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Review Article

Challenges and Perspectives towards the Development of more Effective Influenza Vaccine

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Abstract

Influenza viruses continue to be a major health threat in human and bird populations. The improvements in formulation and production level of the current influenza vaccines are not sufficient to afford complete protection. The continuous antigenic drifts and emergence of endemic and zoonotic strains make influenza vaccine planning difficult. Concern about the emergence of new influenza pandemic provides subject for developing a universal influenza vaccine to be most effective in preventing influenza A either by targeting the HA or other viral proteins. The recombinant and synthetic antigens used in influenza vaccine research and development are generally less immunogenic and need to incorporate novel adjuvants with modified delivery carriers to develop broad-protective vaccines.

Keywords: Influenza virus, vaccine, adjuvant, delivery system.

Introduction

Recombination in RNA viruses involves the exchange of genetic information between two nonsegmented RNA genomes. Since the first detected recombination in poliovirus in the early 1960s, the major evolutionary significance occurs in many RNA viruses that plays an important role in viral biology (1). The influenza A virus genomes comprise eight negative-sense, single-stranded RNA segments, which are numbered in order of decreasing length. The polymerase subunit 2 or PB2, PB1 (in some strains this segment also codes for the accessory protein PB1-F2), PA, the strongly immunogenic surface protein HA, NP, NA, M1 (the M2 ion channel is also expressed from segment 7 by RNA splicing), NS1 and NEP/NS2 by mRNA splicing (2). The segmented genome structure and the error-prone RNA-dependent RNA polymerases enable the influenza viruses to undergo antigenic shift and drift (3-5) the two possible mechanisms for antigenic evolution.

Antigenic drifts or small changes in the genes of viruses are happening continually in the types of influenza viruses. These gradual and subtle mutations produce in the two surface glycoproteins, HA and NA may lead to a loss of immunity, or in vaccine mismatch (6-8).

The antigenic shift is the process by which at least two different strains or different influenza A viruses combine to form a new subtype. The recombine or reassorted virus, which has a mixture of the surface antigens of the two original strains, may follow to enter a new niche and transmit directly from animal to human (9-11). The host shifts have resulted in human pandemics, such the H1N1 “Spanish flu” (1918-1920) killed an estimated 50 million people or more globally (12).

The causative agent was an avian-descended H1N1 virus and a direct progenitor of all of the influenza A viruses circulating in humans today. Along with the Asian flu (1957) and Hong Kong flu (1963) human pandemics, the avian H5N1 viruses caused devastating

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outbreaks in 2004 in domestic poultry worldwide and posed a major challenge to human health (13-16). The recent 2009 H1N1 pandemic virus with 0.03% case fatality rate was derived by reassortment between a triple reassortant lineage of North American swine and a Eurasian H1N1 swine lineage virus (11, 17).

The influenza researches into the genes of the recombined viruses as well as the circulated avian subtypes such as H9N2, H5N1 and H7N9 show they have genes may adapt to human (18-22). Thus, the viruses have to be considered a potentially serious pandemic threat.

Traditional influenza virus vaccines and challenges

Vaccination against influenza is the most effective way to prevent morbidity and mortality in the target risk groups. Influenza virus vaccines comprise whole inactivated virus, split, virosomal, or subunit antigen are used in widespread annual influenza vaccination campaigns (23, 24).

The vaccine production platforms are including embryonated eggs licensed worldwide, Madin Darby canine kidney (MDCK) and monkey kidney (Vero) cells licensed in EU and USA, and trivalent vaccine of recombinant HA produced in baculovirus licensed in USA and Japan. The egg-based production system has been used for more than 60 years, and it is still the most extensively used method to generate the influenza vaccine. In cell-based production system, viral seeds recommended for the seasonal or epidemic vaccination are separately expanded in each of the mammalian cells and pooled to formulate the vaccine (25).

