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REVIEW

Pandemic Influenza H1N1 2009, Innate Immunity, and the Impact of Immunosenescence on Influenza Vaccine

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Seasonal and pandemic strains of influenza have widespread implications for the global economy and global health. This has been highlighted recently as the epidemiologic characteristics for hospitalization and mortality for pandemic influenza H1N1 2009 are now emerging. While treatment with neuraminidase inhibitors are effective for seasonal and pandemic influenza, prevention of morbidity and mortality through effective vaccines requires a rigorous process of research and development. Vulnerable populations such as older adults (i.e., > age 65 years) suffer the greatest impact from seasonal influenza yet do not have a consistent seroprotective response to seasonal influenza vaccines due to a combination of factors. This short narrative review will highlight the emerging epidemiologic characteristics of pandemic H1N1 2009 and focus on immunosenescence, innate immune system responses to influenza virus infection and vaccination, and influenza vaccine responsiveness as it relates to seasonal and H1N1 pandemic influenza vaccines.

PANDEMIC INFLUENZA H1N1 2009: ORIGINS, EPIDEMIOLOGY, AND TREATMENT OPTIONS

Influenza A is a single-stranded negative sense RNA virus that encodes eight major genes, including two major surface antigens: hemagglutinin (HA†) (16 subtypes) and neuraminidase (NA) (nine subtypes). The natural host of influenza A is wild waterfowl, although domesticated poultry and swine also can become infected (creating potential for genetic reassortment of strains from avian and swine origins). Seasonal influenza poses a major global

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†Abbreviations: HA, hemagglutinin; NA, neuraminidase; FDA, U.S. Food and Drug Administration; WHO, World Health Organization; PRR, pattern recognition receptors; PAMP, Pathogen-Associated Molecular Patterns; TLR, Toll-Like Receptors; NLR, Nucleotide-binding domain and Leucine-rich-repeat Receptors; MyD88; myeloid differentiation primary response protein 88; IFN, type I Interferon; mDC, myeloid DC; pDC, plasmacytoid DC.

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health burden annually that is magnified when potential strains create pandemics through point mutations in genes encoding HA and NA (antigenic drift) or viral genomic reassortment of subtypes (especially during interspecies transmission), often resulting in the introduction of novel influenza strains into the human population (antigenic shift). The highly pathogenic "Spanish" influenza was an avian-like H1N1 virus, and H1N1 viruses were dominant in human populations from 1918 until approximately 1956. In 1957, reassortment of H1N1 with avian influenza strains resulted in the formation of a new human pandemic, H2N2 "Asian" strain. In 1968, antigenic shift resulted in the appearance of a pandemic H3N2 "Hong Kong" strain. H1N1 strains reemerged beginning in 1976, when a novel strain causing an influenza outbreak among military personnel at Fort Dix, New Jersey, was reported. This strain did not spread beyond the military base, but concern about a possible pandemic led to a mass vaccination campaign in the United States that resulted in more deaths from vaccine-associated Guillain-Barre syndrome than had occurred from the Fort Dix H1N1 strain. In 1977, a H1N1 strain nearly identical to a strain circulating in the 1950s reemerged in the former Soviet Union and China. Such genetic preservation in the known animal reservoirs for influenza virus is conceivable but would be unexpected, and there remains speculation that the 1977 Eurasian outbreak was the result of a laboratory accident in the region. Human H3N2 and H1N1 strains have remained in co-circulation as seasonal influenza until today [1,2]. Less common strains with pandemic potential include avian H5N1 influenza. These strains are highly pathogenic in humans with inpatient mortality reported as ranging from 24 percent to 100 percent (the wide range reflecting the heterogeneity in affected age groups and viral clades) [3]. Cases usually reflect close exposure to poultry outbreaks (with sick or dead animals) or, in rare cases, are associated with person-to-person transmission [3]. Fortunately, at present, sustained human-to-human transmission of H5N1 strains has not occurred [2,3].

