Published in final edited form as:
Ageing Res Rev. 2011 July ; 10(3): 379–388. doi:10.1016/j.arr.2010.10.008.

Influenza Vaccine Responses in Older Adults

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Abstract

The most profound consequences of immune senescence with respect to public health are the increased susceptibility to influenza and loss of efficacy of the current split-virus influenza vaccines in older adults, which are otherwise very effective in younger populations. Influenza infection is associated with high rates of complicated illness including pneumonia, heart attacks and strokes in the 65+ population. Changes in both innate and adaptive immune function not only converge in the reduced response to vaccination and protection against influenza, but present significant challenges to new vaccine development. In older adults, the goal of vaccination is more realistically targeted to providing clinical protection against disease rather sterilizing immunity. Correlates of clinical protection may not be measured using standard techniques such as antibody titres to predict vaccine efficacy. Further, antibody responses to vaccination as a correlate of protection may fail to detect important changes in cellular immunity and enhanced vaccine-mediated protection against influenza illness in older people. This article will discuss the impact of influenza in older adults, immunologic targets for improved efficacy of the vaccines, and alternative correlates of clinical protection against influenza that are needed for more effective translation of novel vaccination strategies to improved protection against influenza in older adults.

Keywords

influenza vaccine; older adults; T cells; cytokines; granzyme B

1. The Impact of Influenza

1.1. Influenza – a significant cause of morbidity in older adults

Aging is associated with a decline in humoral, innate, and adaptive cell-mediated immunity and a dramatic increase in late-life morbidity and mortality from influenza. The full impact of influenza is increasingly recognized as an illness that goes well beyond pneumonia and influenza statistics. Peak months of mortality due to respiratory illness, ischemic heart disease, cerebrovascular events and diabetes in adults 70 years and older coincide with annual influenza epidemics, suggesting that influenza illness is the major cause of excess mortality in this population during the winter months (1). Hospitalization and death rates from influenza are rising in spite of widespread influenza vaccination programs

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implemented in the 1990's. Influenza on average resulted in 36,000 deaths annually in the U.S. from 1990 to 1999 (2), almost double that of the period from 1976 to 1990. A similar rise in hospitalization rates for acute respiratory illnesses and cardiovascular diseases during the influenza season was also observed over these two time periods (3,4).

1.2. Influenza – impact of pandemic H1N1

The dramatic increase in rates of serious influenza illnes is, in part, due to aging of the U.S. population but also the rising prevalence of high-risk conditions for influenza illness including cardiovascular diseases in the population age 65 years and older (4). Although prior exposure to A/H1N1 strains in the years following the 1918 influenza pandemic explains the lower attack rates among the 65 and older population (5), the case fatality rate from pandemic H1N1 was maintained across adult age groups including those ≥ 70 years old (6). With improved healthcare, advances in medicine, and aging of the "baby boomers", the percentage of the population age 65 years and older is steadily increasing worldwide. Vaccination programs using the current split-virus vaccines are cost-saving in the over 65 population even though these vaccines often fail to provide adequate protection in older adults and influenza illness continues to have devastating consequences in this population. Rising rates of hospitalization are anticipated from seasonal influenza and, in the event of a real pandemic in older people, threaten to paralyze the health and social systems of support.

1.3. Influenza – vaccine preventable disability

The impact of rising hospitalization rates on disability and frailty in older adults is only beginning to be understood (7,8), and this type of data is not captured in the typical databases used to estimate influenza vaccine effectiveness. Influenza and pneumonia and the cardiovascular complications of influenza are among the six leading causes of catastrophic disability (9); rates of long-term morbidity and disability following influenza illness in older people are predictable and will increase in parallel with hospitalization rates, not only impacting on cost to the health care system but on the quality of life of older persons.

Estimates of vaccine efficacy can only be established in randomized, placebo-controlled trials that document laboratory-confirmed influenza illness. In the case of older adults, the only placebo-controlled trial of influenza vaccine conducted in this population provided an estimate of vaccine efficacy of 50% for the prevention of influenza in relatively healthy older adults (10). Such trials as no longer considered ethical in the context of a standard of practice for influenza vaccination in all persons of 65+ years. Estimates of vaccine efficacy have subsequently been based on the ability of influenza vaccines to stimulate an antibody response as a correlate of protection against infection, or "sterilizing immunity". This estimate of vaccine efficacy has significant limitations in older individuals who may become infected in spite of protective levels of antibodies. Once infection occurs, cell-mediated mechanisms are needed for viral clearance and "clinical protection" against disease. As one grows older, the ability to re-stimulate influenza-specific T cell memory through vaccination declines and, thus, different correlates or surrogates of protection against influenza disease are needed.

