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Influenza vaccination in the elderly: seeking new correlates of protection and improved vaccines

Janet E McElhaney, MD

Department of Medicine, University of British Columbia, Vancouver, Canada and, Center for Immunotherapy of Cancer & Infectious Diseases, University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030-1601, USA, Tel.: +1 604 806 9153, Fax: +1 604 806 8390, jmcclhan@interchange.ubc.ca

Abstract

Influenza is foremost among all infectious diseases for an age-related increase in risk for serious complications and death. Determining the benefit of current influenza vaccines is largely limited to epidemiologic studies, since placebo-controlled trials of influenza vaccines are no longer considered ethical in the older adult population. Vaccine effectiveness is calculated from the relative reduction in influenza outcomes in individuals who elect to be vaccinated compared with those who do not, the assumptions for which are diverse and have led to considerable controversy as to the exact benefit of influenza vaccination in older adults. In spite of this controversy, there is no doubt that new influenza vaccine technologies are needed to improve protection and reverse the trend of rising hospitalization and death rates related to influenza in older adults despite widespread influenza vaccination programs. This article will review the challenges to new vaccine development, explore the potential correlates of protection against influenza, and describe how new vaccine technologies may improve protection against complicated influenza illness in the older adult population.

Keywords

benefits of vaccination; granzymeB; immunosenescence; impact of influenza; older adults; T-cell responses

Impact of influenza in older people & the benefits of vaccination

Influenza viruses are a common cause of respiratory illnesses among persons in all age groups, and typically occur during winter months for those living in temperate climates. The complications of influenza are not uncommon and may include secondary bacterial infections and exacerbations of underlying medical conditions. The elderly are among the groups at highest risk for the serious complications of influenza that might result in hospitalization or death. Each year in the USA, approximately 60% of the nearly 300,000 excess respiratory and circulatory hospitalizations [1], and 85–90% or more of the 31,000–51,000 excess deaths from

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all causes [2], are attributable to influenza that occurs among persons aged 65 years and older. Owing to the limitations of epidemiologic studies, an often unrecognized complication is the risk for catastrophic disability (defined as a loss of three or more basic activities of daily living in older adults) [3], and loss of functional independence as a result of an influenza illness [4, 5]. Given their high risk for these serious, influenza-associated complications, the elderly are included among the high-priority groups for annual influenza vaccination in many countries [6].

Annual vaccination is the mainstay of influenza management

Vaccination programs are cost effective in older people and even cost-saving in developed countries, despite being only 40–60% effective in this population [7–11]. The fact that these vaccines also prevent complications of influenza (pneumonia, heart attacks, strokes and exacerbations of congestive heart failure) provides even greater incentive to increase the use of existing vaccines and develop new vaccines that are targeted to the unique immune function of older people [10, 12]. Current influenza vaccination programs reduce hospitalization rates by 30 to 40% [8, 9]. Even so, and despite widespread immunization, hospitalizations due to influenza-related illnesses continue to rise [1]. This observation has been attributed to aging of the population, rising prevalence of chronic diseases that increase the risk for complicated influenza illness, and circulation of other respiratory illnesses that have similar presentations to influenza in older people [13]. In the absence of vaccination, the risk of hospitalization or death from influenza is increased, particularly in those with chronic diseases. The risk of hospitalization or death in the most vulnerable elderly people (specifically, chronic heart and lung conditions, renal diseases or transplant, dementia or stroke, and hematological and nonhematological cancer) is more than 60-times that of healthy elderly people (65–75 years old) [14, 15]. In the institutional setting, vaccination of healthcare workers is also needed to protect residents in that setting [16]. However, current vaccines are not keeping pace with the changing risk profile of an aging population [17].

Immune response to influenza & influenza vaccination

The challenge of improving protection against influenza in the elderly

In association with the dramatic increase in late-life morbidity and mortality from influenza, there is a decline in cell-mediated immune function and diminished influenza vaccine efficacy in the population aged 65 years and older. A multitude of changes in the immune system occur with aging, but the specific mechanisms that increase risk for influenza illness are poorly understood. The gold standard for determining the protective efficacy of influenza vaccination utilizes the hemagglutination inhibition assay to measure serum antibody titers to influenza hemagglutinin. However, the limitations of this assay as a sole measure of vaccine efficacy in older adults are increasingly recognized. Vaccination of healthy young compared with healthy older adults shows no difference in antibody response or affinity to A/H3N2 strains, even though strains of this influenza subtype disproportionately affect older compared with young adults [1, 2, 18]. Many of the assays that evaluate the cell-mediated immune response are too labor intensive or show significant interassay variability to be practical for the development of alternate correlates of protection.

Influenza virus infection stimulates both humoral & cell-mediated immunity

Influenza virus stimulates an antiviral response in bone-marrow-derived lymphocytes (B cells) and thymus-derived lymphocytes (T cells) resulting in humoral and cell-mediated immunity, respectively. Virus-activated T cells, through a variety of cytokine mediators, stimulate B cells to differentiate and produce antibodies that are specific for the infecting virus or vaccine strains [19]. These specific antibodies bind to the surface glycoproteins hemagglutinin (HA) and

neuraminidase (NA) to neutralize the viral particle. The peptide sequences on the outer surfaces of HA and NA change as a result of selective pressure by the immune system against the native virus, a phenomenon known as antigenic drift. Influenza vaccines are updated annually to stimulate antibody-mediated immunity to the predicted circulating strains of influenza A/H3N2 and A/H1N1 and influenza B.

In contrast to the strain-specific response of B cells, the antigenic determinants of helper T cells (T_h) and cytotoxic T lymphocytes (CTLs) are more conserved across different strains of influenza and the T-cell response does not degrade with antigenic drift [20]. Internal peptide sequences of HA and NA are conserved within the subtypes of influenza (A/H3N2 vs A/H1N1) and the internal viral proteins (matrix and nucleoproteins [NP]) within the types of influenza (influenza A vs B) [21]. Peptides derived from these viral proteins stimulate T_h and CTL responses that are crossreactive within the strains of influenza A or influenza B. Thus, influenza vaccination may provide some protection even when there is a mismatch between the vaccine strain and the circulating strain of influenza.

