Published in final edited form as:

Vaccine. 2009 August 27; 27(39): 5344–5348. doi:10.1016/j.vaccine.2009.06.084.

Contrasting Effects of Type I Interferon as a Mucosal Adjuvant for Influenza Vaccine in Mice and Humans

Robert B. Couch,

Department of Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS: BCM280, Houston, TX 77030, 713-798-4474 o, 713-798-8344 f, ikirk@bcm.edu

Robert L. Atmar,

Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-6849 o, 713-798-6802 f, ratmar@bcm.edu

Thomas R. Cate,

Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798- o, 713-798-6802 f, tcate@bcm.edu

John M. Quarles,

Dept. of Microbial and Molecular Pathogenesis, 407 Joe H Reynolds Medical Building, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843-1114, 979-845-1358 o, 979-845-3479 f, quarles@medicine.tamhsc.edu

Wendy A. Keitel,

Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-5250 o, 713-798-6802 f, wkeitel@bcm.edu

Nancy H. Arden,

Dept. of Microbial and Molecular Pathogenesis, 407 Joe H Reynolds Medical Building, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843-1114, 979-845-1358 o, 979-845-3479 f, quarles@medicine.tamhsc.edu

Janet Wells,

Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-5250 o, 713-798-6802 f, jwells@bcm.edu

Diane Niño, and

© 2009 Elsevier Ltd. All rights reserved.

Corresponding Author: Robert B. Couch, M.D., Department of Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza, MS: BCM280, Houston, TX 77030, 713-798-4474 o, 713-798-8344 f, ikirk@bcm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest:

Robert B. Couch, no conflict.

Robert L. Atmar, no conflict

Thomas R. Cate, no conflict

John M. Quarles, no conflict

Wendy A. Keitel, no conflict

Nancy H. Arden, no conflict

Janet Wells, no conflict

Diane Niño, no conflict

Philip R. Wyde, no conflict

Baylor College of Medicine, One Baylor Plaza, MS: BCM 280, Houston, TX 77030, 713-798-5250
o, 713-798-6802 f, dnino@bcm.edu

Philip R. Wyde

5366 River Oaks Drive, Kingsland, TX 78639, 325 388-8692, no fax, pwyde@dishmail.net.

Abstract

To identify an adjuvant that enhances antibody responses in respiratory secretions to inactivated influenza virus vaccine (IVV), a comparison was made of responses to intranasal vaccinations of mice with IVV containing monophosphoryl lipid A (MPL), type I interferon (IFN) or cholera toxin B (CTB). Antibody in nasal secretions and lung wash fluids from mice was increased after vaccination and lung virus was significantly reduced after challenge to a similar level in each adjuvant group. Interferon was selected for a trial in humans. Trivalent inactivated influenza vaccine was given intranasally to healthy adult volunteers alone or with one million units (Mu) or 10 Mu of alpha interferon. Vaccinations were well tolerated but neither serum hemagglutination-inhibiting nor neutralizing antibody responses among the vaccine groups were significantly different. Similarly, neither neutralizing nor IgA antibody responses in nasal secretions were significantly different. Thus, despite exhibiting a significant adjuvant effect in mice, interferon did not exhibit an adjuvant effect for induction of antibody in respiratory secretions of humans to inactivated influenza virus vaccine given intranasally.

Keywords

Inactivated influenza vaccine; mucosal antibody; adjuvants; interferon; mice and humans

1. Introduction

There is a need to improve the efficacy of inactivated influenza vaccines for seasonal influenza (1). Current inactivated vaccines are given intramuscularly (IM) and induce serum antibody that is primarily immunoglobulin G (IgG) (2). Available information indicates that this is the major type of immunoglobulin (Ig) and antibody to influenza virus in lower respiratory tract secretions after vaccination (2,3). Antibody to influenza virus in upper respiratory tract secretions may be mostly IgG after IM vaccination even though the predominant Ig in the upper tract is IgA (4). Because influenza virus infections of humans involve both the upper and lower respiratory tract mucosa, it is desirable to optimize antibody responses to influenza viruses at the mucosal surface of both sites (5). Since serum IgG antibody is the major antibody response to parenteral (IM) immunization and is the major type of antibody in lower respiratory tract secretions, increasing that immune response can best be done by improving parenterally administered influenza vaccines. However, for optimizing immune responses in the upper respiratory tract, it is desirable to increase IgA antibody in secretions and this is best done by administering antigen to the nasopharyngeal mucosa (6,7).