For more than 50 years, World Health Organization (WHO) has been collaborating with influenza vaccine development to identify the dominant circulating strains during the previous and the following vaccination seasons in the northern and southern hemispheres. The traditional “trivalent” vaccines are formulated based on an influenza A (H1N1) virus, an influenza A (H3N2) virus, and an influenza B virus (26). Despite the marketing of influenza vaccines since the 1930s, several limitations still involve their effectiveness include limited efficacy, limited worldwide vaccine availability, and lack of cross-reactivity. The seasonal vaccine is effective in eliciting protection when the vaccine strains and the circulating viral are closely antigenic matched together. Due to frequent antigenic drifts, seasonal influenza vaccines need to be re-formulated regularly to match the circulating strains. During each influenza season, Centers for Disease Control and Prevention (CDC) has estimated the effectiveness of the used vaccine. Based on the data from children and adults enrolled in the U.S. during 2004-2018 (www.cdc.gov/flu), the vaccine effectiveness against influenza A and influenza B virus infection was 36% (95% confidence interval [CI]=27%–44%).

Most (69%) influenza infections were caused by H3N2 viruses. The vaccine effectiveness was estimated to be 25% (CI=13% to 36%) against H3N2 virus, 67% (CI=54%–76%) against H1N1 pdm09 viruses, and 42% (CI=25%–56%) against influenza B viruses. The best overall estimated effectiveness was 60% for 2010-2011 influenza seasons.

However the vaccine effectiveness confirm the value of influenza vaccination as a public health intervention, the results are vary based on study design, outcome measured, population and the season in which the influenza vaccine was studied. Because the seasonal vaccine may provide limited protection in cases of antigenic mismatch, the quadrivalent vaccine consists of H3N2 and H1N1, and two distinct influenza B lineages was also licensed for induction a better immunogenicity (27, 28).

WHO has recommended that quadrivalent vaccines for use in the 2018-2019 northern hemisphere influenza season contain A/Michigan/45/2015 (H1N1) pdm09-like virus; A/Singapore/INFIMH-16-0019/2016 (H3N2) like virus; B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage); and B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage) (www.who.int/influenza/vaccines).

The reformulation is only useful for battling seasonal influenza epidemics and remind

ineffective in preventing unexpected new influenza pandemic.

In the last decade, the emergence of H1N1 pandemic and outbreaks of various avian influenza viruses have been a threat to the effectiveness of the current vaccines and an alarm for public health organizations (29, 30). On the other hand, zoonotic influenza viruses continue to be identified and evolve both genetically and antigenically, leading to the need to select and develop additional candidate vaccine viruses for pandemic preparedness purposes (18). These events as well as the increased demand for effective coverage against the infection are great challenges to vaccine production. Most countries without vaccine production facilities will have no access to the effective vaccines during the first pandemic wave. All these challenges call researchers for the development of the proper influenza vaccines capable of conferring broad cross-protection against multiple subtypes of influenza A viruses (31). The current in development researches can be divided into the various categories: elicit antibody responses to structurally conserved regions of the stalk domain of HA and M2 ectodomains (M2e); induce cross-protective T-cell responses against internal proteins like nucleoprotein (NP) and matrix 1 (M1); and improved adjuvants and delivery systems.

Universal influenza vaccines

Upon influenza infection virus-specific T cell responses including CD4+ T helper cells and CD8+ cytotoxic T cells (CTLs) are induced. These cells are primarily targeted to induce immunity to the HA antigen then directed to conserved proteins (32). Among the viral antigen, HA is considered as most (or highly) immunogenic. The molecule comprises the head, an immuno-dominant and the primary target for currently licensed influenza vaccines, and stalk regions. The 18 HAs are phylogenetically divided into two groups based on similarities in their stalk regions. Thus, the portion displays cross-reactivity with different subtypes (33). The conserved feature of HA stalk domain makes it an attractive target for the induction of a cross-reactive humoral response. However, HA stalk-reactive antibodies prevent infection by inhibiting the virus attachment to host cell membrane or disruption viral membrane fusion it should be determined whether these antibodies are suitable for protection against influenza infection (34-36).