In the absence of established correlates of protection against disease, current estimates of vaccine-mediate protection are based on observational studies comparing vaccinated and unvaccinated older adults to determine the extent to which current influenza vaccines prevent complications arising from influenza like illness (ILI) in community-dwelling older adults, as most cases are not confirmed by culture or PCR. Studies in the 1990s provided estimates of vaccine effectiveness of 30–40% in community-dwelling older adults (11,12). However, there is not uniform agreement on vaccine benefit based on subsequent epidemiologic studies and much controversy exists with respect to the benefits of

vaccination in older adults (13,14). Methodologic differences in selecting the control period for these observational studies, the specificity of the case definition, and the adjustment for functional status and life expectancy has led to estimates ranging from no mortality benefit (14–19) to varying degrees of benefit related to the complications of influenza disease (20–23). Out of this controversy, a common understanding has evolved - what is needed to move the field forward is good, prospective and systematically collected information on functional status and prognosis/life expectancy, and a concerted effort to develop more effective influenza vaccines for the 65+ population (24,25). However, the challenge remains as to how to design a product development plan that will identify the most promising candidate vaccines to advance through the phases of clinical trials and are most likely to demonstrate enhanced efficacy over the currently available vaccines. The rest of this article will focus on how new vaccines may overcome the challenges of immune senescence and protect against the serious complications of influenza in older adults.

2. Loss of Influenza Vaccine Efficacy

2.1. Link to Immune Senescence

A decline in immune function is a hallmark of aging and affects the ability to resist influenza infection and respond to vaccination. It is recognized that multiple components of immune function, particularly cell-mediated immunity, are affected during the aging process. As a consequence, there has been a paradigm shift in understanding the limitations of antibody titers as a sole measure of influenza vaccine efficacy in older people (26–31). In this population, adequate antibody titers may not provide sterilizing immunity, where antibody fails to bind the influenza virus to prevent infection of the cell (32,33). Further, statistically significant increases in antibody titers that correlate with protection in response to vaccination, may not translate to clinically important improvements in influenza outcomes in older adults (34). Thus, the goal of vaccination may be clinical protection against illness when infection occurs and is mediated by both humoral and cellular immune mechanisms. The challenge to new vaccine development is that antibody titers as a sole predictor of vaccine efficacy may fail to detect important changes in cellular immunity that enhance vaccine-mediated protection in older people.

2.2 Antibody Titers and Vaccine Effectiveness – effect of annual repeated vaccination

A multitude of changes in the immune system occur with aging but the specific mechanisms that increase risk for influenza illness and limit the protective effects of vaccination are poorly understood. Govaert et al conducted the only published randomized, placebo-controlled trial of influenza vaccine in older adults providing an estimate of vaccine efficacy of 50% for the prevention of influenza in relatively healthy older adults (10), but antibody responses to the vaccine were not significantly correlated with protection against laboratory-confirmed influenza illness (35). The outcomes of this study also suggested that subjects who had received influenza vaccine in previous years were better protected than first-time vaccinees in the trial. Other observational studies support these results showing increases in vaccine effectiveness with repeated annual vaccination, and a reduction in vaccine effectiveness associated with the presence of co-morbidity (36–38). These results contrast with the expected decline in the antibody response with repeated vaccination; if antibody responses are a reliable correlate of protection, repeated vaccination would be associated with a reduction rather than the observed improvement in vaccine effectiveness. Further, serum antibody responses to influenza vaccination detected by hemagglutination inhibition improve with repeated vaccination in older adults (39) and the quantity and quality is similar to that of young adults (40). Because prior vaccination may affect the antibody response, it is critical that vaccination history be included as a potential confounder in the interpretation

of studies of age-related correlations or associations between the antibody response to influenza vaccination and other aspects of immune function.

2.3. Antibody and cell-mediated immune responses to influenza vaccine

Influenza virus stimulates an antiviral response in both B and T lymphocytes resulting in humoral and cell-mediated immunity, respectively. Virus-activated T-cells, through cytokine mediators, stimulate B-cells to differentiate and produce antibodies specific for a particular vaccine strain. These specific antibodies bind to the surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), to neutralize the virus particle. Influenza vaccines must be updated annually to include the predicted circulating strains of influenza A/H3N2 and A/H1N1 and influenza B. Antibody responses to influenza vaccination have largely been evaluated by the hemagglutination inhibition (HI) assay. While the HI test is convenient and considered a gold standard, virus neutralization assays are gaining more acceptance as they are considered more functional.