Giving inactivated vaccine by the nasal route will induce IgA antibody to influenza viruses in nasal secretions (8). Additionally, increasing influenza vaccine dosages given intranasally (IN) will increase IgA antibody responses at this site (9). Another option for enhancing IgA antibody to influenza viruses in upper respiratory secretions is to administer vaccine IN along with a mucosal adjuvant. A variety of mucosal adjuvants have been shown to enhance IgA antibody responses to antigens administered intranasally in animal model systems and some have been shown to do so in humans (10-20). We compared monophosphoryl lipid A (MPL) and type I interferon (IFN) to cholera toxin B (CTB) as adjuvants for inactivated influenza vaccine in the mouse model of influenza. All three have been shown to be mucosal adjuvants for influenza vaccine in mice (12,13,17). All three exhibited adjuvant effects for inactivated influenza

vaccines of about equal magnitude. We selected type I interferon for evaluation in humans because of its commercial availability and our prior experience with intranasal administrations in studies of rhinovirus infections (21-23). This report presents a summary of experience in mice and in humans.

2. Materials and Methods

2.1 Evaluations in Mice

2.1.1 Vaccines, Viruses and Adjuvants—Vaccine used was an inactivated monovalent A/Texas/91 (H1N1) vaccine (kindly provided by Sanofi Pasteur, Inc.). Before use, the 50% immunogenic dose for two IM vaccinations a month apart was shown to be 0.1 µg of HA. The dosage chosen for IN immunizations was 0.3 µg HA; without adjuvant, only an occasional animal developed serum hemagglutination-inhibiting (HAI) antibody at this dosage. The 50% infection dose (ID₅₀) and 50% lethal dose (LD₅₀) for intranasal challenge with infectious A/Texas/91 virus were 10^{1.5} 50% tissue culture infectious doses (TCID₅₀) in MDCK cultures per 50 µl and 10^{2.5} TCID₅₀/50 µl, respectively. Challenge of vaccinated animals was with 100 ID₅₀ (10 LD₅₀); live virus “vaccination” was with <1 LD₅₀.

Adjuvants selected for comparison were CTB, MPL, and mouse type I IFN (Sigma Chemicals, Inc.). A titration of CTB and MPL was performed with IVV to select an adjuvant dosage that optimally enhanced antibody responses (5 µg CTB, 7.5 µg MPL) in animals; IFN was used undiluted.

2.1.2 Design—Mice were 6-9 week old ICR mice (Charles Rivers Laboratories). In an initial comparative evaluation, IFN did not enhance antibody responses or induce protection against challenge so a repeat experiment was conducted with INF of higher dosage. This experiment involved IN vaccinations on days 0 and 1 and on days 28 and 29 followed by challenge with IN live virus (100 ID₅₀) on day 42. Blood for antibody was obtained prevaccination (day 0), pre boost (day 28) and day 42 (challenge day). Nasal secretions (NS) and lung fluids (LF) were obtained on day 45 from unchallenged mice for antibody assays and LF from challenged animals for virus. Controls included an adjuvant mixture, vaccine without adjuvant and a sublethal dose of live virus given at day 0. Lung fluids were obtained by exsanguinating animals followed by lung removal and lavage; nasal secretions were obtained by removing the lower jaw and rinsing the nasal cavity with PBS.

2.1.3 Laboratory Assays—HAI and neutralizing antibody assays were done on sera as previously described (24). Nasal and lung fluids were tested for antibody in ELISA assays using whole virus as antigen (25).

2.2 Evaluations in Humans

2.2.1 Subjects—Healthy adults between 18 and 40 years were recruited to the study. To be enrolled, they must have been free of any acute or chronic illness that might interfere with reactogenicity or immunogenicity evaluations, be free of any nasal allergy history, not be pregnant if female, and not have received vaccine for at least one year. The protocol was reviewed and approved by the Baylor College of Medicine Institutional Review Board.

2.2.2 Vaccine and Adjuvant—Vaccine was the 2006-2007 formulation of trivalent influenza vaccine (sanofi pasteur) and contained 15 µg of HA of A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/205 (H3N2) and B/Malaysia/2506/2004-like viruses in 0.5 ml. Commercially available lyophilized IFN (Schering Inc., IFN α2b) was obtained as Intron A powder for injection in 10 million (M) and 50M unit vials and diluted with vaccine or sterile

PBS to provide 0.6 ml containing 0.5 ml of vaccine or 0.6 ml containing vaccine and one million units of (Mu) IFN or 0.7 ml containing vaccine and 10 Mu of IFN.