Until recently, H5N1 avian strains of influenza were the predominant focus for surveillance of potential new pandemic viral strains. This obviously changed with the emergence of the swine-related H1N1 strain currently circulating in the United States and around the world. It is noteworthy that occasional transmission of swine-related H1N1 strains to humans, usually in the setting of exposure to pigs, has been reported for decades and results in clinical presentations reminiscent of those being observed in the current pandemic [4]. These earlier swine-origin viruses are the result of a so-called triple re-assortment, with five of the eight influenza virus gene segments of North American swine lineage, two of North American avian lineage, and one from the human H3N2 influenza A strain. In the current human pandemic H1N1 2009 strain, two of the North American swine origin gene segments (encoding the NA and M proteins) have been replaced with segments originating from Eurasian swine. The origin of this novel re-assortment from four different sources remains incompletely understood at present [2,5,6].

Epidemiologic characteristics of patients infected with pandemic H1N1 2009 have begun to emerge from around the globe. In general, both uncomplicated and critically ill presentations are similar to those reported for seasonal influenza. However, in contrast to seasonal influenza, in which 90 percent of the 30,000 to 40,000 annual deaths are associated with individuals over age 65, early data indicates increased mortality primarily in younger individuals 20 to 49 years of age, as well as patients with risk factors such as immunosuppression, pregnancy, or obesity [7-9]. Possible explanations for the increased burden of disease in young compared to older adults (in contrast to seasonal influenza) may be the presence of preexisting cross-protective antibodies against the current pandemic H1N1 2009 strain found in individuals over the age of 59. This presumably reflects exposure earlier in life to related H1N1 strains circulating in the 1950s [10,11].

Murine models lack some of the clinical symptoms described above, but in cases of

influenza-related pneumonia, they mirror many of the changes found in the lower respiratory tracts of humans: vessel thromboses, necrosis and inflammatory infiltrates, formation of hyaline membranes, hemorrhage, and alveolar damage [12]. Early evidence with swine origin H1N1 influenza virus strains has demonstrated high viral titers in lower respiratory tract tissue leading to an increased propensity to cause pneumonia. This has been accompanied by an increase in pro-inflammatory cytokines and inflammatory infiltrate on histological examination [13,14]. Some of these findings have been replicated in macaques and ferrets [13-15]. Notably, in swine origin H1N1 influenza virus-related pneumonia, there are some similarities in the aforementioned pathological changes within lung tissue to influenza H5N1-associated pneumonia [2,16].

As suggested by the work in animal models, the novel pandemic H1N1 2009 strain has resulted in complicated presentations requiring hospitalization. In a recent study reporting on 272 patients in the United States hospitalized with pandemic H1N1 influenza, 25 percent were admitted to the ICU [17]. Among patients presenting to the ICU, the mean Apache II score was 20, mean age 29-44 years, and female sex predominated. Major co-morbidities among critically ill patients in the United States, Canada, and Mexico included chronic lung disease, obesity, hypertension, diabetes, and a smoking history [8,9,17]. The average time to symptom onset was three to four days, with common symptoms including fever, respiratory symptoms, weakness, myalgias, headache, nausea, vomiting, or diarrhea [8,9,17]. Common radiographic presentations in the critically ill were bilateral infiltrates and acute lung injury [9,17]. The 28- and 60-day mortality among critically ill patients was 14 percent and 41 percent, respectively [8,9]. Thus, while this new strain of pandemic influenza results in uneventful recovery for the vast majority of affected individuals, patients requiring hospitalization are at increased risk for morbidity and mortality.