Beside the development of vaccines inducing HA stalk-reactive antibodies, the M2e-targeted vaccines attracted the attention of researchers for developing a universal influenza vaccine or a vaccine that provides robust, long-lasting protection. M2e is a potential target for cross-reactive immune responses among all known influenza A viruses that circulated between 1918 to now. In contract to the stalk domain of HA, M2e vaccines do not prevent viral infection but efficiently inhibit viral replication once inside the host. M2e itself has low immunogenicity and need fused with a carrier vehicle to enhance anti-M2e immune responses. The impact of various viral and bacterial carriers for increasing the immunogenicity of M2e was tested in animal models (37, 38). The studies on the protective efficacies by M2e-based vaccination suggest that M2e immunity-mediated cross protection is relatively weak and need to become sufficiently immunogenic.

T cell-inducing influenza vaccines

Cell-mediated immune responses play an important role in the cross-protective immune response against influenza virus. It is well documented that individuals who possessed preexisting CTLs cells displayed decreased morbidity after infection with pandemic H1N1 influenza, or patients with early CTL responses recovered quickly from H7N9 infection (39). The half-life of circulating T cells specific to influenza calculated at least 2 years, so a central memory response can be maintained for many years following recovery from the infection (40). The induction of influenza-specific cellular responses might be a great addition to current antibody-inducing influenza vaccines. The naturally acquired immune responses to influenza include CTLs recognizing antibodies to HA as well as conserved internal

viral antigens. Most highly conserved T cell epitopes are located on NP and M1 proteins, which role in preventing infection or reducing disease severity (41). As a novel vaccine concept based on the induction of influenza-specific T cells, the efficacy of a recombinant replication-deficient poxvirus vector was assessed in an effort to boost T-cell responses to these internal antigens. However, a large expansion in circulating influenza-specific T cells was observed after a single vaccination (42), it seems that several formulation strategies should utilize to successfully induce specific T cell responses to the non immunogenic antigens. This approach is valuable both for priming and boosting an immune response.

Antigenic peptides-based influenza vaccines

Multiple antigenic peptide constructs which induce both influenza-specific immune B-cell and T cell responses against conserved epitopes are used for enhancing the immunogenicity of peptide antigens. A peptide construct consists of nine linear B cell and T cell epitopes of HA, NP and M1, named Multimeric-001 was able to induce considerable cellular immune responses when administered twice in both adults and elderly (25). This approach has also been used in M2e-derived antigens and in a prime-boost immunization with seasonal traditional inactivated influenza vaccine (43, 44). A prime with Multimeric-001 and subsequently boosting with seasonal vaccine had significantly higher HI titers compared to individuals who received the inactivated vaccine at both prime and boost. Despite these promising results, further formulation with adjuvants might increase the immunogenicity of Multimeric-001 vaccine in the future.

Adjuvants

The HA stalk domain, M2e- and T cell-based influenza vaccine candidates having completed phase II trials and will enter phase III trials in the coming years. But only the covalently bound M2e antigen to a carrier protein or adjuvant could induce potent cross-protective immune responses (45). To overcome limited efficacy of the current influenza vaccines and also the future universal vaccine, advanced formulation with adjuvants both immunopotentiator and delivery system are considered. For more than 70 years adjuvants have been added to vaccine formulations to boost the immune response to the vaccine. The components act by diverse mechanisms which include enhancing the delivery of antigen, improving magnitude of the immune response, directing antigen presentation by the major histocompatibility complex or providing immune stimulatory signals that potentiate the immune response (46, 47). Generally, inactivated vaccines should formulate with adjuvants to augment the potency of the antigen and presentation to the immune system.

The oil-in-water emulsions are approved as suitable adjuvants for influenza vaccines. MF59, the first oil-in-water emulsion adjuvant approved for formulation of influenza vaccines, is biodegradable squalene oil droplets stabilized by non-ionic surfactants. The mechanism actions attribute to MF59 are enhance regulation of genes for cytokines and chemokines, increase influx of macrophages and monocytes to the site of injection, differentiation of monocytes to active dendritic cells, and antigen transportation to draining lymph nodes (48, 49). AS03 is another oil-in-water emulsion based on squalene droplets and only used in pandemic influenza vaccines (50).