2.2.3 Design—Subjects were randomized to receive IVV alone (n= 32), IVV with 1 Mu of IFN (n=32), or IVV with 10 Mu of IFN (n=31). Blood and nasal wash specimens were obtained before and two and four weeks after immunization. A single vaccine or vaccine/interferon combination was administered slowly by the intranasal route (0.3 – 0.35 mL per nostril) not less than four hours after obtaining nasal wash specimens. Following vaccination, each subject was asked to complete a memory aid for seven days and to report any unexpected adverse effects (AEs). The subjects were also contacted six months after vaccination regarding occurrence of any unreported severe adverse effects (SAEs).

2.2.4 Laboratory Assays—Serum samples were evaluated for HAI and neutralizing antibody responses to the A/H1N1, A/H3N2, and B vaccine antigens as described previously (24,26). Nasal secretions were tested for the presence of neutralizing antibody and for IgA and IgG antibody to the A/H1N1 and A/H3N2 HA using ELISA tests and rDNA-produced HA as antigen (4).

2.2.5 Statistics—For mice, Anova (parametric) and Kruskal-Wallis (nonparametric) tests were used for comparing results for the different groups and logistic regression for correlations (Instat, GraphPad software). For humans, comparisons of groups utilized paired t tests, Chi square for trend, Anova, and linear and logistic regression statistics (SPSS, 14.0).

3.0 Results

3.1 Mouse Evaluations

After initial evaluations were conducted to select dosages and conditions, (see M and M) an experiment was done with vaccinations on day 0 and 1 and day 28 and 29 with the vaccine/adjuvant dosage given as one-half of the total on the two successive days (total 0.3 µg HA, 5 µg CTB, 7.5 µg MPL) except for IFN which was given as 10,000 u in each vaccination (total 40,000 u for the four doses). As shown in Table 1, serum neutralizing antibody responses were similar for live virus and the three adjuvant groups and were significantly greater than the GMT for mixed adjuvant and vaccine with PBS ($P < 0.001$, parametric Anova). Serum HAI antibody responses were similar (not shown). Mean O.D. for ELISA antibody in nasal wash specimens were significantly greater than mixed adjuvant for the live virus, CTB, and IFN groups but not for the MPL group ($p < .05$); only the live virus group was significantly greater than the vaccine alone group ($p < .05$; parametric Anova). In lung fluids, significantly more antibody was detected in the live virus and all three adjuvant groups than the mixed adjuvant group ($p < .01$); but, only the live virus and MPL groups were significantly greater than vaccine alone ($p < .01$; parametric Anova). Mean lung virus titers three days after challenge were significantly lower for the live virus and all adjuvant groups than for either the mixed adjuvant or vaccine alone groups ($p < .001$, parametric Anova).

Serum HAI and neutralizing antibody titers were inversely related to lung virus titers ($p < 0.001$; logistic regression, data not shown). Similarly, ELISA antibody concentrations in NS and LF were inversely related to lung virus titers ($p < .001$; logistic regression).

3.2 Human Evaluation

Ninety-five enrolled subjects were randomized to receive IVV, IVV with 1 Mu of IFN or IVV with 10 Mu of IFN intranasally. Reactogenicity in the week following vaccination is shown in Table 2. There were no statistically significant differences in the frequency of moderate local or systemic reactions between groups; however, combined moderate and severe systemic

reactions exhibited an increase with increasing IFN dosage ($p = 0.047$, X^2 for trend; $p = 0.026$, logistic regression).

All vaccination groups developed significant increases in serum antibody to each vaccine component following vaccination (Table 3, $p < .05$, paired t tests). The magnitude is similar to those reported by others for IN vaccinations with aqueous vaccine (8,15,27-29). A suggestion of decreased fold increase in antibody with increasing IFN dosage was not statistically significant when the analysis was controlled for prevaccination antibody titers ($p > 0.05$, linear regression).

Very few IgA or IgG antibody responses were detected in nasal wash samples; neutralization tests yielded more increases (Table 4). The differences between groups were not statistically significant for either HA subtype or assay ($p > 0.05$, X^2 for trend and logistic regression).

