155}$, $NP_{55–69}$, M2-ectodomain$_{2–24}$ consensus sequence (M2e), H5N1 M2e, H7N9 M2e, adenovirus-5 hexon ($Hex_{486–494}$), B/NP peptide pools and recombinant A/NP and B/NP proteins were produced by GenScript.

Statistical analyses were performed using SigmaPlot for Windows version 14.

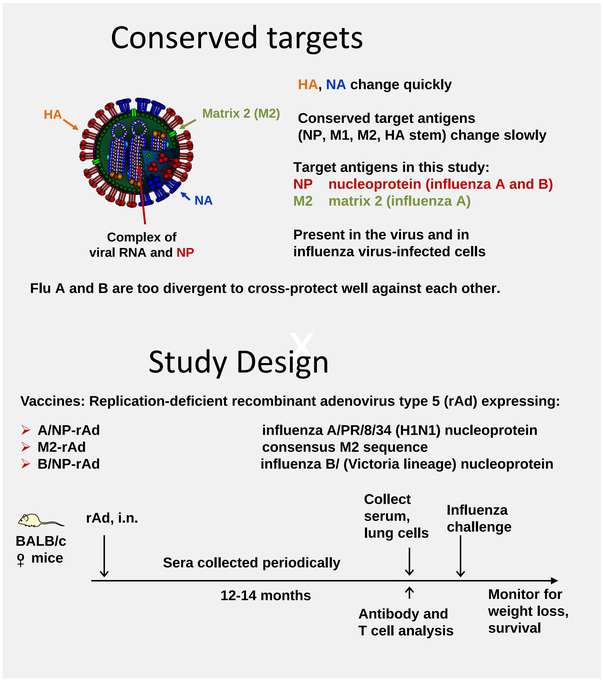


Figure 1. Conserved vaccine antigens and design of these studies

Results and Discussion

One year after a single dose of intranasal rAd expressing influenza antigen, antibodies to the vaccine antigens A/NP, A/M2 and B/NP persisted in serum and BAL. Also, INF-γ secreting cells in the lung specific for these antigens were detected by ELISPOT. This long-term immunity induced by A/NP alone or with M2, protected mice against lethal challenge with diverse influenza A viruses, H1N1 or H3N2. Protection was weaker against highly pathogenic H7N9 avian influenza virus but was improved by boosting one year after priming. Immunization with B/NP from the Victoria lineage protected against mismatched challenge with virus of the Yamagata lineage.

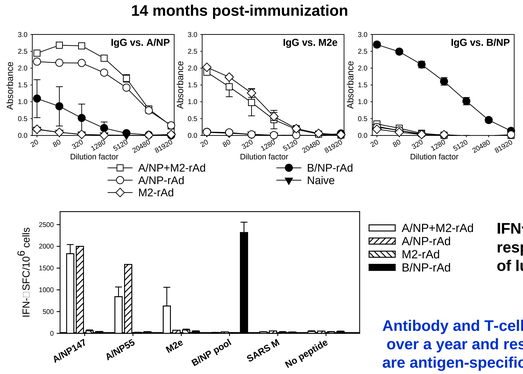


Figure 2. Antibody and T cell responses one year after single-dose, intranasal vaccination

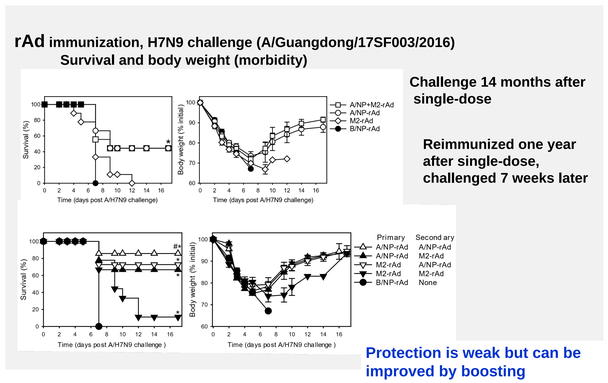
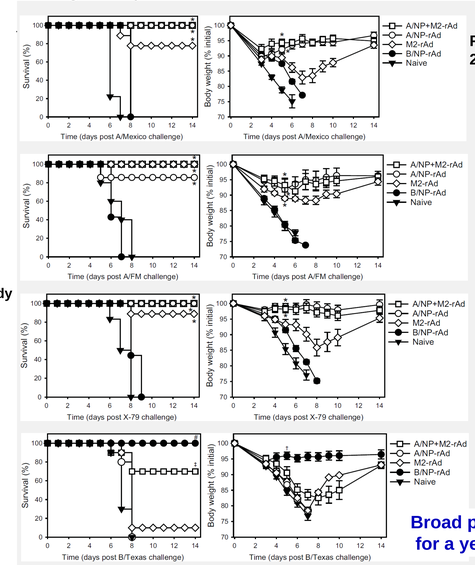


Figure 4. Protection against highly pathogenic H7N9 influenza A virus.
Top panel: Mice were immunized with a single intranasal dose of indicated vaccines and challenged 14 months later with H7N9.
Bottom panel: mice were immunized with an intranasal dose of indicated vaccines, boosted one year later, and challenged 7 weeks after the boost.

Fig 3. Durability and breadth of protection one year after vaccination. Mice were immunized with a single intranasal dose of indicated vaccines and challenged one year later with diverse virus strains.



Conclusion

Universal influenza vaccines could be used “off the shelf” as a first line of defense early in an outbreak, when strain-matched vaccines are not available, and perhaps in conjunction with strain-specific vaccines. They can reduce illness, death, and viral titers. Despite absence of neutralizing antibodies such vaccines might reduce transmission, limiting the size of outbreaks. NP+M of Flu A and B/NP could be combined with an HA-stem component. The ability to boost the response in mice using the same vector as the primary vaccination indicates that at least for this late time point the immune response to vector does not block its ability to induce a response to the influenza antigen. Similar approaches can be explored for other rapidly evolving respiratory viruses with this and other platforms expressing conserved antigens.

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