Immunosenescence and Influenza Vaccine Efficacy

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Abstract A number of protective immune functions decline with age along with physiological and anatomical changes, contributing to the increased susceptibility of older adults to infectious diseases and suboptimal protective immune responses to vaccination. Influenza vaccination is the most cost-effective strategy to prevent complications from influenza viral infections; however, the immunogenicity and effectiveness of currently licensed vaccines in the United States is about 30–50% in preventing complications arising from influenza and preventing death from all causes during winter months in older adults. Hence, it is crucial to understand the molecular mechanisms that lead to immune dysfunction as a function of age so that appropriate strategies can be developed to enhance the disease resistance and immunogenicity of preventive vaccines, including influenza vaccines, for the older adult population.

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1 Introduction

A dramatic increase in the older adult population is occurring globally due to improved sanitation, preventive vaccination, development of effective antimicrobial drugs, and advances in medical sciences. This growth of the older adult population is having a major impact on healthcare, social services, and public health. In the year 2004, older adults accounted for 12.4% of the total population and required $531.5 billion in primary healthcare costs (Hartman et al. 2008). This represented almost 34% of all healthcare spending, as the cost of providing healthcare for an older adult aged 65 or above is 3–5 times greater than the cost for a younger adult. The older adult population in the USA is projected to almost double by 2030 2007(AoA). A decline in immune function leading to increased susceptibility to infectious diseases and poor adaptive immune response to vaccination is a key characteristic of aging (Miller 1996, 1997). For example, increased colonization of bacteria and yeast on the skin and mucosal surfaces, respiratory, and urogenital tracts, increased susceptibility to viral infections, and reactivation of latent viral and bacterial infections are all well documented (Gardner et al. 2006; Worley 2006; Ely et al. 2007; Htwe et al. 2007; Kovaiou et al. 2007; Simmons et al. 2007; van Duin and Shaw 2007). In general, infectious diseases such as severe acute respiratory syndrome (SARS), West Nile virus, respiratory syncytial virus (RSV), influenza, and pneumococcal infections tend to be more severe (with complications), often resulting in unfavorable outcomes among older adults when compared to those in healthy adults. In addition, the efficacy of preventive vaccines against bacterial and viral targets declines dramatically with the progression of age among older adults, clearly indicating that the dysregulated immune status referred to as "immunosenscence" is the consequence of altered physiological and anatomical functions (Ginaldi et al. 2001; Aw et al. 2007). Hence, in this review, we will address the status of innate and adaptive immune functions in aging, the current state of influenza vaccines and their efficacy in older adults, and strategies that need to be considered to protect them against influenza.

2 Immune Status in Aging

2.1 Innate Immunity

Innate immunity was considered "nonspecific" and received secondary importance when compared to antigen-specific adaptive immune functions until the late 1990s. However, with the discovery of Toll-like receptors (TLRs), innate immunity is now recognized to be crucial to the survival of species. Therefore, understanding innate immunity can offer newer insights into the development of novel immunomodulators and antimicrobials (Hoffmann et al. 1999; Medzhitov and Janeway 2000a,b; Imler and Hoffmann 2001). Since the discovery of TLRs, several other pathogen-sensing

receptor families have been identified over the last decade (Bingle and Craven 2002; Kang et al. 2002; Lu et al. 2002; Holmskov et al. 2003; Yoneyama et al. 2004; Martinon and Tschopp 2005; Ting and Davis 2005; Brown 2006; Takaoka et al. 2007). These families evolved to overcome microbial strategies and their metabolic needs in order to eliminate them. TLRs are expressed either as soluble molecules on the cell membrane or in vesicular compartments, or in the cytosol, as shown in Fig. 1. These pathogen sensors recognize structural components of pathogens and activate signal transduction cascades, leading to gene transcription with several outcomes, such as activation of antibacterial and antiviral defenses, secretion of proinflammatory cytokines and chemokines, tissue repair in the event of damage, and activation of adaptive immune responses. In most cases, the precise structure or sequence of the pathogen signature that stimulate the innate immune receptors is not well defined.