4. Discussion

The present studies sought to identify a mucosal adjuvant that would enhance the antibody response to seasonal inactivated influenza vaccines at the respiratory mucosal surface of humans so as to increase protection against influenza. Comparative studies in mice had indicated that type I interferon and an MPL adjuvant could increase mucosal antibody responses over those of vaccine alone and to a level similar to CTB, a known potent mucosal adjuvant (30). MPL, CTB, and INF had all been shown earlier to exhibit mucosal adjuvant activity in mice and both CTB and IFN had been shown to exhibit adjuvant activity with IVV as well as to enhance protection against challenge with influenza virus (12,17). Contributing to the adjuvant selection for a clinical trial was the considerable experience available with Type I IFNs given intranasally to human volunteers in studies of rhinovirus infection by us and others with a variety of dosages (21-23). A consideration of this experience, the demonstrated value of IFN as an adjuvant for IVV in mice, and the availability of preparations suitable for administration to humans caused us to select IFN for a clinical trial for adjuvant effects when given with IVV intranasally.

An increase in dosage of IFN was required for demonstrating an adjuvant effect in mice. We used dosages of 1 to 10 Mu daily in our rhinovirus studies in humans but dosages as high as 40 Mu per day were used (23). Rhinorrhea, sometimes blood-tinged, appeared in those studies but only when higher dosages were continued for several days (21-22). In the present study, there was no significant increase in local reactions but a significant increase in systemic complaints occurred for the 10 Mu dosage for combined moderate and severe reaction frequencies. Nevertheless, the overall reactogenicity was clinically acceptable. However, no increase in either the serum or secretion antibody responses to the vaccine components was demonstrable for either of the IFN groups over those for the vaccine only group. Thus, the mucosal adjuvant effect of IFN given with IW in mice was not seen in this clinical trial.

Previous comparative studies by us had shown adjuvant effects with IVV given IM to mice; the adjuvant QS21 was superior to MPL and incomplete Freund's adjuvant for increasing serum antibody responses in both "primed" and "unprimed" mice (31). However, in a clinical trial, responses to IVV with QS21 IM were not superior to those of vaccine alone (32). Thus, the mouse did not prove to be a reliable animal for predicting adjuvant value for humans for either systemic or mucosal antibody responses to IVV in our studies.

The experience with IFN as a mucosal adjuvant in humans differs from those reported with a toxigenic enterotoxin of *E. coli* given IN with IVV to humans. The heat-labile enterotoxin B subunit reportedly enhanced both serum HAI and salivary IgA antibody following two intranasal vaccinations with a seasonal IVV over that of vaccine alone; vaccine dosage was not given in the report (33). Combining a nontoxigenic *E. coli* enterotoxin with a bioadhesive

delivery system and a trivalent IVV containing an influenza A/H5N3, A/H3N2, and B component led to increases in serum and nasal secretion IgA antibody in the group given 7.5 µg of HA twice IN with the highest adjuvant dosage (34). Potential reasons for success with enterotoxin and not IFN include number of doses, vaccine dosage, and greater potency of *E. coli* enterotoxin as a mucosal adjuvant for humans. We gave vaccine containing 15 µg of the HA of each component in a trivalent vaccine once; the dosage in the nontoxigenic enterotoxin trial was 7.5 µg of HA of each component given twice a week apart. Vaccine was given twice a month apart in the toxigenic enterotoxin trial but vaccine dosage was not given. We considered giving vaccine twice since antibody responses were seen in mice only in the CTB and live infection groups after one dose (data not shown). A priming effect of IFN for increased responses to an inducer is well known; perhaps the HA with IFN dose primed for an increased antibody response that would have been seen after a second dose (35). Furthermore, two doses of IN vaccine have been reported as more immunogenic in humans than one dose (15,19,29, 36). We wished, however, to evaluate a potentially useful regimen and a requirement for two doses of seasonal vaccine did not seem practical. We also thought that the two IN doses with IFN might be required for mice to increase antibody since they are “unprimed” for influenza virus antigens, unlike adults who are “primed.” All study subjects had prevaccination serum antibody to the A/H3N2 virus and only five lacked antibody to the A/H1N1 virus. “Unprimed” to influenza HA antigens is the circumstance for very young children and, apparently, for pandemic vaccines as two doses appear to be required for optimal responses to the HA of potential pandemic viruses (37,38). In the nontoxigenic *E. coli* enterotoxin trial, serum antibody was not increased to the A/H5N3 component but IgA antibody in secretions was increased (34). However, IgA antibody is known to exhibit crossreactivity to different HA subtypes and this could have been the basis for detecting IgA antibody to A/H5N3 (12,39,40).