Elements of a protective immune response

Influenza virus enters the body through the respiratory tract and can be neutralized by influenza strain-specific antibodies on the mucosa. In the absence of adequate levels of neutralizing antibodies, influenza virus is taken up by and replicates in respiratory epithelial cells. Viral replication triggers both the innate and adaptive immune response, the innate immune response producing interferons and antiviral proteins that suppress viral replication until the adaptive response is activated. In the presence of immunologic memory from vaccination or prior exposure to influenza, virus-specific memory T cells can be activated within 1–2 days to more rapidly clear the virus. Once the virus has been cleared, the T-cell enters a memory phase to enable a more rapid response to a subsequent influenza infection. The duration of T-cell memory after influenza vaccination or infection is highly variable, even among younger adults (months to years) [22]. Studies of the response to vaccination in adult populations represent restimulation of previously primed T-cell responses through exposure to natural infection or prior vaccination (reviewed in [23]).

What are the likely correlates of protection against influenza?

Helper T cells and CTLs have different requirements for effective presentation of viral peptides on the major histocompatibility complex (MHC) II and I, respectively [24–27]. Since only T_h and B cells are effectively stimulated by killed or split virus (e.g., the influenza vaccine), vaccination stimulates good antibody responses, but only weak CTL responses in adults; the CTL response results from restimulation of a previously primed response to influenza through natural infection [28–31]. Human studies have confirmed that CTL responses are important for recovery from influenza infection even in the absence of protective antibodies to the infecting virus strain [22]. CTLs combat influenza infection by recognizing MHC I-viral peptide complexes on virus-infected host cells, destroying them before they release infective progeny virus [25]. A direct comparison showed that protection correlates with the virus-specific CTL ($CD8^+$) response in the lungs and associated lymphoid organs. Furthermore, it has been demonstrated that granzyme B (GrzB) released from CTLs is the key cytolytic mediator of apoptotic killing of influenza-infected host cells [32–34]. Although self-renewing populations of virus-specific $CD8$ T cells are maintained for many years after influenza infection, protective cellular immunity is short-lived and disappears within 6 months [35–37]. However, we have demonstrated that memory CTLs from natural infection may be restimulated by influenza vaccination [McElhaney et al., submitted-manuscript], demonstrating the potential for older adults to mount an enhanced CTL (GrzB) response to vaccination.

How do split-virus vaccines stimulate the immune system?

Influenza vaccines are updated annually to include the predicted circulating strains of influenza A/H3N2 and A/H1N1 and influenza B. When influenza vaccine is administered into the deltoid muscle, antigen-presenting cells (APCs, dendritic cells [DCs] and monocytes) and other immune cells are recruited to the site by the inflammatory response to the vaccine (reactogenicity). APCs carrying viral antigens migrate to the regional draining lymph nodes where viral peptides bound to MHC I or II on APC are presented to T_h and CTLs to stimulate both antibody and CTL responses [38]. Virus-specific T-cells bind to the MHC-viral peptide complex and, together with costimulatory and cytokine signals, the T-cell response is activated. MHC II takes up peptides derived from both live and killed virus to stimulate T_h [30,39]. In contrast, MHC I is most effectively loaded with peptides derived from a live virus; although antigen cross-presentation on MHC I does occur in DCs, the process is less effective with killed or split-virus vaccine alone [38]. Thus, while peptides derived from live virus stimulate both T_h and CTLs, peptides derived from split viruses (contained in the current influenza vaccines) stimulate T_h but only weakly stimulate CTLs [25,40,41]. The net result is that current influenza vaccines effectively stimulate the production of vaccine strain-specific antibodies but only weakly boost the crossprotective immunity of pre-existing CTL memory. New adjuvant technologies are being used to facilitate DC activation and presentation of peptides on MHC I, and enhance the CTL responses elicited by the current split-virus vaccines.

Why might differences in the antibody- & cell-mediated immune responses to influenza vaccination be important to protection in older adults?

There has been a paradigm shift in understanding the limitations of antibody titres as a sole measure of influenza efficacy in older people [42]. Aging does not affect the antibody response to influenza vaccination [18,43], and annual repeated vaccination improves protection against influenza [44–46]. Although antibody titres to influenza have been correlated with alterations in T-cell function [47,48], the mechanism for the overall decline in vaccine-mediated protection in older adults has not yet been established. In studies of vaccinated older adults in the community, our work has shown that serum antibody titers measured by the influenza hemagglutination inhibition assay do not distinguish between those who subsequently develop influenza illness from those who do not [41]. This is not to say that antibodies are not an important defense against influenza, but emphasizes the importance of both humoral and cellular immune mechanisms of protection in this population [49]. However, given that age-related changes in immune function mainly impact on T-cell function, assays of this arm of the immune response are needed to evaluate influenza vaccine efficacy in the older population.

It has been well documented that T-cell-mediated immune responses are also protective against influenza infection and illness [50]. In contrast to the strain-specific antibodies that bind to the outer surface of the surface glycoproteins and drive the selection of new influenza strains response of B cells, the protein sequences within HA and NA and internal viral proteins (matrix and nucleoproteins) are more conserved across different strains of influenza and appear not to degrade with antigenic drift [20,51,52]. Thus, T_h and CTL responses to different vaccine strains are largely cross-reactive across the subtypes of influenza A (A/H3N2 vs A/H1N1), and within the influenza A versus influenza B strains [21]. With aging, the memory T-cell response to viral infection may be delayed or diminished and increases the risk for complicated influenza illness. However, the exact mechanism by which immunosenescence leads to an increased risk for respiratory and cardiovascular complications of influenza has yet to be elucidated. Correlates of T-cell-mediated protection following influenza vaccination are key to understanding this increased risk and designing new influenza vaccines to enhance protection in older adults.

How does T_h cytokine regulation impact on the response to influenza vaccination in older adults?