The paradox related to HI titers is that in spite of the high disease burden of influenza A/H3N2 strains in older compared to young adults, the antibody response to A/H3N2 strains is similar. In contrast, older adults are relatively protected from H1N1 strains, while lower antibody titers to these strains in older compared to young adults have been observed (40). These results would suggest comparable antibody-mediated protection against A/H3N2 strains in young and older adults, but are in sharp contrast to the observed outcomes in older adults; influenza A/H3N2 strains have by far the greatest impact on hospitalization rates, relative to influenza B and A/H1N1 strains in this population (4). In addition, although repeated influenza vaccination may limit the antibody response, protection against influenza improves when received annually (36–38) suggesting that cellular immune mechanisms may also be important for protection in older adults. While antibody responses to influenza vaccination have been correlated with alterations in T-cell function (41,42) and a decline in cell-mediated immune response to influenza vaccine (28), the mechanism for the overall decline in vaccine-mediated protection in older adults has not yet been established.

2.4. Immunologic Correlates of Protection

Correlates of protection can be established for “sterilizing immunity” as the serum level of antibody titer that prevents infection and thus, influenza illness. While antibody titers have been shown to correlate with protection in large studies of older adults (34), hemagglutination inhibtion assays may lack the sensitivity to detect differences in risk among older adults especially those with underlying co-morbidities (38). Further, our work has shown that serum antibody titers measured by the influenza hemagglutination inhibition assay do not distinguish between older individuals who subsequently develop influenza illness from those who do not (32,33). T-cell mediated immunity responsible for clearance of the virus once infection occurs, may be a more realistic goal of vaccination to provide “clinical protection” against disease when antibody responses fail to provide sterilizing immunity. Developing novel correlates of protection based on the cell-mediated immune response are being actively pursued but will need to overcome the challenges of technical practicality and inter-assay variability for application in large clinical studies of older people in whom influenza outcomes can be monitored. Studying the impact of immune senescence and the loss of T-cell mediated immunity with aging, on vaccine responsiveness will also be important for a mechanistic approach to establishing these correlates of protection (29).

Other areas of investigation that warrant further study include the use of serum neutralization assays as correlates of protection. These functional assays detect cross-reactive antibodies that may not necessarily be detected in a hemagglutination inhibition assay including those antibodies directed to conserved proteins in the stalk region of the

hemagglutinin (HA) molecule (43–45) and can be stimulated by seasonal influenza vaccine (46). Another evolving area of interest is the potential for cross-reactive antibodies stimulated by vaccination to enhance protection against influenza disease through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-mediated lysis of influenza-infected cells. While cross-reactive antibody to the highly conserved external domain of the M2 protein of influenza A was shown to confer weak protection via an antibody-dependent natural killer cell-mediated mechanism (47), the contribution of neutralizing and cross-reactive antibodies to the stalk region of HA remains an area for future investigation.

2.5. Immmunity in pandemic and seasonal influenza

The current pandemic influenza A/H1N1 virus (pH1N1) is a novel influenza virus strain which spread from Mexico to the United States in the spring of 2009 and circulated through the early winter (48). Lower attack rates during the pH1N1 pandemic among the population age 65 years and older (5), were attributed to pre-existing antibodies from exposure to the A/H1N1 strains that circulated in the years following the 1918 influenza pandemic. However, the case fatality rate in this population remained comparable to younger adults (6), with severe outcomes being more common in those over age 50. For seasonal influenza, H3N2 strains have been responsible for most of the influenza outbreaks and excess morbidity over the last four decades. Although there is significant cross-reactivity between the internal proteins of H1N1 and H3N2 subtypes, seasonal H1N1 has not been a significant cause of illness in older adults. This observation has been attributed to the effect of original antigenic sin (i.e. protection derived from first exposure, for example, in early childhood, to H1N1 strains). However, in contrast to the high antibody titers against pH1N1 in older compared to young adults, antibody titers against seasonal H1N1 are significantly lower in older compared to young adults (32,49). The paradox is that older people appear to be equally protected against infection with pandemic and seasonal H1N1 and better protected against H1N1 strains compared to young adults; protection against H1N1 infection in older adults appears to be antibody-mediated based serum transfer experiments in a mouse model (50). In contrast, A/H3N2 strains show the greatest disparity in influenza attack rates between young and older adults and yet there is no difference in the antibody titers to A/H3N2 following influenza vaccination between the two age groups (32). These observations suggest that cross-reactive T-cell responses are important for protection in older adults.

3. T-cell immunology in the response to influenza

3.1. Cross-reactivity of the T cell response

In contrast to the strain-specific antibody response of B-cells, viral epitopes that stimulate T helper cells (Th) and cytotoxic T lymphocytes (CTL) responses are more conserved across different strains of influenza and appear not to degrade with antigenic drift (51–53). Virus is taken up and processed by antigen-presenting cells (APC) such as macrophages and dendritic cells, and the resulting peptides are presented with the major histocompatability complex (MHC) to activate T-cells (54). Protein sequences within HA and NA and internal viral proteins (matrix and nucleoproteins) that stimulate Th (CD4+) and CTL (CD8+) responses have been identified. Importantly, these peptide epitopes are conserved across subtypes of influenza A (A/H3N2 vs. A/H1N1), and between types of influenza (influenza A vs B)(55), and are cross-reactive between the different strains of influenza. Current killed virus vaccines are poor stimulators of the CTL response (discussed below) and thus are not optimal for stimulating CTL which are necessary for clearing virus once infection occurs. Our work and others have shown that defects in cell-mediated immune responses are associated with increased risk for influenza illness in older adults and that such change cannot be detected by the antibody response alone to influenza vaccination (28,32,33).