Currently available antiviral therapy against influenza targets either the influenza M2 protein (adamantanes) or neuraminidase (neuraminidase inhibitors). Neuraminidase inhibitors in particular interfere with viral neuraminidase-mediated cleavage of host sialic-acid receptor residues, a critical step in the release of newly created influenza virons from infected cells. The overwhelming majority of pandemic H1N1 2009 strains are susceptible to neuraminidase inhibitors such as oseltamivir or zanamivir and resistant to the adamantanes [18]. This contrasts with seasonal influenza, in which circulating H1N1 strains (A/Brisbane/59/2007) from the 2008-2009 influenza season were found to be uniformly resistant to oseltamivir [19]. In very rare cases, resistance to oseltamivir in the pandemic H1N1 strain may arise through a histidine to tyrosine substitution in the active site of neuraminidase (H275Y); additionally, a novel viral mutation found in close contacts receiving oseltamivir also has been reported (I223V) [20]. However, the clinical significance of oseltamivir resistance for pandemic H1N1 influenza remains unclear [21]. In severe hospitalized adult or pediatric patients with suspected or laboratory confirmed pandemic H1N1 2009 strains or infections suspected to be pandemic H1N1 2009 based on community epidemiology, peramivir (an intravenous formulation of a neuraminidase inhibitor currently entering phase III clinical studies) may be available via Emergency Use Authorization from the U.S. Food and Drug Administration (FDA) for individuals unable to take oseltamavir (which can only be administered by mouth) or zanamavir (which can only be given in inhaled form) [22]. Available data on peramivir suggest a prolonged half-life in humans and peak plasma concentrations that are two-fold higher than oral oseltamivir; however, clinical data on efficacy are still lacking [23]. Peramivir should not be used in cases of documented or suspected oseltamivir resistance.

VACCINE DEVELOPMENT

The widespread implications of pandemic H1N1 2009 influenza necessitated the development of an effective vaccine. Epi-

demic models projected savings in excess of $300 million and prevention of 1,468 deaths if 40 percent of the population in a large U.S. city were vaccinated against pandemic influenza during November [24]. Based upon antigen similarities among circulating strains, the World Health Organization (WHO) and FDA recommended a monovalent H1N1 vaccine strain using influenza A/California/7/2009 [25,26]. The pandemic H1N1 vaccine was produced in a manner similar to seasonal influenza vaccine, underwent the same licensing standards in the United States, and is currently available without adjuvant in either a monovalent live attenuated formulation administered intranasally or in an inactivated formulation for intramuscular injection [25]. The live attenuated formulation is a cold adapted virus that optimally grows at lower temperatures in the nasal mucosa and cannot replicate at core body temperatures. In doing so, replication of the attenuated virus in the upper respiratory tract elicits a systemic and local mucosal immune response. While this technology has been safely employed for influenza immunization since 2003, this formulation should not be administered to certain subsets of the population, including those: 1) younger than 2 or older than 50 years of age; 2) with immunosuppressive conditions; 3) with chronic underlying medical conditions; 4) who are pregnant; 5) with a prior history of Guillain-Barre syndrome; 6) with severe egg allergy; or 7) children/adolescents receiving aspirin therapy. Preliminary evidence from a phase II clinical trial enrolling healthy adults 18 to 64 years using an monovalent inactivated pandemic H1N1 vaccine without adjuvant demonstrated 96.7 percent of subjects had HI titer > 1:40 and 70.8 percent patients seroconverted or had a significant increase in antibody titer after one dose containing 15 µg of HA [27]. Combined with data from other FDA approved manufacturers of pandemic H1N1 vaccine, the immunologic response has been similar to the historical seasonal influenza vaccine efficacy rates of 70 percent to 90 percent in young healthy adults [25]. Based upon experiences with seasonal vaccine (see below), manufacturers note the possibility of being unable to replicate such robust immune responses using the pandemic vaccine in immunocompromised populations or older adults. Since pathogen-associated molecular pattern recognition by the innate immune system guides the formation of the adaptive response, it is important to understand the specific innate immune responses to influenza vaccine and how can they be modified to create more effective vaccine, particularly in older adults. In this report, we will further describe: i) immunosenescence and influenza vaccine efficacy/effectiveness and ii) the innate immune response to seasonal and pandemic H1N1 2009 influenza virus vaccines.