The T_h-mediated immune response to influenza virus plays a key role in the generation of both humoral and CTL responses to influenza vaccination. Previously, T_h1 and T_h2 were defined by their cytokine products such that the T_h1 cytokine IFN- γ downregulated T_h2, and IL-10 downregulated T_h1 [53–55]. Furthermore, it has been shown that T_h1 stimulate influenza virus-specific CTLs while the production of T_h2 cytokines directly suppresses the CTL response to influenza virus [55–57]. While this paradigm is generally applicable in the mouse model, recent studies have questioned the validity of the T_h1/T_h2 paradigm in humans, and the contributions of regulatory T cells (T_{reg} or T_h3) and T_h17 subsets to cytokine regulation are only beginning to be understood [58]. Under a revised model, naive CD4⁺ helper T cells are stimulated by IL-12 to produce IFN- γ (i.e., become T_h1); IL-4 stimulates T_h2 to produce IL-4, -5 and -13; and IL-1, -6 and -23 stimulate T_h17 to produce IL-17, -22 and -26. These T_h subsets have counter-regulatory interactions between each other [59]. Our data demonstrated that the IFN- γ :IL-10 ratio correlates with risk for influenza illness [41], but characteristics of the vaccine recipient and peripheral blood mononuclear cell (PBMC) culture conditions may alter this relationship [60–63]. The apparent downregulation of IFN- γ by IL-10 may be T_h3-mediated rather than a shift from a T_h1 to a T_h2 response [64], and the interaction with T_h17 has not been studied at all. T_h17 promotes the recruitment of IFN- γ -producing T cells and, as such, is regulated by the tissue level of IFN- γ [65]. Recent studies in human PBMCs have demonstrated that T_h17 can simultaneously produce IL-17 and IFN- γ , suggesting that the two cytokines may work synergistically in the adaptive immune response [66]. Given the centrality of the T_h17 subset in immune regulation, their role in the response to influenza vaccination in older adults may also need to be considered.

Implications of a T_h1 versus T_h2 response to influenza vaccination

The T_h-mediated immune response to influenza virus plays a key role in the generation of both humoral- and CTL-mediated responses to influenza vaccination [67]. In addition to the cytokines produced by T_h1 versus T_h2, these T-cell subsets respectively promote IgG2a antibody production and CTL memory, and IgG1. Thus, anti-influenza antibodies produced in response to vaccination may be of the IgG2a and IgG1 subtypes but do not distinguish between a T_h1 (IgG2a) or a T_h2 (IgG1) response or how effectively CTLs are stimulated. As discussed below, the distinction between T_h1 and T_h2 responses may be an important determinant of both the CTL response and outcomes of vaccination in older people. For this reason, antibody titers measured by the traditional hemagglutination inhibition assays may fall short as a sole predictor of vaccine efficacy in older people.

Aging causes a shift toward T_h2 cytokine production & loss of CTL activity

Aging leads to a reduction in IL-2 production. T_h1 responses shift to the production of T_h2 cytokines (IL-4, -5 and -10) with an associated decline in CTL activity [68] and increased risk of pneumococcal pneumonia (when IL-10 is elevated following influenza infection) [69]. However, a review of the literature suggests that the many inconsistencies in these results are due to differences in the health status of the older adults studied and the conditions under which peripheral blood mononuclear cells are stimulated (reviewed in [64]). Studies of the cytokine response to influenza vaccination are prime examples of the significant variability related to characteristics of the vaccine recipient, the vaccine and the PBMC culture conditions [61, 62]. Although absolute cytokine levels may not distinguish between those who will develop influenza illness and those who will not [63], the ratio of IFN- γ :IL-10 reflecting the balance of T_h1 and T_h2 cytokines in the supernatants of *ex vivo* influenza-stimulated PBMCs does appear to correlate with protection against influenza in older adults [41].

Cytotoxic T lymphocytes are critical for clearance of influenza virus & recovery from illness

Cytotoxic T lymphocytes are important for recovery from influenza infection even in the absence of protective antibodies to the infecting virus strain [22]. CTLs combat influenza viral infections by recognizing and destroying virus-infected host cells that become the factories for viral replication. Infected cells expressing on their surfaces the MHC I-viral peptide complex are recognized by and activate virus-specific CTLs. Activated CTLs kill virus-infected cells primarily by perforin/granule-mediated killing, although fas-mediated killing may provide an alternative but less specific mechanism [70]. Self-renewing populations of virus-specific CTLs are maintained in the lymphoid organs for many years after influenza infection, and may be the reason why split-virus vaccines containing internal proteins (killed virus) can stimulate a CTL response. However, this response is probably similar to the duration of CTL-mediated immunity provided by live virus infection [35] and may be even more short-lived in older individuals. Aging results in diminished CTL responses to influenza challenge, delays in peak CTL activity and a loss of cross-reactive responses that delay recovery from illness [71]. Although influenza vaccines stimulate a CTL response in older and even chronically ill adults [72], this response is diminished in older compared with young adults [73]. The cross-reactivity of CTLs between subtypes of influenza is further impaired in chronically ill compared with healthy older adults [72,⁷³].

How is granzyme involved in CTL-mediated apoptosis?

Virus-specific killing is mediated by granzymes and perforin contained in granules within CTLs. Granules migrate to the 'immune synapse' between the activated CTLs and the virus-infected target cell, are transported across the cell membrane into the cytoplasm of the target cell, and are involved in an enzymatic cascade that leads to apoptotic cell death [74]. GrzB is a key element of the T-cell response to influenza in the lung [32,³⁴]. Indeed, an assay of the GrzB activity correlates with cytolytic activity by standard ⁵¹Cr-release assays [75,⁷⁶]. Studies of the GrzB response to influenza vaccination have also demonstrated a progressive decline in GrzB levels from young to older and frail older adults [77], and low levels of GrzB are associated with increased risk of influenza illness [63]. *Ex vivo* studies have shown no difference in influenza-specific CTL frequencies [78]. Taken together, these results suggest that in influenza-susceptible older persons, there is a defect in the quality more than the quantity of CTL responding to influenza virus challenge. The GrzB assay incorporates a relatively simple method for challenging PBMCs with live virus, a 'test tube' assay of a critical component of antiviral immunity. Using a proposed threshold level of GrzB activity in this assay as a correlate of protection, potential defects in the cell-mediated immune response that lead to loss of vaccine efficacy in older adults can be explored. Novel strategies for stimulating CTLs are key to the development of more effective influenza vaccines for older people.

Advances in vaccine technology & delivery

Strategies for improving influenza vaccines

A variety of new influenza vaccines are in various stages of the development pipeline to directly address the unmet need of improved vaccines in older adults for both seasonal and pandemic influenza. These include live-attenuated and intranasal vaccines, oil-in-water emulsion adjuvants added to split-virus vaccines, virosomal and virus-like particle vaccines, Toll-like receptor (TLR) ligands and heat-shock proteins. Live-attenuated vaccines contain cold-adapted viruses that infect and are limited to replication in the nasal mucosa to stimulate both humoral and cell-mediated immunity. Only one formulation (Flumist[®], Medimmune, Gaithersburg, MD, USA) is currently registered for use in children and adults 2–49 years of age; other live-attenuated formulations currently under development are still many years from registration. However, in spite of the theoretical advantage of overcoming age-related changes in T-cell-mediated immune responses, current live-attenuated intranasal vaccines may not offer

enhanced protection over split-virus vaccines in the older adults [79,⁸⁰] and, thus, have not been approved for use in this population.