Impact of saponin-based adjuvants such as immune stimulating complexes (ISCOMs) and Matrix-M has been evaluated in clinical studies in combination with influenza vaccines. The ISCOMs-adjuvanted influenza split vaccines revealed increase of influenza-specific in humoral and CD8+T cell responses in vaccinated individuals. The addition of Matrix-M to virosomal influenza vaccine resulted in increased vaccine-induced T cell responses (51). The third generation of saponin based adjuvant was used for a H7N9 virus-like particle vaccine and showed significantly higher seroconversion rates after vaccination.

The co-administration of bacterial-derived components, flagellin and heat-labile entero-

toxin are potent adjuvants for influenza vaccines and able to induce protective hemagglutination inhibition (HI) titers after immunization with influenza vaccines (52).

Peptide antigens generally suffer from poor immunogenicity and need adding adjuvant to induce influenza-specific cellular responses. NP peptide encapsulated in cationic liposomes could induce potent T cell responses (53) and HLA-A2.1 and HLA-A24.2 restricted peptides conjugated to liposomes were able to induce CD8+ memory T cells in influenza-infected mice (54). The same results were found in conjugation of PA-derived peptide to Pam2Cys (a bacterial lipopeptide) (55), and influenza peptides conjugated to phosphatidylserine (56).

With the advent of novel concepts for immunity against influenza, novel types of adjuvants such as cytokines, type I interferon, the tumor necrosis factor, and bacterial derivatives have been evaluated to induce stronger responses as well as CTL-mediated immunity against influenza infection (57, 58) are under development. Several studies suggested that molecular adjuvants such as myxovirus resistance (Mx) protein (35), Hemokinin-1 (HK-1) (59, 60), unmethylated bacterial CpG motifs (61), Mycobacterium-dependent protein-1 (62), HSP 70 (63), and ESAT-6 (64) might act better by enhanced antigen persistence, increased antigen uptake, and recruitment of antigen presenting cells (APCs) compared with the current adjuvants. These experimental approaches support improvement of adjuvant may be an important route to obtaining better protecting influenza vaccines.

Nano particles delivery system

The delivery of vaccine antigens to dendritic cells is critical to the improvement of a protective immune response. Most current inactivated vaccines are usually administered via intramuscular injection may fail to reach the APCs and induction of immune responses (65).

To overcome the difficulty in targeting the APCs new approaches are being investigated to apply more effective vaccine delivery methods (66). Many antigen and adjuvant delivery carriers have been considered for enhancing the efficiency of the application of vaccines.

Recent studies have demonstrated that antigen delivery via nanoparticle formulations can significantly improve immunogenicity of vaccines due to either intrinsic immunostimulatory properties of the components or by co-entrapment of molecular adjuvants. Encapsulating the antigen within the nanoparticles decreases the risk of degradation of antigen (67, 68). A variety of polycationic polymeric nanoparticles and their derivatives explored to stimulate immune system. Among them, chitosan is preferred due to its ability in enhancing antigen uptake by mucosal lymphoid tissues, and inducing strongly immune responses against the antigens (69-71).

Utilization of chitosan nanoparticles facilitate the delivery of a vaccine to the targeted cells but some researches focus on the ability of the polymer as a novel adjuvant to induce specific antibody titer against influenza antigens. For instance, reduced markedly the influenza morbidity and also complete protection against challenge mice vaccinated with chitosan nanoparticle encapsulated HA-split vaccine (72) or enhanced immunogenicity of H9N2 influenza nanovaccine in chickens in comparison to an oil adjuvant (73).

There is no doubt that chitosan nanoparticles can stimulate early immune response and enhance the specific HI titer against influenza antigens, but the boost delivery system role of the polysaccharide is also extremely recommended. The highly induction of immune system and long-lived response are invaluable properties of a vaccine thus adding a number of adjuvants to nanovaccines are highly suggested to enhance the induction of the immune responses (74, 75). Influenza is still one of the most wide-reaching and deadly infectious diseases and development of a more effective vaccine imply that novel adjuvants correlate of the delivery system need to be established.