IMMUNOSENESCENCE OF THE ADAPTIVE AND INNATE IMMUNE SYSTEM

It is generally accepted that the seasonal influenza vaccine has decreased efficacy and effectiveness in older adults compared to younger patients. In combined analyses of community dwelling older adults, seasonal vaccines have an effectiveness ranging from 17 percent to 53 percent [28-30]. Biological plausability arises from the multifactorial combination of antigenic match of vaccine and virus, nutritional status, frailty, co-morbid conditions, and immunosenescence of the adaptive and innate immune systems [31]. Broadly, the adaptive immune system protects the host through antigen specific responses and the development of immunologic memory. Changes in the adaptive immune system with aging include decreased numbers of naïve B and T cells, oligoclonality of T and B cell receptors and reduced signaling, decreased replicative ability of T cells, and decreased immunoglobulin class switching capability of B cells [32-34].

The earliest immunologic responses to seasonal and non-seasonal influenza A viruses are led by activation of the innate immune system. In contrast to the adaptive immune system, the innate responses lack

the exceptional antigen specificity and immunologic memory associated with the adaptive immune system. The innate immune system is activated by germline encoded invariant pattern recognition receptors (PRR) that recognize cellular damage or a broad array of pathogens by recognizing unique conserved sequences known as Pathogen-Associated Molecular Patterns (PAMP); important examples of PRR include Toll-Like Receptors (TLR), Nucleotide-binding domain and Leucine-rich-repeat Receptors (NLR), and Retinoic acid-inducible gene-I Like Receptors (RLR). Toll-like receptors are one of the key components of the innate immune system and are found primarily in antigen presenting cells within the cell membrane or endosome. TLRs are important in triggering the host response against PAMP from gram positive and negative bacteria, mycobacteria, and viruses, including lipopeptides and lipoproteins, lipoteichoic acid, unmethylated nucleic acid sequences, lipopolysaccharide, and flagellin. Activation of TLRs result in proinflammatory signaling and is an important link in forming the adaptive immune response. TLR7 is an important endosomal PRR for the ssRNA of influenza virus [35-37]. Once TLR7 recognizes ssRNA, the adaptor protein myeloid differentiation primary response protein 88 (MyD88), via down-stream signal activation of transcription factors, results in the production of type I Interferons (IFNs) and inhibition of viral replication.

Among several cytoplasmic PRR, the NLR family member NLRP3 is involved in recognizing molecules such as alum or viral nucleic acid [38-42]. Upon sensing these ligands, protein interactions among domains of NLRs, the adaptor protein ASC, and Caspase-1 (a cysteine protease) ultimately form the multi-protein inflammasome structure. The inflammasome is responsible for cleaving immature forms of proinflammatory cytokines in a caspase-1 dependent manner to their mature forms protecting the host against a viral infection. Another group of cytoplasmic PRR are the RLR, which include RNA helicases such as RIG-I that recognize 5' triphosphate ssRNA and dsRNA. After infection with influenza A in human macrophages, reorganization of the actin and tubulin skeleton brings RIG-I in proximity to its adaptor molecule Mitochondrial Antiviral Signaling Protein (MAVS). This interaction leads to downstream activation of several nuclear transcription factors ultimately resulting in type I IFN production and the host's antiviral response [43].

In addition to the adaptive immune system, concomitant dysregulation of the innate immune system with aging plays a major role in the response to influenza vaccine and infection in older adults in several ways, including: 1) altered expression, regulation, and function of PRR and costimulatory molecules; 2) altered secretion of inflammatory chemokines, cytokines, and anti-microbial peptides; and 3) decreased function of NK cells, macrophages, and neutrophils [31,32]. Since the innate immune system is linked to subsequent development of adaptive immunity, the combination of these innate defects can lead to reduced adaptive immune responses against a viral pathogen [31,44,45].