The dynamic barrier against infectious diseases, the epithelial lining of skin, gastrointestinal, respiratory, and urogenital systems, prevents the colonization and entry of potential pathogens into the body's interior, which is sterile (Ganz 2002). These epithelial cells express several pattern recognition receptors and, upon recognition of the molecular signatures of pathogens, secrete antimicrobial substances that aid in the destruction of pathogenic microbes (Ganz et al. 1992; Schittek et al. 2001; Zanetti 2004). In addition to epithelial cell turnover that reduces the microbial load, mucosal secretions of respiratory, urogenital, and gastrointestinal tracts have antibacterial substances that also facilitate the elimination of colonization. Similarly, the antibacterial components of sweat and skin secretions reduce colonization of microbial load (Schittek et al. 2001; Zanetti 2004). Limited information is available on the status and functionality of skin, lung, and other mucosal epithelial layers among older adults. The epithelial cell turnover rate in skin slows down among the older adults, with a reduced secretion of sweat and sebum resulting in dryness and increased microbial colonization, especially with *Pseudomonas* and *Proteus* species

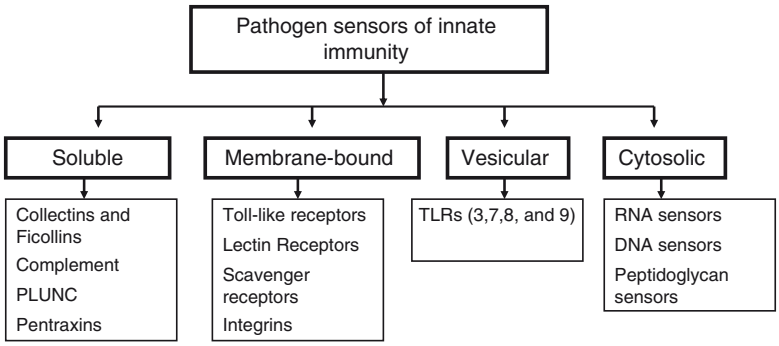

Fig. 1 Pathogen sensors of the innate immune system. Several families of pathogen-sensing receptors that recognize conserved molecular signatures of pathogens are localized in various compartments within the cell as well as in body fluids. Engagement of these receptors leads to the activation of the innate immune system and the elimination of the pathogens

(Laube 2004). These altered physiological states may contribute to delayed skin wound healing (Thomas 2001; Reed et al. 2003; Sorensen et al. 2003; Gosain and DiPietro 2004; Laube 2004). Altered physiological and anatomical changes in the lungs also contribute to poor innate immunity, thereby increasing microbial colonization and the incidence of pneumonia (Meyer 2001). These changes include reduced elasticity and function of lung muscles, reduced mucociliary clearance rates, decreased oropharyngeal clearance of bacteria, decreased phagocytic activity of alveolar macrophages, and decreased mucosal secretions (Meyer et al. 1996; Meyer 2004, 2005). Similarly, there is increased localization of *Candida* species on the oral and urogenital mucosal surfaces with age among older adults (Shay and Ship 1995; Sobel 1997). In the case of influenza infection, no data are available on the status of innate immune responses at the epithelial barriers in aging. Hence, detailed studies addressing the statuses of pathogen-sensing mechanisms with age are required and will enable us to come up with the strategies to reduce microbial load at epithelial surfaces and to enhance disease resistance.

2.2 Pathogen Sensing and Antigen-Presenting Cells

The primary role of innate immunity is to prevent the entry of pathogens into the tissues; however, a number of factors such as dose of infecting pathogen and the immune and nutritional status of the individual determine if innate immunity is able to prevent colonization and infection. Once pathogens overcome the epithelial defenses and gain access into tissues, myeloid lineages of hemopoietic stem cells from bone marrow, namely tissue-resident macrophages and dendritic cells, recognize the pathogens. Innate immune receptors, either directly or through scavenger receptors or pathogens bound to soluble innate immune receptors, initiate phagocytosis and an inflammatory response. These interactions lead to the secretion of proinflammatory cytokines such as IL-6, TNF-α, and IL-8, which attract neutrophils and natural killer cells to the site of infection, thus creating an optimal priming environment to initiate an adaptive immune response. Dendritic cells (DCs) capture antigens from pathogens, mature, differentiate, and migrate to regional draining lymph nodes to stimulate antigen-specific T and B cells, the lymphoid lineages that originate from hemopoietic stem cells. Following antigen-specific clonal expansion of B and T cells, the invading pathogen is or the pathogen-infected cells are removed by specific antibody and T cells. Tissue-resident macrophages play a major role in pathogen sensing, elimination, and tissue repair. We have demonstrated previously that the expression and function of TLRs on peritoneal as well as splenic macrophages decline with age using a murine model or peripheral blood mononuclear cells (PBMCs) from humans (Renshaw et al. 2002; van Duin et al. 2007b; van Duin and Shaw 2007). These findings are consistent with the previous observations that macrophage function declines with age, although the molecular mechanisms were not clear (Plowden et al. 2004a,b; Sebastian et al. 2005). Not only does macrophage function decline with age, but so does their ability to process and present antigens, secrete proinflammatory cytokines and chemokines, provide costimulatory signals,