Novel strategies being explored to augment a T_H1 response include the addition of adjuvants to split-virus vaccines. Alum adjuvants are the only approved adjuvants, but alum, when combined with split-virus vaccine, stimulates a T_H2 response to increase antibody production and is, thus, not suitable for vaccines in older adults. Newer adjuvants, such as MF59-adjuvanted [81] and other proprietary adjuvanted split-virus vaccines, have demonstrated enhanced immunogenicity over conventional split-virus vaccines in older adults. The increased reactogenicity of these adjuvanted vaccines at the site of injection theoretically enhances the recruitment of immune cells and activation of DCs for more effective antigen pres- and CTLs. Whole-virus influenza entation to T_H vaccines have also been previously demonstrated to activate DCs and stimulate higher levels of T_H1 cytokines [82] in comparison with split-virus vaccines, and potentially have a similar capacity to stimulate broad protection in older adults. The potential clinical benefits of these new technologies, beyond that provided by intramuscular administration of current split-virus vaccines, await randomized, controlled trials to determine differences in clinical outcomes of these different vaccination strategies.

Virosomal vaccines are reconstituted virus envelopes that, after DC uptake and fusion with the endosomal membrane, deliver viral proteins into the cytoplasm, the peptides from which are loaded onto MHC I to stimulate CTLs (reviewed in [83]). Virosomal influenza vaccines containing HA, currently marketed in Europe, have also shown similar serologic responses to conventional split-virus vaccines in older adults, but potentially with increased tolerability to other adjuvanted split-virus vaccines [84]. These virosomes have the potential to be engineered to also contain internal proteins of the influenza virus and stimulate both humoral and CTL responses [85,⁸⁶]. Cytokines added to these virosomes have also been demonstrated to enhance the immunogenicity of these vaccines [87]. Influenza virosomal vaccines also offer the possibility of combining them with adjuvants to stimulate the broader immune response necessary for protection against influenza in older people.

Influenza virus-like particles (VLPs) are a new generation of non-egg or non-mammalian cell culture-based candidate influenza vaccines and have similar properties to virosomal vaccines, but offer an advantage over the egg-based manufacturing of the influenza virus required for production of the virosomes. These vaccines are produced from baculovirus vectors encoding HA, NA and matrix 1 protein. VLPs stimulate antibody responses consistent with a T_H1 cytokine response in animal models [88], but have yet to be tested in people. As with virosomes, these vaccines may be combined with adjuvants to improve and broaden protection against different strains of influenza.

The discovery of TLRs has opened the door to an entirely new strategy for vaccine development. Conserved microbial products, termed pathogen-associated molecular patterns (PAMPs) are recognized by TLRs expressed on DCs and macrophages [89]. Pathogen sensors for influenza play a crucial role in the early innate immune response to influenza A virus in the lungs, and initiate the transition from innate to the antigen-specific adaptive immune response. Pathogen-associated molecular patterns recognized by TLRs include the replicative intermediate double-stranded RNA of influenza virus recognized by TLR3, and the negative single-stranded RNA genome of influenza virus recognized by TLR7 [90]. TLRs have been shown to mediate the type I IFN-dependent and proinflammatory response to influenza virus; IL-12 and other proinflammatory cytokines stimulate T_H1 cytokine production, creating the necessary microenvironment to activate CTLs. Although defects at the level of TLR expression and function may increase the susceptibility to influenza in older adults [91], one of the most promising approaches is the combination of agonistic TLR ligands (such as poly I:C and CpG) with influenza proteins/peptides. These approaches have been developed in the mouse model

[92], and stimulation of both heterologous and heterotypic immunity has been reported [93]. Early results in our laboratory demonstrated that TLR ligands combined with split-virus vaccine may improve the Th1:Th2 and CTL response to influenza challenge in older adult PBMCs [McElhaney JE, Unpublished Data].

Novel delivery systems are also in development and may provide access to a different compartment of the immune system. Although a previous study suggested that split-virus vaccine delivered by intradermal injection was not effective as a vaccine dose-sparing strategy in older adults, this may have been owing to inconsistent delivery of the vaccine to the intradermal compartment. However, a newly designed intradermal delivery system has demonstrated that this new microinjection system appears to enhance the antibody response to vaccination in older adults [94]. Whether or not this enhanced response correlates with improved protection against influenza in this population remains to be determined [95].

Future perspective

Future vaccine development will require a multifaceted approach to reverse age-related changes at multiple levels of the cellular immune response that are critical to protect against influenza. A better understanding of how age and the chronic diseases that commonly affect older adults and increase influenza risk affect immune responses to vaccination and protection from influenza are needed. The challenge to new vaccine development for older adults is to stimulate the senescent immune response in ways that may not be measured using standard techniques such as antibody titres to predict vaccine efficacy. Comparing antibody responses alone to different vaccine formulations may fail to detect important changes in cellular immunity that will enhance protection against influenza illness in older people, and potentially eliminate more potent vaccine candidates in the early phases of clinical development. Future efforts to develop alternative correlates of clinical protection against influenza are needed for more effective translation of novel vaccination strategies to improved protection against influenza in older adults. In the absence of additional immunologic surrogates of clinical outcomes, large-scale clinical trials will be required to adequately screen new vaccines for potential improved efficacy in older people.

Conclusion

Influenza vaccination is a cost-saving intervention in older adults, but aging of the population and particularly the increasing prevalence of chronic diseases, has led to increasing hospitalization and death rates despite widespread influenza vaccination programs. A lack of understanding of how aging and common chronic diseases in older adults affect the immune response to influenza presents a significant barrier to the development of new influenza vaccines. Novel influenza vaccines and delivery systems offer renewed hope that more effective vaccines can be developed for older adults. However, clinical trials of these new vaccines in older adults remain a high-risk venture in the absence of alternate correlates of protection as adjuncts to antibody titers to fully evaluate the immune response in the early phases of clinical trials.