There are important age-related defects in several TLR and co-stimulatory molecules that have an impact on a host's response to bacterial or viral PAMP. During the course of an influenza infection, bacterial super-infection may play a major role in morbidity/mortality [9,17]. For example, over a four-month period, 29 percent of 77 post-mortem lung samples from confirmed cases of pandemic H1N1 2009 demonstrated evidence of coexistent bacterial infection [46]. In animal models, bacterial PAMP, such as lipopolysaccharide (a TLR4 ligand), have been used to stimulate macrophages in older mice; the results have shown decreased expression of proinflammatory cytokines: TNF-α and IL-6 [47]. Stimulation of TLR1/2 (which recognize triacylated lipopeptides) in monocytes from older adults yielded a blunted production of TNF-α and IL-6; additionally, TLR1 had decreased surface expression [48]. In normal immunologic responses, TLR activation of monocytes and DCs results in activation of costimulatory molecules CD80 and CD86.

In older adults however, there has been age-related altered expression of these costimulatory molecules that subsequently predicted a seroprotective response to influenza vaccine [49]. More recent studies have indicated more extensive defects in TLR function in specific classes of human dendritic cells from older individuals compared to young individuals that are predictive of antibody response to influenza immunization [50]. Whether there are any age-related defects in NLR and RLR function remains poorly understood; however, the available evidence indicates that alterations in innate immune function have major implications in the response of older adults to seasonal influenza vaccine and related bacterial infections.

INNATE IMMUNE RESPONSE TO ADJUVANT

Adjuvants are compounds co-administered with antigens that alter the physical delivery of antigens or improve the biological activity against the antigen. This clinical application enhances the immunoprotective response (both potency and duration) in populations with traditionally poor responses to vaccines — such as older adults. A significant proportion of the initial response to vaccine adjuvants is facilitated by components of the innate immune system. Although currently not used in U.S.-based formulations of any influenza virus vaccine, adjuvants such as MF59 (a proprietary oil in water emulsion), CpG (a known TLR9 ligand), or alum into muscle results in local infiltration of antigen presenting cells, proinflammatory cytokines, and chemokines [51]. Of note, MF59 adjuvanted vaccines are currently used in Europe. Alum has been shown to recruit cells important in the innate immune response and assist in T cell priming [52]. The innate immune system's response to adjuvant is important in directing the formation of the adaptive immune response. Similar to MF59, the use of alum adjuvanted antigens resulted in improved antigen presentation from monocytes, myeloid DC (mDC), and plasmacytoid DC (pDC) via improved antigen uptake. Additionally, adjuvant increased the expression of costimulatory markers and the differentiation of monocytes into dendritic cells. The combination of these changes in the innate immune system led to more robust adaptive immune responses as measured by proinflammatory cytokines and rate of T cell clonal expansion [53].

Clinical implications of the use of adjuvant recognition by the innate immune system have been improved adaptive immunity in humans immunized with influenza vaccine. The H5N1 pre-pandemic strain with the adjuvant MF59 had a more robust adaptive immunologic response compared to non-adjuvanted version in preclinical trials [54]. The application of adjuvants has had preliminary evidence of a tolerable safety profile for both pandemic and seasonal influenza vaccines [55-57]. Results from a phase 1 clinical trial of inactivated monovalent pandemic H1N1 2009 vaccine with or without MF-59 demonstrated an improved seroprotective response within two weeks of a single dose of adjuvanted 7.5 μg of HA; the geometric mean titers of adjuvanted vaccine were similar at half the standard influenza dose and at an earlier point in time compared to an unadjuvanted inactivated pandemic H1N1 2009 monovalent vaccine [27,55]. Interestingly, a pandemic H1N1 2009 inactivated strain adjuvanted with alum did not enhance the immunogenicity as MF-59 appears to be a more potent inducer of the innate immune system compared to alum [57]. These clinical trials highlight the importance of further studying adjuvanted formulations of influenza vaccine, especially in populations (i.e., older adults) who classically have reduced immunologic responses to vaccines (as discussed above).