Figure 1 illustrates the “in vivo” response to influenza vaccination wherein vaccine is taken up by dendritic cells or macrophages (antigen-presenting cells, APC) at the site of vaccination. Inflammation stimulated by the injection and potentially enhanced by adjuvant facilitates the activation of the APC and uptake of the virus. The APC then migrates to the local lymph node to interact with B cells, T helper cells (Th) and cytotoxic T lymphocytes (CTL). Presentation of vaccine-derived peptides on the Major Histocompatibility Complexes, MHC I and MHC II, drives the T cell response to stimulate B cells for antibody production and produce a variety of cytokines and cytolytic mediators, as will be discussed. Most importantly, this interaction stimulates T cell memory that can be activated during a subsequent exposure to natural infection to protect against the development of influenza illness and its serious complications. Antibodies are generated through T-cell dependent mechanisms and their production can be stimulated by a Th1 or Th2 response to vaccination. Antibodies mediate protection through virus neutralization on the mucosal surface and hemaglutination inhibition of virus attachment to the epithelial cell to prevent infection, or through NK-mediated cytotoxity or complement-mediated lysis that are dependent on antibody binding to virus-infected cells.

Ex vivo stimulation of peripheral blood mononuclear cells (PBMC) simulates the activation of the T cell response during natural infection. In this case, uptake of live virus results in effective presentation of viral peptides on both MHC I and MHC II, respectively activating CTL and Th1/Th2. Natural infection in the lungs activates Th1/Th2 in the adjacent lymph nodes and the resulting cytokine response may be regulated to favor a Th1 or Th2 response. A Th1 response is needed for effective activation of the memory CTL needed to clear virus from the lungs. The magnitude of the T cell response to natural influenza infection is simulated in ex vivo PBMC by measuring cytokines expressed in T cells, by flow cytometry, or levels in the culture supernatants, and the cytolytic function or activity (granzyme B) in lysates of stimulated PBMC.

3.2. The role of T helper cells in the response to influenza

It has been well documented that T-cell-mediated immune responses are also protective against influenza infection and illness (56). Th (T helper cell)-mediated immune responses to influenza virus play a key role in both humoral and CTL responses to influenza vaccination. T helper type 1 (Th1) effectively stimulate antibody responses, IFN-γ production, and CTL memory, while Th2 stimulate antibody responses and IL-10 production, which suppresses the Th1 response. Anti-influenza antibodies are stimulated by Th1 and Th2 to respectively produce IgG2a and IgG1 responses to influenza vaccination. With aging, a decline in Th1 relative to Th2 cytokine production occurs. Also important to vaccine development, Th and CTL recognize viral peptides on MHC II and MHC I, respectively, and thus have different requirements for effective antigen presentation. Peptides derived from replicating live virus are effectively presented on both MHC I and MHC II. Split-virus vaccines are killed viruses and are effectively presented on MHCII but presentation on MHC I requires antigen cross-presentation within the dendritic cell, and generally stimulate a weak CTL response (57) that depends on previous priming from natural infection (58). Human studies have confirmed that CTL responses effectively clear influenza virus even in the absence of protective antibodies to the infecting virus, and are important for recovery from influenza infection (59). Of note, recruitment of CTL into infected lung tissue is dependent on Th1 cytokine production and thus the shift from Th1 to Th2 cytokine production may further compromise the immunologic defense mechanisms against influenza in older adults. These changes are reflected in the finding that a reduced IFN-γ:IL-10 ratio and lower levels of cytolytic mediators in PBMC responding to influenza challenge correlates with risk for influenza illness in older adults (32). Thus, influenza vaccines that could produce a shift toward a Th1 response and more effectively stimulate

both antibody production and CTL memory, would improve protection in older adults. These are the types of vaccines that are needed for improved protection in older adults. However, it should noted be that the formulation of the vaccine is also important as the delivery of viral antigens for MHC Class I presentation depends on cross-presentation of antigens derived from the vaccine. Thus, the induction of Th1 cytokines alone is insufficient for stimulating a CD8 T cell memory response to the vaccine. Further, CD8 T cell responses are directed against internal components of the viruses and, hence, provide cross-protection. However, influenza vaccines that are currently marketed in the US and Canada vary in the amount of internal proteins (60) and may vary in their ability to recall or induce CD8 T cells, even if they are formulated for Class I processing and presentation.