In considerations of IFN for a clinical use, it should be acknowledged that the type I IFN pathways are proposed for a role in the induction of autoimmune disease in humans (41). Type I IFNs exhibit multiple biological activities, including serving as an intermediary in the adjuvant effects of well known adjuvants such as complete Freund's adjuvant and CpG (17). Thus, any demonstration of a beneficial effect must also entail careful evaluations for a detrimental effect. Since no beneficial effect was seen in the present study, a search for detrimental effects of using IFN becomes moot.

The experience with IN vaccinations with inactivated influenza vaccine with and without adjuvants has been varied. Other trials in humans with an adjuvant have also failed to identify an adjuvant effect (42,43). Nevertheless, the overall experience with intranasal administration of inactivated influenza virus vaccines has yielded some characteristics of the approach: 1) IN vaccination is a well tolerated procedure in all age groups, 2) serum antibody is regularly elicited although generally to a lesser degree than by IM vaccinations, 3) the IN route is superior to the IM route for inducing secretory IgA antibody in respiratory secretions, 4) increasing dosage of antigen and number of vaccine doses of vaccines with ≤ 20 µ of HA frequently induce greater antibody responses, 5) some adjuvants given IN with vaccine appear capable of enhancing mucosal antibody responses. However, it seems appropriate to conclude at present that an optimal vaccine preparation and regimen for IN vaccinations with inactivated vaccine has not yet been identified. Nevertheless, the potential value of enhancing immune responses at the mucosal level for prevention of influenza is sufficient to support continued efforts in development of IN vaccinations.

5. Conclusions

In summary, preclinical screening in mice for selection of adjuvants for use with IM or IN administered seasonal IVV to humans was not useful for predicting value of the adjuvants compared for humans. Thus, translation of adjuvant effects for IVV in mice to humans must

be regarded as unpredictable. A more reliable preclinical system for predicting value in humans is desirable because improved inactivated influenza virus vaccines are needed and adjuvants are one option for achieving this goal.

Acknowledgments

Financial support: Research performed by the authors and summarized in this report was supported by Public Health Service Contract NO1-AI-30039 from the National Institute of Allergy and Infectious Diseases.

References

1. Couch RB. Seasonal inactivated influenza virus vaccines. *Vaccine* 2008;26S:D5–D9. [PubMed: 18602728]
2. Couch, RB. An overview of serum antibody responses to influenza virus antigens. In: Brown, F.; Haaheim, LR.; Wood, JM.; Schild, GC., editors. *Developments in Biologicals: Laboratory Correlates of Immunity to Influenza*. Vol. 115. Karger; New York: 2003. p. 25-30.
3. Couch, RB.; Kasel, JA.; Six, HR.; Cate, TR.; Zahradnik, JM. Immunological reactions and resistance to infection with influenza virus. In: Stuart-Harris, C.; Potter, C., editors. *Molecular Virology and Epidemiology of Influenza*, Beecham Symposium. Academic Press; London: 1983. p. 119-153.
4. Keitel WA, Couch RB, Cate TR, Hess KR, Baxter B, Quarles JM, Atmar RL, Six HR. High doses of purified influenza A virus hemagglutinin significantly augment serum and nasal antibody responses in healthy adults. *J Clin Micro* 1994;32(10):2468–73.
5. Treanor, JJ. Influenza virus. In: Mandell, GL.; Bennett, JE.; Dolin, R., editors. *Principles and Practice of Infectious Diseases*. Vol. Sixth edition. Elsevier Churchill Livingstone; Philadelphia: 2005. p. 2060-85.
6. Ogra PL, Karzon DT, Righthand F, MacGillivray M. Immunoglobulin response in serum and secretions after immunization with live and inactivated poliovaccine and natural infection. *New Engl J Med* 1968;279:893.
7. Ogra PL, Karzon DT. Poliovirus antibody response in serum and nasal secretions following intranasal inoculation with inactivated poliovaccine. *J Immunol* 1969;102:15. [PubMed: 4303877]
8. Kasel JA, Rossen RD, Fulk RV, Fedson DS, Couch RB, Brown P. Human influenza: aspects of the immune response to vaccination. *Ann Intern Med* 1969;71:369–98. [PubMed: 4184389]
9. Atmar RL, Keitel WA, Cate TR, Munoz FM, Ruben F, Couch RB. A dose-response evaluation of inactivated influenza vaccine given intranasally and intramuscularly to healthy young adults. *Vaccine* 2007;25:5367–73. [PubMed: 17559990]
10. Tamura S, Asanuma H, Tomita T, Komase K, Kawahara K, Danbara H, et al. *Escherichia coli* heat-labile enterotoxin B subunits supplemented with a trace amount of the holotoxin as an adjuvant for nasal influenza vaccine. *Vaccine* 1994;12:1083–9. [PubMed: 7998417]
11. Levi R, Aboud-Pirak E, Leclerc C, Lowell GH, Arnon R. Intranasal immunization of mice against influenza with synthetic peptides anchored to Proteosomes. *Vaccine* 1995;13:1353–9. [PubMed: 8585293]
12. Tamura S-I, Funato H, Hirabayashi Y, Kikuta K, Suzuki Y, Nagamine T. Functional role of respiratory tract haemagglutinin-specific IgA antibodies in protection against influenza. *Vaccine* 1990;8(5): 479–85. [PubMed: 2251874]
13. Baldrige JR, Yorgensen Y, Ward JR, Ulrich JT. Monophosphoryl lipid A enhances mucosal and systemic immunity to vaccine antigens following intranasal administration. *Vaccine* 2000;18:2416–25. [PubMed: 10738099]
14. McCluskie MJ, Davis HL. Oral, intrarectal and intranasal immunizations using CpG and non-CpG oligodeoxynucleotides as adjuvants. *Vaccine* 2000;19:413–22. [PubMed: 11027803]
15. Glück U, Gebbers J-O, Glück R. Phase 1 evaluation of intranasal virosomal influenza vaccine with and without *Escherichia coli* heat-labile toxin in adult volunteers. *J Virol* 1999;73:7780–6. [PubMed: 10438868]