Executive summary

Introduction

- Each year in the USA, approximately 60% of the nearly 300,000 excess hospitalizations and more than 85–90% of the 31,000–51,000 excess deaths attributable to influenza illness, occur among persons aged 65 years and older.

- Rising rates of hospitalization due to complications of influenza may be attributed to the increased prevalence of chronic diseases and frailty as the population ages.
- Increased risk for complicated influenza illness and diminished influenza vaccine efficacy are related to immunosenescence – the decline in immune function with aging.

Immune response to influenza & influenza vaccination

- The current split-virus influenza vaccines stimulate helper T-cell responses to support antibody production, but are poor stimulators of cytotoxic T lymphocytes (CTLs).
- CTL function declines with age, thus limiting the ability of older persons to clear the virus from their lungs.
- Antibody responses to influenza vaccination in older adults may fail to result in protection against influenza in older adults.
- Novel correlates of protection based on the T-cell response to vaccination as adjuncts to measures of the antibody response are needed in the early phases of clinical trials in order to select the most promising new influenza vaccines to advance to Phase III clinical trials.

Advances in vaccine technology & delivery

- Vaccine adjuvants, virosomal/virus-like particle vaccines and Toll-like receptor ligands are strategies being used in novel vaccine technologies to improve protection against influenza in older adults.
- Alternate delivery systems to intramuscular injection are also being tested.

Future perspective

- A greater understanding of the basic mechanisms of immunosenescence is needed to determine which components of the immune response need to be targeted in vaccine development in order to achieve improved protection against influenza in older adults.
- Alternate correlates of protection can be developed using a mechanistic approach, but require studies of the immune response to influenza vaccination that are linked to influenza outcomes in this population.

Conclusion

- Demonstrating that new influenza vaccines offer improved protection over current split-virus vaccines in older adults will continue to be a high-risk venture in the absence of alternate correlates of protection as adjuncts to antibody titers.

Bibliography

Papers of special note have been highlighted as:

- of interest
- of considerable interest

- 1 ••. Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. JAMA 2004;292:1333–1340. Hospitalizations increased over the past 2 decades despite widespread influenza vaccination programs. [PubMed: 15367555]

- 2 ••. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;289:179–186. A/H3N2 strains are most virulent in older adults, followed by RSV, influenza B and influenza A/H1N1; these results contrast with differences in antibody responses in young and older adults. [PubMed: 12517228]
- 3 •. Ferrucci L, Guralnik JM, Pahor M, Corti MC, Havlik RJ. Hospital diagnoses, Medicare charges, and nursing home admissions in the year when older persons become severely disabled (see comments). *JAMA* 1997;277:728–734. Defines catastrophic disability and association with influenza and pneumonia as well as other potential complications of influenza. [PubMed: 9042845]
4. Barker WH, Borisute H, Cox C. A study of the impact of influenza on the functional status of frail older people. *Arch Intern Med* 1998;158:645–650. [PubMed: 9521230]
- 5 •. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 2005;352:1749–1759. Impact of influenza on hospital length of stay in a highly vaccinated population, and impact of influenza illness on activities of daily living. [PubMed: 15858184]
6. van Essen GA, Palache AM, Forleo E, Fedson DS. Influenza vaccination in 2000: recommendations and vaccine use in 50 developed and rapidly developing countries. *Vaccine* 2003;21:1780–1785. [PubMed: 12686094]
- 7 •. Govaert TM, Thijs CT, Masurel N, Sprenger MJ, Dinant GJ, Knottnerus JA. The efficacy of influenza vaccination in elderly individuals: a randomized double-blind placebo-controlled trial. *JAMA* 1994;272:1661–1665. The only published placebo-controlled, randomized trial of influenza vaccination in older adults, estimating vaccine efficacy at 50–60%; annually vaccinated older adults were better protected than first-time vaccinees. [PubMed: 7966893]
8. Nichol KL, Margolis KL, Wuorenma J, Von Sternberg T. The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community. *N Engl J Med* 1994;331:778–784. [PubMed: 8065407]
9. Vu T, Farish S, Jenkins M, Kelly H. A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community. *Vaccine* 2002;20:1831–1836. [PubMed: 11906772]
10. Nichol KL. The efficacy, effectiveness and cost-effectiveness of inactivated influenza virus vaccines. *Vaccine* 2003;21:1769–1775. [PubMed: 12686092]
- 11 ••. Nichol KL, Nordin JD, Nelson DB, Mullooly JP, Hak E. Effectiveness of influenza vaccine in the community-dwelling elderly. *N Engl J Med* 2007;357:1373–1381. Analysis of ten influenza seasons comparing hospitalizations and deaths during the influenza season to a control period that follows the influenza season to demonstrate vaccine effectiveness. [PubMed: 17914038]
12. Nichol KL, Nordin J, Mullooly J, Lask R, Fillbrandt K, Iwane M. Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. *N Engl J Med* 2003;348:1322–1332. [PubMed: 12672859]
- 13 •. Glezen WP, Greenberg SB, Atmar RL, Piedra PA, Couch RB. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000;283:499–505. Increasing hospitalization rates may be attributable to the increasing prevalence of chronic diseases in older adults, in addition to aging of the population. [PubMed: 10659876]
14. Hak E, Nordin J, Wei F, et al. Influence of high-risk medical conditions on the effectiveness of influenza vaccination among elderly members of 3 large managed-care organizations. *Clin Infect Dis* 2002;35:370–377. [PubMed: 12145718]
15. Hak E, Wei F, Nordin J, Mullooly J, Poblete S, Nichol KL. Development and validation of a clinical prediction rule for hospitalization due to pneumonia or influenza or death during influenza epidemics among community-dwelling elderly persons. *J Infect Dis* 2004;189:450–458. [PubMed: 14745702]
16. Potter J, Stott DJ, Roberts MA, et al. Influenza vaccination of health care workers in long-term-care hospitals reduces the mortality of elderly patients. *J Infect Dis* 1997;175:1–6. [PubMed: 8985189]
- 17 •. Simonsen L, Taylor RJ, Viboud C, Miller MA, Jackson LA. Mortality benefits of influenza vaccination in elderly people: an ongoing controversy. *Lancet Infect Dis* 2007;7:658–666. The benefit of influenza vaccination for preventing mortality in older adults is questioned in an analysis comparing deaths during the winter months to a control period in the preceding summer months. [PubMed: 17897608]