3.3. Immunosenescence: The loss of T-cell adaptive immune function impacts on vaccine responsiveness

Thymic involution and a decline in naïve T cell output with increasing age, together with a lifetime of exposure to a variety of pathogens, leads to a dramatic reduction in the naïve T cell pool and a relative increase in the proportion of memory T cells. While the reduction in naïve T cells with aging may be important to overall immunity, prior exposure to influenza and the generation of memory T cells may mean that function in this T cell compartment is most important for protection against influenza. Within the total memory pool, the most dramatic functional changes occur in the CD8+ T cell subset; progressive exhaustion of this compartment leads to the loss of costimulatory molecules (CD28), shortening of telomeres, and terminal differentiation to end stage cells that are resistant to the usual apoptotic mechanisms that control the size of memory T cell clones responding to a particular pathogen (61). It has been shown that most of these CD8+CD28- memory T cells are part of large clonal expansions specific for persistent viruses, mainly CMV (62), but also Epstein-Barr virus (EBV) and varicella zoster virus (VZV) (63). Although these viruses typically establish asymptomatic latent infection with intermittent subclinical episodes of reactivation, suppression of disease activity is related to CD8+ T lymphocyte presence and function. By old age, excessive accumulation of these virus-specific CD8+ T lymphocytes eventually overgrows the T lymphocyte pool, compromising immune function and restricting the overall immune repertoire (64). Because the Th and CTL responses are cross-reactive between the different strains of influenza A or influenza B, memory from previous exposure to the virus is re-stimulated with vaccination. Thus, the impact of age-related changes in memory T subsets is likely to have the greatest impact on the response to influenza vaccination and protection from influenza illness.

3.4. Age-related changes in T cells are much greater in CD8+ relative to CD4+ subsets

In contrast to what has been observed within the CD8+ T cell population during aging, CD4+ T cells are relatively less affected by replicative senescence. Indeed, CD28 expression is preserved within this subset during aging (65). Although a normal CD4+ T cell response to influenza vaccination is observed in older adults, over the long-term, the memory CD4+ T cell response is impaired (66). These long-term memory changes do not appear to affect the duration of the serum antibody response to influenza vaccination, which is similar in young and older adults (67), but may contribute to dysregulated cytokine responses that promote virus replication once infection occurs.

3.5. Age-related increase in inflammatory cytokines and dysregulation of T-cell cytokines

Cultures of senescent CD8+ T lymphocytes produce high levels of certain pro-inflammatory cytokines, such as TNFα and IL-6 (68), cytokines that are associated with frailty (69). Thus, CD8+ T cells may directly contribute to elevated serum levels of inflammatory cytokines (IL-1, IL-6, TNF-α) causing dysregulation of the cell-mediated immune response (70). While a cocktail of TNF-α, IL-1 and IL-6 has been shown in mouse splenocytes to reverse

age-related changes in cognate helper T cell function for antibody production (71), we have shown that this inflammatory cytokine cocktail suppresses the CTL response to influenza virus (unpublished observations). A greater understanding is needed as to how with aging, T helper cell production of cytokines is dysregulated and defects in the CTL response to influenza contribute to loss of vaccine-mediated protection. These studies are critical to development of influenza vaccines with improved clinical protection against serious disease in older people, and will complement the antibody responses, which are currently the only available measure of influenza vaccine efficacy in clinical trials.

3.6. T helper cytokine regulation impacts on the response to influenza in older adults

The Th-mediated immune response to influenza virus plays a key role in the generation of both humoral and CTL responses to influenza vaccination. Previously, Th1 and Th2 were defined by their cytokine products such that the Th1 cytokine, IFN-γ, down-regulated Th2, and IL-10 down-regulated Th1 (72–74). While this paradigm is generally applicable in the mouse model, recent studies have questioned the validity of the Th1/Th2 paradigm in humans, and the contributions of regulatory T cells (Treg) and Th17 subsets to cytokine regulation are only beginning to be understood (75). Under a revised model, naïve CD4+ helper T cells are stimulated by IL-12 to produce IFN-γ i.e. become Th1);IL -4 stimulates Th2 to produce IL-4, IL-5, IL-10, IL-13; and IL-1, IL-6 and IL-23 to stimulate Th17 to produce IL-17, IL-22 and IL-26. Treg produce IL-10 and TGF-β, which downregulate inflammatory responses including the production of cytokines such as IFN-γ and IL-6. All of these Th subsets have counter-regulatory interactions between each other (76) but the impact of aging on the dysregulation of these interactions is only beginning to be understood.