and migrate to the site of infection, as documented in aged animal models (Plowden et al. 2004a,b). Although an age-related decline in the acute proinflammatory response of monocytes has been identified, other studies have demonstrated increased levels of proinflammatory cytokines in serum and in culture supernatants of in vitro stimulated monocyte cultures from healthy older adults compared to younger adults. These observations led Franceschi and colleagues to coin the term "inflammaging" indicating a low-grade chronic inflammatory state as a hallmark of aging (Franceschi et al. 2000; Franceschi 2007) and increased risk for adverse changes in health in older adults. This would predict high levels of proinflammatory cytokines in frail older adults, but just the opposite has been found; low levels of the cytokines have been associated with frailty. Differences in the observations may be accounted for based on the type (polyclonal vs. antigen- or ligand-specific) and duration (acute vs. chronic) of stimulus (van den Biggelaar et al. 2004). Although additional studies need to clarify the observed differences in the secretion of proinflammatory cytokines between aged animal models, healthy older adults and frail older adults, it is clear that there are alterations in pro- and anti-inflammatory cytokine secretion and their balance with aging (Alberti et al. 2006). These alterations will affect both innate and adaptive immune functions (Fig. 2). In addition, the migration of antigen-bearing DCs is severely affected in aged animals, indicating that the priming environment for adaptive immune responses is suboptimal (Linton et al. 2005). Although careful studies are yet to be performed, Langerhans cells in skin appear to decline in numbers with age, and their function also declines with age (Meyerson 1966; Laube 2004). In contrast, bone marrow-derived DCs generated with a cocktail of cytokines from aged animals or humans are found to be

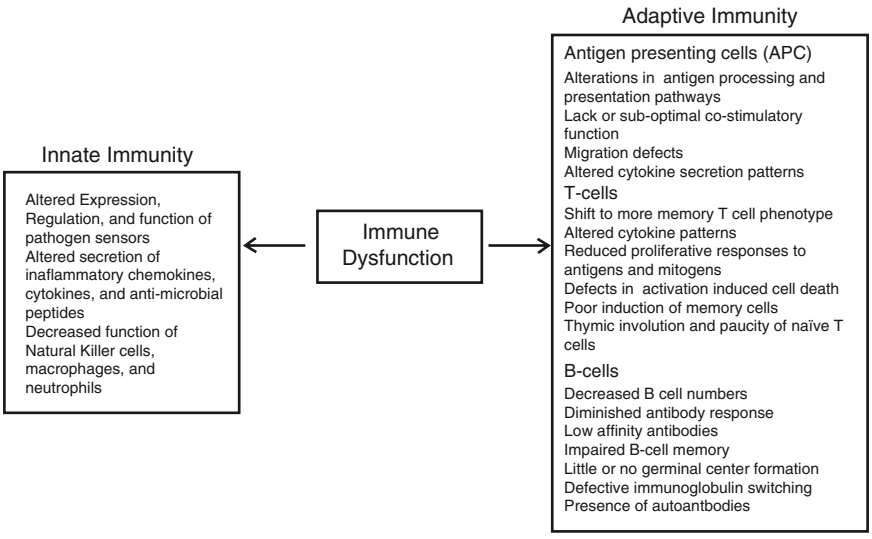

Fig. 2 Immune dysfunction in older adults. The characteristics of the alterations observed in both the innate and adaptive immune compartments