- 18 • de Bruijn IA, Remarque EJ, Jolvan der Zijde CM, van Tol MJ, Westendorp RG, Knook DL. Quality and quantity of the humoral immune response in healthy elderly and young subjects after annually repeated influenza vaccination. *J Infect Dis* 1999;179:31–36. No difference in antibody response to influenza vaccination between young and older adults was found when vaccination history could be included in the analysis. [PubMed: 9841819]
19. Virelizier JL, Postlethwaite R, Schild GC, Allison AC. Antibody responses to antigenic determinants of influenza virus hemagglutinin I Thymus dependence of antibody formation and thymus independence of immunological memory . *J Exp Med* 1974;140:1559–1570. [PubMed: 4139233]
- 20 •. Effros RB, Doherty PC, Gerhard W, Bennink J. Generation of both cross-reactive and virus-specific T-cell populations after immunization with serologically distinct influenza A viruses. *J Exp Med* 1977;145:557–568. Cross-reactivity of T-cell responses between different strains and subtypes of influenza A demonstrated over 30 years ago, but correlates of T-cell mediated protection have yet to be established. [PubMed: 233901]
21. Mitchell DM, Callard RE. Fine specificity of the *in vitro* antibody response to influenza virus by human blood lymphocytes. *J Immunol* 1983;131:1229–1233. [PubMed: 6350452]
- 22 ••. McMichael AJ, Gotch FM, Noble GR, Beare PA. Cytotoxic T-cell immunity to influenza. *N Engl J Med* 1983;309:13–17. Demonstrates cytotoxic T lymphocyte (CTL)-mediated protection in people even in the absence of protective antibody titers. [PubMed: 6602294]
23. Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines . *Scand J Immunol* 2004;59:1–15. [PubMed: 14723616]
24. Hackett CJ, Eisenlohr LC. Virus entry and antigen biosynthesis in the processing and presentation of class-II MHC-restricted T-cell determinants of influenza virus . *Immunol Res* 1990;9:103–114. [PubMed: 2140852]
- 25 •. Ennis FA, Rook AH, Qi YH, et al. HLA restricted virus-specific cytotoxic T-lymphocyte responses to live and inactivated influenza vaccines. *Lancet* 1981;2:887–891. Early studies demonstrated differences in the CTL response to live and inactivated influenza virus. [PubMed: 6117682]
26. Demotz S, Grey HM, Sette A. The minimal number of class II MHC-antigen complexes needed for T cell activation. *Science* 1990;249:1028–1030. [PubMed: 2118680]
27. Rock KL, Shen L. Cross-presentation: underlying mechanisms and role of immune surveillance. *Immunol Rev* 2005;207:166–183. [PubMed: 16181335]
28. Hackett CJ, Yewdell JW, Bennink JR, Wysocka M. Class II MHC-restricted T cell determinants processed from either endosomes or the cytosol show similar requirements for host protein transport but different kinetics of presentation. *J Immunol* 1991;146:2944–2951. [PubMed: 1673146]
29. Lamb CA, Yewdell JW, Bennink JR, Cresswell P. Invariant chain targets HLA class II molecules to acidic endosomes containing internalized influenza virus. *Proc Natl Acad Sci USA* 1991;88:5998–6002. [PubMed: 2068076]
30. Askonas BA, Mullbacher A, Ashman RB. Cytotoxic T-memory cells in virus infection and the specificity of helper T cells. *Immunology* 45:79–84.
31. Hosaka Y, Sasao F, Yamanaka K, Bennink JR, Yewdell JW. Recognition of noninfectious influenza virus by class I-restricted murine cytotoxic T lymphocytes. *J Immunol* 1982;140:606–610. 1988. [PubMed: 2826596]
32. Johnson BJ, Costelloe EO, Fitzpatrick DR, et al. Single-cell perforin and granzyme expression reveals the anatomical localization of effector CD8⁺ T cells in influenza virus-infected mice. *Proc Natl Acad Sci USA* 2003;100:2657–2662. [PubMed: 12601154]
33. Lord SJ, Rajotte RV, Korbitt GS, Bleackley RC. Granzyme B: a natural born killer . *Immunol Rev* 2003;193:31–38. [PubMed: 12752668]
34. Lawrence CW, Ream RM, Braciale TJ. Frequency, specificity, and sites of expansion of CD8⁺ T cells during primary pulmonary influenza virus infection. *J Immunol* 2005;174:5332–5340. [PubMed: 15843530]
35. McMichael AJ, Gotch FM, Dongworth DW, Clark A, Potter CW. Declining T-cell immunity to influenza, 1977–1982 . *Lancet* 1983;2:762–764. [PubMed: 6137602]