References

1. Dermody T, Parker J, Sherry B. Orthoreoviruses. Fields Virology. 2013; 2:1304-46.
2. Simon-Loriere E, Holmes EC. Why do RNA viruses recombine? Nat Rev Microbiol 2011; 9(8): 617–626.
3. Landolt GA, Olsen CW. Up to new tricks—A review of cross-species transmission of influenza A viruses. Anim Health Res Rev. 2007; 8:1–21.
4. Rabadan R, Robins.H. Evolution of the influenza A virus: some new advances. Evol Bioinform Online. 2007;3:299–307.
5. Boni MF. Vaccination and antigenic drift in influenza. Vaccine. 2008; 26 (Suppl 3): C8–14.
6. Schweiger B, Bruns L, Meixenberger K. Reassortment between human A (H3N2) viruses is an important evolutionary mechanism. Vaccine. 2006;24:6683–90.
7. Gamblin SJ, Skehel JJ. Influenza hemagglutinin and neuraminidase membrane glycoproteins. J Biol Chem. 2010;285(37):28403-28409.
8. Ren XW, Ju LW, Yang JX, Lv XH, Jiang LF, Zhao NQ, Jiang QW. Antigenic and genetic variation in the hemagglutinins of H1N1 and H3N2 human influenza a viruses in the Shanghai area from 2005 to 2008. J Med Virol. 2011;83(7):1113-20.
9. Doherty PC, Turner SJ, Webby RG, Thomas PG. Influenza and the challenge for immunology. Nat Immunol. 2006;7(5):449-455.
10. Taubenberger JK, Kash JC. Influenza virus evolution, host adaptation and pandemic formation. Cell Host Microbe. 2010; 7: 440–451.
11. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, et al. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. Science. 2009;325(5937):197–201.
12. Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918–1920 "Spanish" influenza pandemic. Bull Hist Med. 2002;76(1): 105–115.
13. Kilbourne ED. Influenza pandemics of the 20th century. Emerg Infect Dis. 2006;12(1):9–14.
14. Morens DM, Taubenberger JK, Fauci AS. The persistent legacy of the 1918 influenza virus. N Engl J Med. 2009; 361(3):225–229.
15. Kash JC, Tumpey TM, Proll SC, Carter V, Perwitasari O, Thomas MJ, et al. Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. Nature. 2006; 443(7111):578–581.
16. Taubenberger JK, Morens DM. Pandemic influenza-including a risk assessment of H5N1. Rev Sci Tech. 2009;28(1):187–202.
17. Mak GC, Wong, AH, Ho WY, Lim W. The impact of pandemic influenza A (H1N1) 2009 on the circulation of respiratory viruses 2009–2011. Influenza Other Respir Viruses. 2012;6(3):e6-10.