Our data has shown that the IFN-γ:IL-10 ratio correlates with risk for influenza illness (32) but characteristics of the vaccine recipient and PBMC culture conditions may alter this relationship (77–80). The apparent downregulation of IFN-γ by IL-10 may be Th2 or Treg-mediated (81), and the interaction with Th17 may also be important. Studies in human PBMC show that Th17 promotes the recruitment of IFN-γ producing T cells and as such, is regulated by the tissue level of IFN-γ (82). While IFN-γ appears to be important in immune defense mechanisms against influenza, aberrant IL-17A production has been shown to stimulate a neutrophil-dependent increases in pro-inflammatory cytokines in response to systemic viral infection that contributes to death in aged mice (83). Further, we have found that from a panel of Th1/Th2/Treg/Th17 cytokines, IL-17 is the only cytokine that showed an age-dependent increase in the cytokine response to live influenza challenge while all other cytokines declined with age (unpublished observations). Since IL-2 has been shown to constrain the formation of Th17, the decline in the IL-2 response with aging may explain the increase in IL-17 in influenza-stimulated PBMC. Further, age-related increases in IL-17 responses to influenza virus may be relevant to poor outcomes in this population. The effect of vaccination on Th17 remains to be studied.

3.7 What are the likely cell-mediated immune correlates of protection against influenza?

Th and CTL have different requirements for effective presentation of viral peptides on MHC II and MHC I, respectively (57,84–86). Because only Th and B-cells are effectively stimulated by killed virus (as in the trivalent inactivated split virus influenza vaccine), vaccination stimulates good antibody responses, but only weak CTL responses in adults; the CTL response most likely results from restimulation of a previously primed response to influenza through natural infection (58,87–89). 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 (59). CTL combat influenza infection by recognizing MHC I-viral peptide complexes on virus-infected host cells, and destroy them

before infective progeny virus can be released (85). A direct comparison showed that protection correlates with the virus-specific CTL (CD8+) response in the lungs and associated lymphoid organs. 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 six months (90–92). We have demonstrated that memory CTL from natural infection may be restimulated by influenza vaccination (33) and demonstrated the potential for older adults to mount an enhanced CTL response to vaccination. Novel vaccines designed to stimulate this enhanced response could be more effective in older adults.

3.8. The key function of granzyme B in CTL-mediated Apoptosis

Virus-specific killing is mediated by granzymes contained in granules within CTL. Granules containing granzymes and perforin migrate to the “immune synapse” between the activated CTL and the virus-infected target cell. Granzymes are transported across the cell membrane by perforin into the cytoplasm of the target cell, and granzyme B (GrzB) is involved in an enzymatic cascade that leads to apoptotic cell death of the virus-infected cell (93). GrzB is a key element of the T-cell response to influenza in the lung (94,95). The development and validation of the GrzB assay (96), which correlates with cytolytic activity by standard ^{51}Cr-release assays (97,98), quantifies the amount of GrzB activity and complements measures of influenza-specific CTL frequencies. Ex vivo studies have shown no difference in influenza-specific CTL frequencies between young and older adults (99) and suggest that the defect in influenza susceptible older persons is the amount of GrzB produced on a per CTL basis. Importantly, ex vivo levels of GrzB in lysates of influenza-stimulated PBMC correlate with risk for influenza illness in our studies (32,33).

4. Improving influenza vaccines for older adults

4.1. High-dose vs. adjuvanted influenza vaccines

Two main strategies are being used to improve the response to influenza vaccination in older adults and include changing the content of the vaccine and evaluating alternate routes of administration to the current practice of intramuscular injection. Increasing the amount of influenza viral antigen (standardized according to HA content), adding an adjuvant to seasonal influenza vaccine, or using live-attenuated influenza strains are some of the strategies being pursued. In the case of influenza vaccine trials, “immunogenicity” is limited to antibody responses and thus may not measure some of the important contributions of cell-mediated immune responses, to vaccine-mediated protection in older adults.

Numerous trials of high-dose influenza vaccine have demonstrated their ability for improved immunogenicity based on antibody responses in older adults (Reviewed in (100)) and culminated in the Phase III clinical trial of influenza trivalent, split-virus vaccine containing four times the antigen content of the standard-dose comparator vaccine (101). In December 2009, the US FDA approved Fluzone® High Dose through the accelerated program for influenza vaccine development, which expedites the process of approval of vaccines with demonstrated safety and immunogenicity. Fluzone® High Dose is included in the recommendations of the Advisory Committee for Immunization Practices (ACIP) as another approved vaccine in persons aged 65 and older (102). Under the program for accelerated influenza vaccine development, the manufacturer is expected to conduct post-licensure studies to demonstrate vaccine effectiveness. The planned trial to compare Fluzone® High Dose to Fluzone® standard-dose vaccine has an anticipated enrollment of 33,000 subjects to demonstrate differences in laboratory-confirmed influenza cases between the two vaccine groups (ClinicalTrials.gov NCT00976027). Studies demonstrating enhanced protection against hospitalization and death would require more than double the number of subjects and

clearly would not be feasible. Thus, novel correlates or surrogates of vaccine effectiveness are needed in the ongoing quest to develop vaccines with a demonstrated ability to provide enhanced protection against the serous outcomes of influenza.