16. Westerink MAJ, Smithson SL, Srivastava N, Blonder J, Coeshott C, Rosenthal GJ. ProJuvant™ (Pluronic F127®/chitosan) enhances the immune response to intranasally administered tetanus toxoid. *Vaccine* 2002;20:711–23. [PubMed: 11738734]
17. Proietti E, Bracci L, Puzelli S, Di Pucchio T, Sestili P, De Vincenzi E, et al. Type I IFN as a natural adjuvant for a protective immune response : Lessons from the influenza vaccine model. *J Immunol* 2002;169:375–83. [PubMed: 12077267]
18. Halperin SA, Smith B, Clarke K, Treanor J, Mabrouk T, Germain M. Phase I, randomized, controlled trial to study the reactogenicity and immunogenicity of a nasal, inactivated trivalent influenza virus vaccine in healthy adults. *Human Vac* 2005;1:37–42.
19. Treanor J, Nolan C, O'Brien D, Burt D, Lowell G, Linden J, et al. Intranasal administration of a proteosome-influenza vaccine is well-tolerated and induced serum and nasal secretion influenza antibodies in healthy human subjects. *Vaccine* 2006;24:254–62. [PubMed: 16129526]
20. Mizuno D, Ide-Kurihara M, Ichinomiya T, Kubo I, Kido H. Modified pulmonary surfactant is a potent adjuvant that stimulates the mucosal IgA production in response to the influenza virus antigen. *J Immunol* 2006;176:1122–30. [PubMed: 16394001]
21. Samo TC, Greenberg SB, Couch RB, Quarles J, Johnson PE, Hook S, et al. Efficacy and tolerance of intranasally applied recombinant leukocyte A interferon in normal volunteers. *J Infect Dis* 1983;148(3):535–42. [PubMed: 6619578]
22. Samo TC, Greenberg SB, Palmer JM, Couch RB, Harmon MW, Johnson PE. Intranasally applied recombinant leukocyte A interferon in normal volunteers. II. Determination of minimal effective and tolerable dose. *J Infect Dis* 1984;150(2):181–8. [PubMed: 6381611]
23. Greenberg, SB.; Couch, RB. *The Interferon System, A Current Review*. University of Texas Press; Galveston, TX: 1986. Interferon for respiratory virus infections in man.
24. Robinson, RQ.; Dowdle, WR. Influenza viruses. In: Lennette, EHSNJ., editor. *Diagnostic Procedures for Viral and Rickettsial Infections*. American Public Health Association, Inc.; New York: 1969. p. 414-33.
25. Chen D, Periwal SB, Larrivee K, Zuleger C, Erickson CA, Endres RL, et al. Serum and mucosal immune responses to an inactivated influenza virus vaccine induced by epidermal powder immunization. *J Virol* 2002;75:7956–65. [PubMed: 11483740]
26. Frank AL, Puck J, Hughes BJ, Cate TR. Microneutralization test for influenza A and B and parainfluenza 1 and 2 viruses that uses continuous cell lines and fresh serum enhancement. *J Clin Microbiol* 1980;12:426–32. [PubMed: 6260835]
27. Greenbaum E, Furst A, Kiderman A, Stewart B, Levy R, Schlesinger M, et al. Serum and mucosal immunologic responses in children following the administration of a new inactivated intranasal anti-influenza vaccine. *J Med Virol* 2001;65:178–84. [PubMed: 11505461]
28. Greenbaum E, Furst A, Kiderman A, Stewart B, Levy R, Schlesinger M, et al. Mucosal [SIgA] and serum [IgG] immunologic responses in the community after a single intra-nasal immunization with a new inactivated trivalent influenza vaccine. *Vaccine* 2002;20:1232–9. [PubMed: 11803086]
29. Greenbaum E, Engelhard D, Levy R, Schlesinger M, Morag A, Zakay-Rones Z. Mucosal (SIgA) and serum (IgG) immunologic responses in young adults following intranasal administration of one or two doses of inactivated, trivalent anti-influenza vaccine. *Vaccine* 2004;22:2566–77. [PubMed: 15193382]
30. Cox E, Verdonck F, Vanrompay D, Goddeeris B. Adjuvants modulating mucosal immune responses or directing systemic responses towards the mucosa. *Vet Res* 2006;37:511–39. [PubMed: 16611561]
31. Wyde, PR.; Guzman, E.; Gilbert, BE.; Couch, RB. Immunogenicity and protection in mice given inactivated influenza vaccine, MPL, QS-21 or QS-7. In: Osterhaus, ADME.; Cox, N.; Hampson, AW., editors. *Options for the Control of Influenza IV*. Excerpta Medica; New York: 2001. p. 999-1005.
32. Mbawuike IM, Zang Y, Couch RB. Humoral and cell-mediated immune responses of humans to inactivated influenza vaccine with or without QS21 adjuvant. *Vaccine* 2007;25:3263–9. [PubMed: 17280748]
33. Hashigucci K, Ogawa H, Ishidate T, Yamashita R, Kamiya H, Watanabe K, et al. Antibody responses in volunteers induced by nasal influenza vaccine combined with *Escherichia coli* heat-labile