18. Shahsavandi S, Salmanian A-H, Ghorashi SA, Masoudi S, Ebrahimi MM. Evolutionary characterization of hemagglutinin gene of H9N2 influenza viruses isolated from Asia. Res Vet Sci. 2012;93 (1):234-249.
19. Xu G, Zhang X, Gao W, Wang C, Wang J. Prevailing PA mutation K356R in avian influenza H9N2 virus increases mammalian replication and pathogenicity. J Virol. 2016;90(18):8105-81014.
20. Arai Y, Kawashita N, Daidoji T, Ibrahim MS, El-Gendy EM, Takagi T, et al. Novel Polymerase Gene Mutations for Human Adaptation in Clinical Isolates of Avian H5N1 Influenza Viruses. PLoS Pathog. 2016;12(4): e1005583.
21. Crusat M, Liu J, Palma AS, Childs RA, Liu Y, Wharton SA. Changes in the hemagglutinin of H5N1 viruses during human infection–influence on receptor binding. Virology. 2013;447(1-2):326–37.
22. Imai M, Watanabe T, Kiso M, Nakajima N, et al. A highly pathogenic avian H7N9 influenza virus isolated from a human is lethal in some ferrets infected via respiratory droplets. Cell Host Microl; 22:615–626.
23. Kreijtz JH1, Fouchier RA, Rimmelzwaan GF. Immune responses to influenza virus infection. Virus Res. 2011;162(1-2):19-30.
24. Wong S-S, Webby RJ. Traditional and new influenza vaccines. Clin Microbiol Rev.2013;26 (3):476-492.
25. Soema PC, Kompier R, Amorij JP, Kersten GFA. Current and next generation influenza vaccines: Formulation and production strategies. Eur J Pharma Biopharma. 2015;94:251-263.
26. Palese P, García-Sastre A. Influenza vaccines: present and future. J Clin Invest. 2002;110(1):9–13.
27. Treanor JT, Albano FR, Sawlwin DC, Graves Jones A, Airey J, Formica N, et al. Immunogenicity and safety of a quadrivalent inactivated influenza vaccine compared with two trivalent inactivated influenza vaccines containing alternate B strains in adults: A phase 3, randomized noninferiority study. Vaccine. 2017;35(15):1856-1864.
28. Beran J, Peeters M, Dewé W, Raupachová J, Hobzová L, Devaster J-M. Immunogenicity and safety of quadrivalent versus trivalent inactivated influenza vaccine: a randomized, controlled trial in adults. BMC infectious diseases. 2013;13:224.
29. Beyer WEP, Nauta, JJP, Palache AM, Giezeman KM, Osterhaus ADME. Immunogenicity and safety of inactivated influenza vaccines in primed populations: A systematic literature review