The role of an adjuvant is to increase the level of inflammatory mediators at the site of injection to activate dendritic cells and enhance their antigen-presenting capacity (Reviewed in (103)). Influenza vaccines containing oil-in-water emulsions such as M59 (ClinicalTrials.gov NCT00841763) are at various stages of approval for older adults, and have reported enhanced immunogenicity but, to date, trials of these vaccines have only included antibody titers to estimate vaccine efficacy. An ongoing Phase III trial of a GSK adjuvanted influenza vaccine in the age 65 and older population has enrolled 43,000 subjects, and is yet another example of the size of clinical trials needed to demonstrate enhanced efficacy in this population. In spite of the advanced phases in the development of adjuvanted influenza vaccines, there is limited data on CD4 T cell responses to adjuvanted influenza vaccines (104), particularly in older adults (105), and no publications that this author is aware of on CD8+ T cells responses to adjuvanted influenza vaccines in people. The potential for TLR ligands as influenza vaccine adjuvants to improve cell-mediated immune responses to influenza is being studied (discussed below) but these investigations have yet to progress beyond the pre-clinical phase of testing in older adults.

Virus-like particles (VLP) and virosomal vaccine preparations may also have adjuvant activity but there have been limited studies especially in older adults to determine their advantages over currently available vaccines in older adults (Reviewed in (106)). Studies comparing a virosomal vaccine preparation with MF59-adjuvanted vaccine and conventional influenza vaccines have shown comparable immunogenicity but with the potential for less reactogenicity compared to the MF59-adjuvanted vaccine (107,108). Studies of VLP-based influenza vaccines in older adults have yet to be reported. The challenge with both VLP and virosomal technologies is the incorporation of internal proteins to generate the necessary epitopes for stimulating CD8 T cell memory. It has previously been demonstrated that the internal protein content varies in the currently available influenza vaccines and is proportionate to their ability of to stimulate a T cell response (60). Thus, in addition to enhancing antibody responses, novel influenza vaccines should be designed to enhance the cell-mediated immune response; the dose, type (live attenuated, killed, split, particle or subunit viral proteins, with or without adjuvants) or route of delivery of the viral antigens will need to consider the influenza internal protein content of these vaccines.

4.2. TLR ligands as influenza vaccine adjuvants

Novel vaccines in the early phases of development contain ligands of Toll-like receptors [TLR] as vaccine adjuvants. TLR on the surface of APC recognize structural components of pathogens and activate signal transduction cascades leading to gene transcription with several outcomes including the secretion of pro-inflammatory cytokines and chemokines, and activation of adaptive immune responses. In mouse models, antigen-presenting cells including monocyte/macrophages and DC produce lower levels of proinflammatory cytokines in response to ligation of TLR in aged compared to young mice (109). This diminished inflammatory cytokine response may impact on the usual synergy of innate and adaptive immune responses, and limit the utility of TLR ligands as influenza vaccine adjuvants in older adults (110). However, experiments in age mouse models show that poly I:C (TLR3 ligand) appears to possess a unique mechanism among other TLR agonists in its ability to stimulate antigen-presenting cells and the production of proinflammatory cytokines to enhance cognate CD4+ T cell help in aged animals (111). More recent studies have shown age-related defects in TLR expression and a dysregulation of cytokine production in senescent plasmacytoid and myeloid dendritic cells (112). These defects in TLR expression may limit the response to TLR ligands used as influenza vaccine adjuvants

in older people. As a model for pre-clinical testing of vaccine-adjuvant combinations, we have shown a dose-response improvement in the T-cell response to influenza challenge (IFN-γ:IL-10 ratio and GrzB activity) in PBMC from older adults when a TLR4 ligand was added to split-virus vaccine (SVV) (McElhaney, manuscript in preparation). This response was associated with an increase in the proportion of DC that produced IL-6 and TNF-α in response to the TLR4 ligand. These results suggest that a TLR4 ligand may also be effective for reversing age-related changes in cognate CD4+ T cell help for improved CTL responses influenza.

4.3. Alternate Routes of Vaccine Delivery

Most vaccines for older adults are delivered by intramuscular or subcutaneous injection and the response mainly bypasses the mucosal immune compartment. Age-related changes in this compartment are relatively unknown and may have a significant impact on the response to vaccines administered by alternate routes of delivery. Intranasal and intradermal routes for influenza vaccination are in clinical trials and include intranasal live-attenuated influenza vaccines (113), and intradermal injection of trivalent split-virus influenza vaccine (114).