36. Nguyen HH, Moldoveanu Z, Novak MJ, et al. Heterosubtypic immunity to lethal influenza A virus infection is associated with virus-specific CD8⁽⁺⁾ cytotoxic T lymphocyte responses induced in mucosa-associated tissues. *Virology* 1999;254:50–60. [PubMed: 9927573]
37. Ray SJ, Franki SN, Pierce RH, et al. The collagen binding $\alpha\beta 1$ integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection. *Immunity* 2004;20:167–179. [PubMed: 14975239]
38. Ennis FA, Yi-Hua Q, Schild GC. Antibody and cytotoxic T lymphocyte responses of humans to live and inactivated influenza vaccines. *J Gen Virol* 1982;58:273–281. [PubMed: 7038041]
39. Blazevic V, Trubey CM, Shearer GM. Analysis of the costimulatory requirements for generating human virus-specific *in vitro* T helper and effector responses. *J Clin Immunol* 2001;21:293–302. [PubMed: 11506200]
- 40 • Powers DC, Belshe RB. Effect of age on cytotoxic T lymphocyte memory as well as serum and local antibody responses elicited by inactivated influenza virus vaccine. *J Infect Dis* 1993;167:584–592. Demonstrates that a CTL response could be elicited by inactivated influenza vaccine in healthy older adults, but the response was significantly lower compared with young adults. [PubMed: 8440930]
- 41 • McElhaney JE, Xie D, Hager WD, et al. T cell responses are better correlates of vaccine protection in the elderly. *J Immunol* 2006;176:6333–6339. Prior to onset of influenza illness, *ex vivo* GrzB levels and IFN- γ :IL-10 ratios, and not antibody titers, distinguish those who will develop influenza illness from those who do not. [PubMed: 16670345]
42. Effros RB. Role of T lymphocyte replicative senescence in vaccine efficacy. *Vaccine* 2007;25:599–604. [PubMed: 17014937]
43. de Bruijn IA, Remarque EJ, Beyer WE, le Cessie S, Masurel N, Ligthart GJ. Annually repeated influenza vaccination improves humoral responses to several influenza virus strains in healthy elderly. *Vaccine* 1997;15:1323–1329. [PubMed: 9302738]
44. Keitel WA, Cate TR, Couch RB. Efficacy of sequential annual vaccination with inactivated influenza virus vaccine. *Am J Epidemiol* 1988;127:353–364. [PubMed: 3337087]
- 45 • Ahmed AE, Nicholson KG, Nguyen-Van-Tam JS. Reduction in mortality associated with influenza vaccine during 1989–1990 epidemic. *Lancet* 1995;346:591–595. Repeated annual vaccination is more effective than first-time vaccination for protecting against influenza illness in older adults. [PubMed: 7651002]
46. Voordouw AC, Sturkenboom MC, Dieleman JP, et al. Annual revaccination against influenza and mortality risk in community-dwelling elderly persons. *JAMA* 2004;292:2089–2095. [PubMed: 15523069]
- 47 • Goronzy JJ, Fulbright JW, Crowson CS, Poland GA, O’Fallon WM, Weyand CM. Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. *J Virol* 2001;75:12182–12187. Cellular immune markers predict the antibody response to influenza vaccination in older adults. [PubMed: 11711609]
- 48 • Saurwein-Teissl M, Lung TL, Marx F, et al. Lack of antibody production following immunization in old age: association with CD8⁽⁺⁾CD28⁽⁻⁾ T cell clonal expansions and an imbalance in the production of T_H1 and T_H2 cytokines. *J Immunol* 2002;168:5893–5899. Age-related changes in cellular immune markers predict a lack of antibody response to influenza vaccination. [PubMed: 12023394]
49. Murasko DM, Bernstein ED, Gardner EM, et al. Role of humoral and cell-mediated immunity in protection from influenza disease after immunization of healthy elderly. *Exp Gerontol* 2002;37:427–439. [PubMed: 11772530]
50. Thomas PG, Keating R, Hulse-Post DJ, Doherty PC. Cell-mediated protection in influenza infection. *Emerg Infect Dis* 2006;12:48–54. [PubMed: 16494717]
51. Butchko GM, Armstrong RB, Ennis FA. Specificity studies on the proliferative response of thymus-derived lymphocytes to influenza viruses. *J Immunol* 1978;121:2381–2385. [PubMed: 82584]
52. Russell SM, Liew FY. T cells primed by influenza virion internal components can cooperate in the antibody response to haemagglutinin. *Nature* 1979;280:147–148. [PubMed: 317881]

53. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone I Definition according to profiles of lymphokine activities and secreted proteins . J Immunol 1986;136:2348–2357. [PubMed: 2419430]
54. Scherle PA, Gerhard W. Differential ability of B cells specific for external vs. internal influenza virus proteins to respond to help from influenza virus-specific T-cell clones *in vivo*. Proc Natl Acad Sci USA 1988;85:4446–4450. [PubMed: 3260034]
55. Palladino G, Scherle PA, Gerhard W. Activity of CD4⁺ T-cell clones of type 1 and type 2 in generation of influenza virus-specific cytotoxic responses *in vitro*. J Virol 1991;65:6071–6076. [PubMed: 1920626]
56. Feltquate DM, Heaney S, Webster RG, Robinson HL. Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization . J Immunol 1997;158:2278–2284. [PubMed: 9036975]
57. Zhang Y, Acuna CL, Switzer KC, Song L, Sayers R, Mbawuike IN. Corrective effects of interleukin-12 on age-related deficiencies in IFN- γ production and IL-12R β 2 expression in virus-specific CD8⁺ T cells. J Interferon Cytokine Res 2000;20:235–245. [PubMed: 10714560]
58. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1b and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol 2007;8:942–949. [PubMed: 17676045]
59. Laurence A, O'Shea JJ. TH-17 differentiation: of mice and men . Nat Immunol 2007;8:903–905. [PubMed: 17712339]
60. McElhaney JE, Gravenstein S, Upshaw CM, Hooton JW, Krause P, Drinka P. Immune response to influenza vaccination in institutionalized elderly: effect on different T-cell subsets. Vaccine 1998;16:403–409. [PubMed: 9607063]
61. McElhaney JE, Upshaw CM, Hooton JW, Lechelt KE, Meneilly GS. Responses to influenza vaccination in different T-cell subsets: a comparison of healthy young and older adults . Vaccine 1998;16:1742–1747. [PubMed: 9778750]
- 62 • Bernstein E, Kaye D, Abrutyn E, Gross P, Dorfman M, Murasko DM. Immune response to influenza vaccination in a large healthy elderly population. Vaccine 1999;17:82–94. Demonstrated an age-related decline in the cytokine response to influenza vaccination in a comparison between healthy young and older adults. [PubMed: 10078611]
63. McElhaney JE, Gravenstein S, Upshaw CM, et al. Granzyme B: a marker of risk for influenza in institutionalized older adults . Vaccine 2001;19:3744–3751. [PubMed: 11395209]
64. Gardner EM, Murasko DM. Age-related changes in Type 1 and Type 2 cytokine production in humans . Biogerontology 2002;3:271–290. [PubMed: 12237564]
65. Khader SA, Bell GK, Pearl JE, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. Nat Immunol 2007;8:369–377. [PubMed: 17351619]
66. Acosta-Rodriguez EV, Rivino L, Geginat J, et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells . Nat Immunol 2007;8:639–646. [PubMed: 17486092]
- 67 • Yewdell JW, Bennink JR, Hosaka Y. Cells process exogenous proteins for recognition by cytotoxic T lymphocytes. Science 1988;239:637–640. Early demonstration of antigen cross-presentation suggested that peptides derived from killed virus could be presented on MHC I and, thus, stimulate a CTL response to influenza vaccination. [PubMed: 3257585]
68. Castle S, Uyemura K, Wong W, Modlin R, Effros R. Evidence of enhanced type 2 immune response and impaired upregulation of a type 1 response in frail elderly nursing home residents. Mech Ageing Dev 1997;94:7–16. [PubMed: 9147356]
69. van der Sluijs KF, van Elden LJ, Nijhuis M, et al. IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection . J Immunol 2004;172:7603–7609. [PubMed: 15187140]
- 70 • Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD4⁺ and CD8⁺ T-cell mechanisms in the control of respiratory virus infections. Immunol Rev 1997;159:105–117. Review of effector mechanisms that control influenza infection. [PubMed: 9416506]