and meta-analysis. Vaccine. 2011;29(34):5785-5792.
30. Castilla J, Navascués A, Fernandez-Alonso M, Reina G, Pozo F, Casado I, et al. Effectiveness of subunit influenza vaccination in the 2014–2015 season and residual effect of split vaccination in previous seasons. Vaccine. 2016;34(11):1350-1357.
31. de Vries RD, Altenburg AF, Rimmelzwaan GF. Universal influenza vaccines, science fiction or soon reality? Expert Rev Vaccines. 2015;14(10): 1299-1301.
32. Kreijtz JH, Fouchier RA, Rimmelzwaan GF. Immune responses to influenza virus infection. Virus Res. 2011;162(1-2):19-30.
33. Shahsavandi S, Salmanian AH, Ghorashi SA, Masoudi S, Fotouhi F, Ebrahimi MM. Specific subtyping of influenza A virus using a recombinant hemagglutinin protein expressed in baculovirus. Mol Biol Rep. 2011;38(5):3293–3298.
34. Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. Curr Opin Virol. 2013;3(5):521-530.
35. Soleimani S, Shahsavandi S, Madadgar O. Improvement influenza HA2 DNA vaccine cellular and humoral immune responses with Mx bio adjuvant. Biologicals. 2017;46:6-10.
36. Krammer F, Pica N, Hai R, Margine I, Palese P. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. J Virol. 2013;87(12):6542-50.
37. Rappazzo CG, Watkins HC, Guarino CM, Chau A, Lopez JL, Delisa MP, et al. Recombinant M2e outer membrane vesicle vaccines protect against lethal Influenza A challenge in BALB/c mice. Vaccine. 2016;34(10):1252-1258.
38. Lee YN, Kim MC, Lee YT, Kim YJ, Kang SM. Mechanisms of cross-protection by influenza virus M2-based vaccines. Immune Netw. 2015;15(5): 213-221.
39. Gilbert SC. T-cell-inducing vaccines – what's the future. Immunology. 2012;135(1):19–26.
40. Wang Z, Wan Y, Qiu C, Quiñones-Parra S. Recovery from severe H7N9 disease is associated with diverse response mechanisms dominated by CD8+ T cells. Nat Commun. 2015;6:6833.
41. Ben-Yedidia T, Arnon R. Epitope-based vaccine against influenza. Expert Rev Vaccines 2007;6(6):939-48.
42. Antrobus RD, Lillie PJ, Berthoud TK, Spencer AJ, McLaren JE, Ladell K, et al. A T Cell-Inducing Influenza Vaccine for the Elderly: Safety and Immunogenicity of MVA-NP+M1 in Adults Aged over 50 Years. PLoS One. 2012;7(10): e48322.
43. Atsmon J, Caraco Y, Ziv-Sefer S, Shaikevich D, Abramov E, Volokhov I, et al. Priming by a novel universal influenza vaccine (Multimeric-001)-a gateway for improving immune response in the elderly population. Vaccine. 2014;32(44):5816-5823.
44. Atsmon J, Kate-Ilovitz E, Shaikevich D, Singer Y, Volokhov I, Haim KY, et al. Safety and immunogenicity of multimeric-001-a novel universal influenza vaccine. J Clin Immunol. 2012;32(3): 595-603.
45.Oyarzún P, Kobe B. Recombinant and epitope-based vaccines on the road to the market and implications for vaccine design and production. Hum Vaccin Immunother. 2016;12(3):763–767.
46. Even-Or O, Samira S, Ellis R, Kedar E, Barenholz Y. Adjuvanted influenza vaccines. Expert Rev Vaccines. 2013;12(9):1095-1108.
47. Fox CB, Kramer RM, Barnes L, Dowling QM, Vedvick TS. Working together: interactions between vaccine antigens and adjutants. Ther Adv Vaccines. 2013;1(1):7-20.
48. Banzhoff A, Haertel S, Praus M. Passive surveillance of adverse events of an MF59-adjuvanted H1N1 vaccine during the pandemic mass vaccinations. Human vaccines.2011;7(5):539-548.
49. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med. 2009;361(25):2424-2435.
50. Wilkins AL, Kazmin D, Napolitani G, Clutterbuck EA, Pulendran B, Siergrist C-A, et al. AS03 and MF59 adjuvanted influenza vaccines in children. Front Immunol. 2017;8:1760.
51. Lövgren K, Morein B, Osterhaus AD. ISCOM technology-based Matrix M™ adjuvant: Success in future vaccines relies on formulation. Expert Rev Vaccines. 2011;10(4):401-403.
52. Wang B-Z, Xu R, Quan F-S, Kang S-M, Wang L, Compans RW. Intranasal Immunization with Influenza VLPs Incorporating Membrane-Anchored Flagellin Induces Strong Heterosubtypic Protection. PLoS One. 2010; 5(11): e13972.
53.Taneichi M, Tanaka Y, Kakiuchi T, Uchida T. Liposome-coupled peptides induce long-lived memory CD8 T cells without CD4 T cells. PLoS One. 2010;5(11):e15091.
54. Matsui M, Kohyama S, Suda T, Yokoyama S, Mori M, Kobayashi A, et al. A CTL-based liposomal vaccine capable of inducing protection against heterosubtypic influenza viruses in HLA-A∗0201 transgenic mice. Biochem Biophys Res Commun. 2010;391(3):1494-1499.