- enterotoxin B subunit containing a trace amount of the holotoxin. *Vaccine* 1996;14:113–9. [PubMed: 8852406]
34. Stephenson I, Zambon MC, Rudin A, Colegate A, Podda A, Bugarini R, et al. Phase I evaluation of intranasal trivalent inactivated influenza vaccine with nontoxigenic *Escherichia coli* enterotoxin and novel biovector as mucosal adjuvants, using adult volunteers. *J Virol* 2006;80:4962–70. [PubMed: 16641287]
 35. Havell EA, Vilček J. Production of high-titered interferon in cultures of human diploid cells. *Antimicrob Agents Chemother* 1972;2:476–84. [PubMed: 4670440]
 36. Muszkat M, Greenbaum E, Ben-Yehuda A, Oster M, Yeu E, Heimann S, et al. Local and systemic immune response in nursing-home elderly following intranasal or intramuscular immunization with inactivated influenza vaccine. *Vaccine* 2003;21:1180–6. [PubMed: 12559796]
 37. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N Engl J Med* 2006;354(13):1343–51. [PubMed: 16571878]
 38. Leroux-Roels I, Borkowski A, Vanwolleghem T, Dramé M, Clement F, Hons E, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. *Lancet* 2007;370:580–89. [PubMed: 17707753]
 39. Liew FY, Russell SM, Appleyard G, Brand CM, Beale J. Cross-protection in mice infected with influenza A virus by the respiratory route is correlated with local IgA antibody rather than serum antibody or cytotoxic T cell reactivity. *Eur J Immunol* 1984;14:350–56. [PubMed: 6609824]
 40. Waldman RH, Wigley FM, Small PA Jr. Specificity of respiratory secretion antibody against influenza virus. *J Immunol* 1970;108:1477–83. [PubMed: 4320734]
 41. Baccala R, Kono DH, Theofilopoulos AN. Interferons as pathogenic effectors in autoimmunity. *Immunol Rev* 2005;204:9–26. [PubMed: 15790347]
 42. Boyce TG, Hsu HH, Sannella EC, Coleman-Dockery SD, Baylis E, Zhu Y, et al. Safety and immunogenicity of adjuvanted and unadjuvanted subunit influenza vaccines administered intranasally to healthy adults. *Vaccine* 2001;19:217–26. [PubMed: 10930676]
 43. Samdal HH, Bakke H, Oftung F, Holst J, Haugen IL, Korsvold GE, et al. A non-living nasal influenza vaccine can induce major humoral and cellular immune responses in humans without the need for adjuvants. *Human Vac* 2005;1:85–90.