as effective as those generated from their younger counterparts in recalling memory T cell responses. This would suggest that in vitro generation bypasses age-related defects that are seen with ex vivo DCs (Lung et al. 2000; Tesar et al. 2006). However, primary CD4 T-lymphocyte responses remain impaired in spite of normal DC function, suggesting increased antigen and costimulation thresholds of aged, naïve T-lymphocytes, consistent with our earlier published data (Haynes et al. 2000; Sambhara et al. 2001; van Duin et al. 2007a). Unpublished findings from our laboratory indicate a substantial delay in the mobilization of DCs and macrophages into lungs and regional draining lymph nodes following infection with influenza virus in aged compared to younger animals, suggesting an altered microenvironment. A decline in the expression of pathogen sensors (specifically TLRs) and the secretion of cytokines and chemokines was also observed that may influence the migration, activation, differentiation, and function of macrophages and dendritic cells. Indeed, a recently published study demonstrated poor induction of costimulatory molecules on monocytes of older adults following TLR stimulation, consistent with our observations in the murine model (Renshaw et al. 2002; van Duin and Shaw 2007). Using adjuvants that stimulate the innate immune system, providing costimulation, or supplementing with a cocktail of cytokines along with antigen at the time of immunization significantly improved adaptive immune responses by stimulating antigen-presenting cells (APCs) (Sambhara et al. 1998, 2001; Haynes et al. 2004). Hence, it is logical to formulate vaccines for older adults with adjuvants to induce an optimal priming environment for adaptive immune responses.

2.3 Adaptive Immunity

Hematopoietic stem cells (HSC) in the bone marrow give rise to both myeloid- and lymphoid-committed stem cells. While the myeloid lineage gives rise to monocytes, macrophages, and dendritic cells, lymphoid-committed stem cells give rise to T lymphocytes and B lymphocytes which go through "education and selection" in the thymus (in the case of T cells) and bone marrow (in the case of B cells), a process that removes potentially autoreactive clones. These cells are further educated in the periphery to be tolerant to self-antigens. Although there is some indirect evidence that HSC numbers decline with age, detailed studies are yet to be done to determine if HSC numbers, function or migration alter with age (Wang et al. 1995; Lamberts et al. 1997; de Haan and Van Zant 1999). Changes in the cellularity of bone marrow with aging have been clearly documented, and may alter the local cytokine milieu, thus affecting the proliferation, differentiation, and seeding of secondary lymphoid organs by lineage-specific stem cells (Liang et al. 2005).

2.3.1 Humoral Immunity in Aging

Antigen-specific adaptive immune responses against influenza virus infection or vaccination are mediated by B lymphocytes and T lymphocytes; both contribute to

humoral and cellular immunity to influenza. T helper cells secrete cytokines for B lymphocyte differentiation and class switching. Following the recognition of antigens with their surface immunoglobulin receptors, B lymphocytes undergo differentiation to become plasma cells that secrete antibody. Antibodies against the major surface glycoprotein of influenza viruses, the hemagglutinin (HA), neutralize the virus by binding to conformational determinants on HA, and prevent infection. Antibodies directed against the second major surface glycoprotein, the neuraminidase (NA), can limit virus release from an infected cell and can therefore reduce virus replication. The functionality of anti-HA antibodies is usually determined by the hemagglutination-inhibition (HAI) test, and in some cases by virus-neutralization tests. A HAI titer of ≥1:40 is correlated with a 50% protection rate in a population against influenza viral infections (Wood et al. 1997). Due to the high mutation rate of this RNA virus and the selection pressure of pre-existing antibody in humans that acts on circulating viruses, influenza viruses accumulate mutations in HA and NA genes, leading to antigenic drift, which requires that the strains of influenza contained in the vaccine must be updated every year to antigenically match the circulating strains. In general, it is known that humoral immune responses induced by influenza vaccination decline with age. However, humoral immune responses as measured by HAI titers in community-dwelling "healthy older adults" and centenarians are similar to those observed in younger adults, indicating that aging alone does not affect antibody responses against influenza vaccination. Other contributing factors to the decline in antibody responses include comorbid conditions such as chronic diseases and frailty, as well as poor nutrition, stress, and limited physical activity. Pre-existing humoral immunity due to annual vaccination of older adults does not appear to impact the antibody responses to subsequent vaccinations and does not explain the poor vaccine efficacy. One possibility is that the quality and duration, rather than the magnitude of the antibody response, may be affected; however, results from a recent study indicate that this may not be the case (de Bruijn et al. 1999; Gardner et al. 2001; Iorio et al. 2007). Hence, additional markers of the immune response may be needed to predict vaccine efficacy in the older adult population. Earlier studies from our laboratory have shown that serum antibody titers did not correlate with the susceptibility to influenza virus infection among older adults, suggesting that both antibodies and cellular immunity contribute to clinical protection against influenza illness. Although the antibody responses are strain-specific within a subtype, they do provide cross-protection against viruses of the same subtype via antibody-dependent cell-mediated cytotoxicity carried out by NK cells or macrophages.