55. Day EB, Zeng W, Doherty PC, Jackson DC, Kedzierska K, Turner SJ. The context of epitope presentation can influence functional quality of recalled influenza A virus-specific memory CD8+ T cells. J Immunol. 2007;179(4):2187-2194.
56. Ichihashi T, Satoh T, Sugimoto C, Kajino K. Emulsified phosphatidylserine, simple and effective peptide carrier for induction of potent epitope-specific T cell responses. PLoS One. 2013;8(3): e60068.
57. Singh M, O'hagan DT. Recent advances in vaccine adjuvants. Pharm Res. 2002;19(6):715-728.
58. Tovey MG, Lallemand C. Adjuvant activity of cytokines. Methods Mol Biol. 2010;626:287-309.
59. Shahsavandi S, Ebrahimi MM, Mahravani H, Saedghi K. Design of a heterosubtypic epitope-based peptide vaccine fused with hemokinin-1 against influenza viruses. Virol Sin. 2015;30(3): 200-207.
60. Sadghi K, Shahsavandi S, Ebrahimi MM, Mahravani H, Fazel H. Hemokinin-1 molecular adjuvant: an approach to enhance the efficacy of influenza vaccine. Arak Med Univ J. 2014;17:62-69.
61. Wu F, Yuan XY, Li J, Chen YH. The co-administration of CpG-ODN influenced protective activity of influenza M2e vaccine. Vaccine. 2009; 27(32):4320-4324.
62. Rasoli M, Omar AR, Aini I, Jalilian B, Syed Hassan SH, Mohamed M. Fusion of HSP70 gene of Mycobacterium tuberculosis to hemagglutinin (H5) gene of avian influenza virus in DNA vaccine enhances its potency. Acta Virol. 2010;54(1):33-39.
63. Jalilian B, Omar A, Bejo M, Alitheen N, Rasoli M, Matsumoto S. Development of avian influenza virus H5 DNA vaccine and MDP-1 gene of mycobacterium bovis as genetic adjuvant. Genet Vaccines Ther. 2010;8:4-10.
64. Oveissi S, Omar AR, Yusoff K, Jahanshiri F, Hassan SS. DNA vaccine encoding avian influenza virus H5 and Esat-6 of mycobacterium tuberculosis improved antibody responses against AIV in chickens. Comp Immunol Microbiol Infect Dis. 2010;33(6):491-503.
65. Sridhar S, Brokstad KA, Cox RJ. Influenza vaccination strategies: comparing inactivated and live attenuated influenza vaccines. Vaccines. 2015; 3(2):373-389.
66. Peek LJ, Middaugh CR, Berkland C. Nanotechnology in vaccine delivery. Adv Drug Deliv Rev. 2008;60(8):915-928.
67. Zhao L, Seth A, Wibowo N, Zhao CX, Mitter N, Middelberg AP. Nanoparticle vaccines. Vaccine. 2014;32(3):327-337.
68. Storni T, Kündig TM, Senti G, Johansen P. Immunity in response to particulate antigen delivery systems. Adv Drug Deliv Rev. 2005;57 (3):333-355.
69. Sanchez MV, Ebensen T, Schulze K, Cargnelutti D, Blazejewska P, Scodeller EA, et al. Intranasal delivery of influenza rNP adjuvanted with c-di-AMP induces strong humoral and cellular immune responses and provides protection against virus challenge. PLoS One. 2014;9(8):e104824.
70. Dobhal A, Bangde P, Dey A, Dandekar P, Jain R. Chitosan-based nanoparticulate systems: implication towards therapeutics application. In: Particulate Technology for Delivery of Therapeutics. Springer. 2017;p167-225.
71. Hajizade A, Ebrahimi F, Salmanian A-H, Arpanae A, Amani J. Nanoparticles in vaccine development. J Applied Biotech Rep. 2015;1:125-134.
72. Sawaengsak C, Mori Y, Yamanishi K, Mitrevej A, Sinchaipanid N. Chitosan nanoparticle encapsulated hemagglutinin-split influenza virus mucosal vaccine. AAPS PharmSciTech 2014;15 (2):317-325.
73. Khalili I, Ghadimipour R, Sadigh Eteghad S, Fathi Najafi M, Ebrahimi MM, Godsian N, et al. Evaluation of immune response against inactivated avian influenza (H9N2) vaccine, by using chitosan nanoparticles. Jundishapur J Microbiol 2015;8(12): e27035.
74. Dehghan A., Shahsavandi S., Jabalameli L. Improvement efficacy of H9N2 influenza nanovaccine in combination with hemokinin-1 molecular adjuvant. AJMB. 2018;10 (in press).
75. Sawaengsak C, Mori Y, Yamanishi K, Mitrevej A, Sinchaipanid N. Chitosan nanoparticle encapsulated hemagglutinin-split influenza virus mucosal vaccine. AAPS PharmSciTech. 2014;15 (2):317-325.