Table 1Responses of Mice to Intranasal Immunizations with Inactivated Influenza A/Texas/91 (H1N1) Vaccine¹

Group ²	Serum GMT Neut Ab (log ₂)	NS Mean Antibody ³	Lung Fluid Mean Ab ³	Lung Virus Mean Titer ⁴
Mixed adj	<2	0.25	0.25	6.7
Vac + PBS	2.6	0.68	0.65	6.2
Vac + CTB	6.0	1.15	1.18	2.6
Vac + MPL	5.6	0.94	1.45	2.2
Vac + IFN	5.6	1.18	1.22	2.0
Live virus	5.8	2.05	1.76	<1.8

¹ 10 mice/group, 30 microliter dose; vac – 0.15 microgram on day 1, 2, and 28, 29, with PBS or 2.5 microgram CTB, 3.75 microgram MPL or 1X10⁴ IFN u; serum antibody on day 42, nasal secretions and lung fluid antibody on day 45 on nonchallenged mice; challenge on day 42 with lung virus on day 45

² adj = adjuvant, vac = vaccine, PBS = phosphate buffered saline, CTB = cholera toxin B, MPL = monophosphoryl lipid A, IFN = interferon

³ Mean optical density in Elisa assay; NS = nasal secretions

⁴ log₁₀/lung

Table 2
Reactogenicity of Humans after Inactivated Influenza Vaccine With or Without Alpha Interferon¹

IFN Group ²	No. (%) with Local Reaction			(No. (%) with Systemic Reaction			
	No.	Any	Moderate	Severe	Any	Moderate	Severe
0	32	31 (97)	4 (12.5)	0	21 (66)	3 (9)	1 (3) ³
1 M	32	22 (69)	7 (22)	0	21 (66)	4 (12.5)	0
10 M	31	20 (65)	6 (19)	0	20 (65)	8 (26)	2 (6) ⁴

¹ Solicited AEs for 7 days post vaccination

² Inactivated vaccine plus indicated interferon dosage (million units)

³ Report of malaise, myalgias, feverish

⁴ One was headache plus malaise and one malaise only

Table 3

Mean Serum Antibody of Humans Before and After Inactivated Influenza Vaccine With or Without Alpha Interferon¹

Vaccine Virus and Assay ¹	Interferon Group ³ (N)		
	0 (32)	1M (32)	10M (31)
H1			
HAI Pre ⁴	3.56	4.41	4.19
2 wks	6.00	6.22	5.48
4 wks	6.06	6.22	5.61
Neut Pre	3.66	4.22	4.19
2 wks	5.56	5.78	5.44
4 wks	5.84	6.23	5.69
H3			
HAI Pre ⁴	3.78	3.81	4.68
2 wks	5.84	5.25	5.58
4 wks	5.97	5.66	5.74
Neut Pre	5.25	5.20	6.40
2 wks	7.38	6.78	7.61
4 wks	7.61	6.59	7.44
B			
HAI Pre ⁴	2.75	3.16	3.19
2 wks	3.97	4.50	4.00
4 wks	4.28	4.78	4.32
Neut Pre	2.50	3.41	3.02
2 wks	4.08	4.48	3.92
4 wks	4.53	4.95	4.37

¹log₂

¹ H1 = A/New Caledonia/20/99; H3 = A/Wisconsin/67/05; B = B Malaysia/2506/4

³ Inactivated vaccine plus indicated interferon dosage (million units)

⁴ Time in relation to vaccination

Table 4
ELISA and/or Neutralizing Antibody Responses in Nasal Secretions of Humans to Inactivated Influenza Vaccine Given Intranasally with or without Alpha Interferon

IFN Group ¹	No.	No. (%) ↑ to H1 ²			No.	No. (%) ↑ to H3 ²		
		EIA	Neut	Either		EIA	Neut	Either
0	32	5 (16)	9 (28)	11 (34)	32	7 (22)	12 (37.5)	14 (44)
1M	30	4 (13)	5 (17)	7 (23)	32	5 (16)	6 (19)	8 (25)
10M	30	5 (17)	4 (13)	7 (23)	31	11 (35)	9 (29)	16 (52)

¹Inactivated vaccine plus indicated interferon dosage (million units)

²H1 = A/New Caledonia/20/99; H3 = A/Wisconsin/67/05