CONCLUSIONS AND OUTLOOK

Approaches aimed at improving the immunogenic response to seasonal influenza vaccine in older adults range from higher doses containing 60 μg of HA in the inactivated vaccine to creating vaccines directed

against universal influenza antigens, recombinant trivalent HA antigen alone, DNA vaccines encoding HA, or the development of monoclonal antibodies against highly conserved regions of HA among many influenza strains [58-61]. Since the use of adjuvants in seasonal influenza vaccine improves immunogenicity (especially in vulnerable populations like older adults) through interactions with the innate immune system, future areas of research should concentrate on developing new classes of vaccine adjuvant to improve host protection [62].

The typical seasonal influenza burden on the health care system and global economy have become magnified with the rapid transmission of the pandemic H1N1 2009 strain. Increased mortality in younger patients necessitated the need for an effective vaccine. By translating knowledge of innate immunobiologic responses against vaccines, future strategies will develop more effective vaccines against influenza.

REFERENCES

1. Khiabanian H, Trifonov V, Rabadan R. Reassortment patterns in Swine influenza viruses. PLoS One. 2009;4(10):e7366.
2. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature. 2009;459(7249):931-9.
3. Uyeki TM. Human infection with highly pathogenic avian influenza A (H5N1) virus: review of clinical issues. Clin Infect Dis. 2009;49(2):279-90.
4. Shinde V, Bridges CB, Uyeki TM, et. al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. N Engl J Med. 2009;360(25):2616-25.
5. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science. 2009;325(5937):197-201.
6. Zimmer SM, Burke DS. Historical perspective-Emergence of influenza A (H1N1) viruses. N Eng J Med. 2009;361(3):279-85.
7. Archer B, Cohen C, Naidoo D, et al. Interim report on pandemic H1N1 influenza virus infections in South Africa, April to October 2009: epidemiology and factors associated with fatal cases. Euro Surveill. 2009;14(42):193639.
8. Dominguez-Cherit G, Lapinsky SE, Macias AE, et al. Critically Ill Patients With 2009 Influenza A(H1N1) in Mexico. JAMA. 2009;302(17):1880-7.
9. Kumar A, Zarychanski R, Pinto R, et al. Critically Ill Patients With 2009 Influenza A(H1N1) Infection in Canada. JAMA. 2009;302(17):1872-9.
10. Centers for Disease Control and Prevention. Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. MMWR Morb Mortal Wkly Rep. 2009;58(19):521-4.
11. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med. 2009;361(20):1945-52.
12. Barnard DL. Animal models for the study of influenza pathogenesis and therapy. Antiviral Res. 2009;82(2):A110-22.
13. Itoh Y, Shinya K, Kiso M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature. 2009;460(7258):1021-5.
14. Maines TR, Jayaraman A, Belser JA, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. Science. 2009;325(5939):484-7.
15. Munster VJ, de Wit E, van den Brand JM, et al. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science. 2009;325(5939):481-3.
16. Taubenberger JK, Morens DM. The pathology of influenza virus infections. Annu Rev Pathol. 2008;3:499-522.
17. Jain S, Kamimoto L, Bramley AM, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med. 2009;361(20):1935-44.
18. Centers for Disease Control and Prevention. FluView: 2009-2010 Influenza Season Week 42 ending October 24, 2009 [Internet]. 2009 [cited 2009 Nov 3]; Available from: http://www.cdc.gov/flu/weekly/.
19. Centers for Disease Control and Prevention. FluView: 2008-2009 Influenza Season Week 23 ending June 13, 2009 [Internet]. 2009 [cited 2009 Nov 25]; Available from: http://www.cdc.gov/flu/weekly/weeklyarchives2008-2009/weekly23.htm.
20. Centers for Disease Control and Prevention. Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis — North Carolina, 2009. MMWR Morb Mortal Wkly Rep. 2009;58(35):969-72.
21. Dharan N, Fry AM, Kieke BA, et al., editors. Description of 19 Cases of Oseltamivir-resistant A(H1N1) Enrolled in a Randomized Placebo-controlled Oseltamivir Efficacy Trial, January-February, 2009. 47th Annual Meeting of Infectious Diseases Society of America; 2009 Oct 29-Nov 1; Philadelphia, PA.
22. Centers for Disease Control and Prevention. Emergency Use Authorization of peramivir: fact sheet for health care providers. 2009. [cited 2009 Nov 4]; Available from: http://www.cdc.gov/h1n1flu/eua/Final%20HCP%20Fact%20sheet%20Peramivir%20IV_CDC.pdf.