71. Bender BS, Small PA Jr. Heterotypic immune mice lose protection against influenza virus infection with senescence . *J Infect Dis* 1993;168:873–880. [PubMed: 8376832]
72. Gorse GJ, Belshe RB. Enhancement of anti-influenza A virus cytotoxicity following influenza A virus vaccination in older, chronically ill adults . *J Clin Microbiol* 1990;28:2539–2550. [PubMed: 2123886]
73. Powers DC. Influenza A virus-specific cytotoxic T lymphocyte activity declines with advancing age (see comments) . *J Am Geriatr Soc* 1993;41:1–5. [PubMed: 8418115]
74. Darmon AJ, Nicholson DW, Bleackley RC. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B . *Nature* 1995;377:446–448. [PubMed: 7566124]
75. McElhaney JE, Pinkoski MJ, Upshaw CM, Bleackley RC. The cell-mediated cytotoxic response to influenza vaccination using an assay for granzyme B activity . *J Immunol Methods* 1996;190:11–20. [PubMed: 8601703]
76. Ewen C, Kane KP, Shostak I, et al. A novel cytotoxicity assay to evaluate antigen-specific CTL responses using a colorimetric substrate for Granzyme B . *J Immunol Methods* 2003;276:89–101. [PubMed: 12738362]
77. McElhaney JE. The unmet need in the elderly: designing new influenza vaccines for older adults . *Vaccine* 2005;23 (Suppl 1):S10–S25. [PubMed: 15908062]
78. Boon AC, Fringuelli E, Graus YM, et al. Influenza A virus specific T cell immunity in humans during aging . *Virology* 2002;299:100–108. [PubMed: 12167345]
79. Treanor JJ, Mattison HR, Dumyati G, et al. Protective efficacy of combined live intranasal and inactivated influenza A virus vaccines in the elderly . *Ann Intern Med* 1992;117:625–633. [PubMed: 1530193]
80. Gorse GJ, O'Connor TZ, Young SL, et al. Efficacy trial of live, cold-adapted and inactivated influenza virus vaccines in older adults with chronic obstructive pulmonary disease: a VA cooperative study . *Vaccine* 2003;21:2133–2144. [PubMed: 12706704]
81. Giudice GD, Hilbert AK, Bugarini R, et al. An MF59-adjuvanted inactivated influenza vaccine containing A/Panama/1999 (H3N2) induced broader serological protection against heterovariant influenza virus strain A/Fujian/2002 than a subunit and a split influenza vaccine . *Vaccine* 2006;24:3063–3065. [PubMed: 16464520]
82. Saurwein-Teissl M, Zisterer K, Schmitt TL, Gluck R, Cryz S, Grubeck-Loebenstien B. Whole virus influenza vaccine activates dendritic cells (DC) and stimulates cytokine production by peripheral blood mononuclear cells (PBMC) while subunit vaccines support T cell proliferation. *Clin Exp Immunol* 1998;114:271–276. [PubMed: 9822287]
83. Gluck R, Metcalfe IC. Novel approaches in the development of immunopotentiating reconstituted influenza virosomes as efficient antigen carrier systems . *Vaccine* 2003;21:611–615. [PubMed: 12531327]
84. de Bruijn IA, Nauta J, Gerez L, Palache AM. The virosomal influenza vaccine Invivac[®]: Immunogenicity and tolerability compared with an adjuvanted influenza vaccine (Fluad[®]) in elderly subjects. *Vaccine* 2006;24:6629–6631. [PubMed: 16901593]
85. Bungener L, Idema J, ter Veer W, Huckriede A, Daemen T, Wilschut J. Virosomes in vaccine development: induction of cytotoxic T lymphocyte activity with virosome-encapsulated protein antigens. *J Liposome Res* 2002;12:155–163. [PubMed: 12604050]
86. Huckriede A, Bungener L, Stegmann T, et al. The virosome concept for influenza vaccines . *Vaccine* 2005;23(Suppl 1):S26–S38. [PubMed: 16026906]
87. Ben-Yehuda A, Joseph A, Barenholz Y, et al. Immunogenicity and safety of a novel IL-2-supplemented liposomal influenza vaccine (INFLUSOME-VAC) in nursing-home residents . *Vaccine* 2003;21:3169–3178. [PubMed: 12804845]
88. Bright RA, Carter DM, Daniluk S, et al. Influenza virus-like particles elicit broader immune responses than whole virion inactivated influenza virus or recombinant hemagglutinin . *Vaccine* 2007;25:3871–3878. [PubMed: 17337102]
89. Medzhitov R, Janeway C Jr. The Toll receptor family and microbial recognition. *Trends Microbiol* 2000;8:452–456. [PubMed: 11044679]
90. 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;101:5598–5603. [PubMed: 15034168]

- 91 • Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. *J Immunol* 2002;169:4697–4701. Toll-like receptor expression and function decline with aging and, thus, a potential mechanism that could be targeted in the design of new influenza vaccines for older adults. [PubMed: 12391175]
92. Takeshita F, Tanaka T, Matsuda T, et al. Toll-like receptor adaptor molecules enhance DNA-raised adaptive immune responses against influenza and tumors through activation of innate immunity. *J Virol* 2006;80:6218–6224. [PubMed: 16775309]
93. Seo SU, Lee KH, Byun YH, Kweon MN, Seong BL. Immediate and broad-spectrum protection against heterologous and heterotypic lethal challenge in mice by live influenza vaccine. *Vaccine* 2007;25:8067–8076. [PubMed: 17919786]
94. Holland D, Booy R, De Looze F, et al. Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. *J Infect Dis* 2008;198:650–658. [PubMed: 18652550]
95. McElhaney JE, Dutz JP. Better influenza vaccines for older people: what will it take? *J Infect Dis* 2008;198:632–634. [PubMed: 18652548]