The abundance and function of antigen presenting cells within the skin is an attractive target of immunization strategies. A randomized trial comparing a new microinjection system for intradermal delivery of seasonal influenza vaccine, to intramuscular injection of the same vaccine, showed enhanced immunogenicity in older adults (115). In a separate trial, similar results were obtained when a 60% dose of seasonal influenza vaccine was delivered intradermally and compared to the full-dose given by intramuscular injection (116); despite the potential for intradermal vaccines to more effectively stimulate T cell responses, a comparison of the response in the intradermal vs. intramuscular vaccine groups was inconclusive with respect to T cells expressing IFN-γ and TNF-α. The mechanisms of improved efficacy is still unknown but presumably involves the activation of the dermal dendritic cells and/or dendritic-like macrophages and the efficient shuttling of antigen to the draining lymph nodes where B cells reside. As dendritic cells are the prime activators of naïve T cells, more efficient induction of cell-mediated immune responses by intradermal vaccine administration is anticipated, but remains to be demonstrated.

Intranasal influenza vaccines have the potential for a broadened immune response (Reviewed in (117)). In a randomized trial of live-attenuated influenza virus (LAIV) vs. placebo, in which all subjects received split-virus virus vaccine, the benefit of LAIV for the reduction in influenza illness could not be demonstrated (113). These results correspond to a relatively weak stimulus to the influenza-specific cytolytic response following live-attenuated intranasal vaccine (LAIV) in older adults (34). This lack of efficacy has been speculated to be due to inadequate intranasal infection due to the presence of preexisting antibodies (118). Thus, LAIV are not approved for use in persons age 50 years and older.

4.4. Increasing Thymic Output

Thymic involution is a key element of changes that occur in the immune system with aging. Thymic rejuvenation techniques are in early stages of clinical trials, the most promising of which are keratinocyte growth factor (KGF), IL-7, and ghrelin (Reviewed in (119)). KGF enhances IL-7 production in the thymus by binding to the KGF receptors on thymic epithelial cells (120,121) and may have multiple beneficial effects, given the critical role of IL-7 in the development and maintenance of long-lived memory T cells following vaccination (122). IL-7 treatment increases thymic output and numbers of central memory T cells (CD4+ and CD8+) and improves the antibody response to influenza vaccination in aged rhesus macaques (123); KGF may be a strategy to locally increase IL-7 levels to avoid potential side effects of systemic IL-7 treatment. Ghrelin, a peptide hormone that binds to

the receptors for growth hormone secretagogues is another evolving treatment strategy to promote thymic output in older animals (124) and reduce pro-inflammatory cytokine levels (125).

4.5. Telomerase-based enhancement of CD8+ T cell anti-viral activity

One of the approaches to prevent or retard the generation of senescent CD8+ T cells is based on the well-documented link between telomere shortening and overall replicative potential and function of T lymphocytes (68,126). Telomerase activity that would normally elongate telomeres with T cell activation, is completely turned off in chronically stimulated CD8+ T cells (127). Influenza vaccines aimed at eliciting strong cellular immunity might be enhanced by the incorporation of adjuvants that increase telomerase activity (29).

4.6. New tests are needed for new vaccine technologies

The challenge to new vaccine development for older adults is to stimulate and detect improvements in the senescent immune response to influenza in ways that may not be measured by standard assays of antibody titres to predict vaccine efficacy. As discussed, antibody responses to vaccination as a correlate of protection may fail to detect important changes in cellular immunity and enhanced vaccine-mediated protection against influenza illness in older people. 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.

5. Summary

Rising hospitalization and death rates due to influenza over the last two decades in spite of widespread influenza vaccination programs, call for more effective influenza vaccines in the older population. A greater understanding of how age-related changes and their interaction with common chronic diseases in older persons is to needed to develop new influenza vaccines with enhanced protection in this population. There have been major advances in vaccine technology but the phases of clinical development are entirely dependent on antibody responses as correlates of protection and measures of vaccine efficacy. Although the importance of T-cell mediated protection in older adults is increasingly recognized, T cell correlates or surrogates of clinical protection are needed to select more potent influenza vaccines earlier in the development pipeline. Identification of these correlates of protection will expedite the process and minimize the risk of failure in the development of more effective influenza vaccines for older adults.

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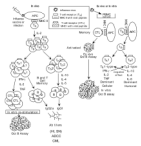

Figure 1.

The response to vaccination is summarized (left diagram) and the response to virus stimulation in PBMC cultures is illustrated (right diagram).

PBMC - peripheral blood mononuclear cells

APC - antigen presenting cell

T_h - helper T cell; type 1 - T_h1; type 2 - T_h2

CTL - cytotoxic T lymphocyte; B - B cell

IL - interleukin; IFN - interferon; TNF tumor necrosis factor

Grz B - granzyme B

Ab - antibody; HI - hemaglutination inhibition; SN - serum neutralization

ADCC - antibody dependent cell-mediated cytotoxicity

CML – complement-mediated lysis