23. Hayden F. Developing new antiviral agents for influenza treatment: what does the future hold? Clin Infect Dis. 2009;48(Suppl 1):S3-13.
24. Khazeni N, Hutton DW, Garber AM, et al. Effectiveness and Cost-Effectiveness of Vaccination Against Pandemic Influenza (H1N1) 2009. Ann Intern Med [Internet]. 2009 Oct 5. Available from: http://www.annals.org/content/early/2009/10/05/0003-4819-151-12-200912150-00157.full?sid=4553bbc7-8f34-4907-ac2f-ff74c25ca10d.
25. Centers for Disease Control and Prevention. Update on influenza A (H1N1) 2009 monovalent vaccines. MMWR Morb Mortal Wkly Rep. 2009;58(39):1100-1.
26. Global influenza surveillance network:laboratory surveillance and response to pandemic H1N1 2009. Wkly Epidemiol Rec. 2009;84(36):361-5.
27. Greenberg ME, Lai MH, Hartel GF, et al. Response after One Dose of a Monovalent Influenza A (H1N1) 2009 Vaccine — Preliminary Report. N Engl J Med [Internet]. 2009 Sep 10. Available from: http://content.nejm.org/cgi/content/full/NEJMoa0907413?resourcetype=HWCIT.
28. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine. 2006;24(8):1159-69.
29. Jefferson T, Rivetti D, Rivetti A, et al. Efficacy and effectiveness of influenza vaccines in elderly people: a systematic review. Lancet. 2005;366(9492):1165-74.
30. Nichol KL, Nordin JD, Nelson DB, et al. Effectiveness of influenza vaccine in the community-dwelling elderly. N Engl J Med. 2007;357(14):1373-81.
31. Sambhara S, McElhaney JE. Immunosenescence and influenza vaccine efficacy. Curr Top Microbiol Immunol. 2009;333:413-29.
32. Panda A, Arjona A, Sapey E, et al. Human innate immunosenescence: causes and consequences for immunity in old age. Trends Immunol. 2009;30(7):325-33.
33. Grubeck-Loebenstein B, Della Bella S, Iorio AM, et al. Immunosenescence and vaccine failure in the elderly. Aging Clin Exp Res. 2009;21(3):201-9.
34. Kumar R, Burns EA. Age-related decline in immunity: implications for vaccine responsiveness. Expert Rev Vaccines. 2008;7(4):467-79.
35. Diebold SS, Kaisho T, Hemmi H, et al. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004;303(5663):1529-31.
36. Heil F, Hemmi H, Hochrein H, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 2004;303(5663):1526-9.
37. Lund JM, Alexopoulou L, Sato A, et al. Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc Natl Acad Sci USA. 2004;101(15):5598-603.
38. Li H, Willingham SB, Ting JP, et al. Cutting edge: inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. J Immunol. 2008;181(1):17-21.
39. Eisenbarth SC, Colegio OR, O'Connor W, et. al. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminum adjuvants. Nature. 2008;453(7198):1122-6.
40. Thomas PG, Dash P, Aldridge JR Jr., et al. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity. 2009;30(4):566-75.
41. Ichinohe T, Lee HK, Ogura Y, et al. Inflammasome recognition of influenza virus is essential for adaptive immune responses. J Exp Med. 2009;206(1):79-87.
42. Allen IC, Scull MA, Moore CB, et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009;30(4):556-65.
43. Ohman T, Rintahaka J, Kalkkinen N, et al. Actin and RIG-I/MAVS signaling components translocate to mitochondria upon influenza A virus infection of human primary macrophages. J Immunol. 2009;182(9):5682-92.
44. Plowden J, Renshaw-Hoelscher M, Gangappa S, et al. Impaired antigen-induced CD8+ T cell clonal expansion in aging is due to defects in antigen presenting cell function. Cell Immunol. 2004;229(2):86-92.
45. van den Biggelaar AH, Huizinga TW, de Craen AJ, et al. Impaired innate immunity predicts frailty in old age. The Leiden 85-plus study. Exp Gerontol. 2004;39(9):1407-14.
46. Centers for Disease Control and Prevention. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) — United States, May-August 2009. MMWR Morb Mortal Wkly Rep. 2009;58(38):1071-4.
47. Boehmer ED, Goral J, Faunce DE, et al. Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. J Leukoc Biol. 2004;75(2):342-9.
48. van Duin D, Mohanty S, Thomas V, et al. Age-associated defect in human TLR-1/2 function. J Immunol. 2007;178(2):970-5.
49. van Duin D, Allore HG, Mohanty S, et al. Prevaccine determination of the expression of costimulatory B7 molecules in activated monocytes predicts influenza vaccine responses in young and older adults. J Infect Dis. 2007;195(11):1590-7.
50. Panda A, Qian F, Wang P, et al. Age-associated Alteration in TLR-induced Cytokine Production and Alterations in Baseline TLR Protein Expression in DC populations. J Immuno. In Revision.
51. Mosca F, Tritto E, Muzzi A, et al. Molecular and cellular signatures of human vaccine adjuvants.

Proc Natl Acad Sci USA. 2008;105(30):10501-6.
52. McKee AS, Munks MW, MacLeod MK, et al. Alum induces innate immune responses through macrophage and mast cell sensors, but these sensors are not required for alum to act as an adjuvant for specific immunity. J Immunol. 2009;183(7):4403-14.
53. Kool M, Soullié T, van Nimwegen M, et. al. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. J Exp Med. 2008;205(4):869-82.
54. Galli G, Medini D, Borgogni E, et al. Adjuvanted H5N1 vaccine induces early CD4+ T cell response that predicts long-term persistence of protective antibody levels. Proc Natl Acad Sci USA. 2009;106(10):3877-82.
55. Clark TW, Pareek M, Hoschler K, et al. Trial of Influenza A (H1N1) 2009 Monovalent MF59-Adjuvanted Vaccine — Preliminary Report. N Engl J Med [Internet]. 2009 Sep 10. Available from: http://content.nejm.org/cgi/content/full/NEJMoa0907650?resourcetype=HWCIT.
56. Pellegrini M, Nicolay U, Lindert K, et al. MF59-adjuvanted versus non-adjuvanted influenza vaccines: Integrated analysis from a large safety database. Vaccine. 2009;27(49):6959-65.
57. Zhu FC, Wang H, Fang HH, et al. A Novel Influenza A (H1N1) Vaccine in Various Age Groups. N Engl J Med [Internet]. 2009 Oct 21. Available from: http://content.nejm.org/cgi/content/full/NEJMoa0908535?resourcetype=HWCIT.
58. Ekiert DC, Bhabha G, Elsliger MA, et al. Antibody recognition of a highly conserved influenza virus epitope. Science. 2009;324(5924):246-51.
59. Jones S, Evans K, McElwaine-John H, et al. DNA vaccination protects against an influenza challenge in a double-blind randomised placebo-controlled phase 1b clinical trial. Vaccine. 2009;27(18):2506-12.
60. Treanor JJ, Schiff GM, Hayden FG, et al. Safety and immunogenicity of a baculovirus-expressed hemagglutinin influenza vaccine: a randomized controlled trial. JAMA. 2007;297(14):1577-82.
61. Couch RB, Winokur P, Brady R, et al. Safety and immunogenicity of a high dosage trivalent influenza vaccine among elderly subjects. Vaccine. 2007;25(44):7656-63.
62. Monto AS, Ansaldi F, Aspinall R, et al. Influenza control in the 21st century: Optimizing protection of older adults. Vaccine. 2009;27(37):5043-53.