2.3.2 Cellular Immunity in Aging

Unlike B lymphocytes, T lymphocytes recognize peptide fragments derived from the antigens that are presented with major histocompatibility complex molecules by professional antigen-presenting cells such as dendritic cells. T lymphocytes consist of CD4 T helper cells and CD8 cytotoxic T cells. While CD4 T lymphocytes recognize

peptides that are processed from exogenous antigens (e.g., killed virus) presented with class II MHC molecules, CD8 T lymphocytes recognize peptide fragments derived from endogenous antigens (e.g., peptides derived from virus replicating inside the cell) that are presented with class I MHC molecules. Depending on the pattern of cytokines they secrete, CD4 T lymphocytes are further classified as T helper 1 (Th1), T helper 2 (Th2), T helper 3 (Th3), and T helper 17 (Th17) cells. T lymphocytes recognize peptide fragments derived from both surface glycoproteins and internal proteins. While surface glycoproteins (HA and NA) vary due to antigenic drift or shift, the internal proteins, namely the nucleoprotein, matrix protein and others, are fairly conserved within the subtype of influenza viruses. It has been shown that although T lymphocytes will not prevent infection, cytotoxic T lymphocytes kill virus-infected cells and aid in viral clearance, thus contributing to clinical protection against influenza illness (Yap et al. 1978). Hence, the activation of both CD4 and CD8 T lymphocytes will provide cross-protection against variant viruses within a subtype. Virus infection induces robust T lymphocyte responses, which persist for a very long time and provide cross-protection in mice. The magnitude and durability of T lymphocyte responses depend on the route of infection/immunization, whether or not the vaccine is formulated to induce or recall especially CD8 T lymphocyte responses. The current inactivated split-virus influenza vaccines provide only exogenous antigens for stimulation of T lymphocytes and thus are poor inducers of CD8 T lymphocyte responses. Activating or recalling CD8 T lymphocyte responses by formulating vaccines with adjuvants which will stimulate antigen-presenting cells creates an optimal priming environment and activates T lymphocytes to provide broader protection against serologically distinct viruses. CD4 T helper cells provide growth factors for B and CD8 T lymphocytes, thereby occupying a central role in the induction of humoral and cellular immune responses. Th1 and Th2 cells were defined based on the secretion of IFN-γ. While Th1 cells secrete IFN-γ following stimulation by IL-12, Th2 cells stimulated by IL-4 secrete IL-4, IL-5, and IL-13. The decline of naïve T cells in the repertoire due to thymic involution and accumulation of dysfunctional memory T cells is well established, but the mechanism for these observations goes beyond that which can be explained by thymic involution alone. Interleukin 7 appears to play an important role in T cell survival in thymic recombination events, and in expanding positively selected thymocytes (Hare et al. 2000; Huang et al. 2001). An age-related reduction in production of IL-7 within the thymus may be responsible for the age-related decline in thymic output of naïve T cells (Andrew and Aspinall 2002; Ortman et al. 2002). In humans, accumulation of an anergic CD28− T cell population with age, especially among the CD8 T cell subset, has been documented (Boucher et al. 1998; Sansoni et al. 2008). The molecular mechanisms leading to the loss of CD28 are not known (Boucher et al. 1998; Sansoni et al. 2008). The CD28− T cells are anergic to stimulation with antigen or mitogen. In murine studies, it has been clearly shown that the clonal expansion and function of naïve CD4 or CD8 T cells is significantly reduced when compared to their younger counterparts (Plowden et al. 2004a,b; Jiang et al. 2007).

In addition to T helper and cytotoxic T cells, Th3 or Treg cells that are CD4+CD25+ Fox3+ have been shown to play an important role in regulating immune responses (Dejaco et al. 2006; Hill et al. 2007). A recently published report and our unpublished findings show a significant increase in the Treg population and function with age, which may be contributing to poor adaptive immune responses (Zhao et al. 2007). However, a direct demonstration of the role of Tregs in the decline in immune responsiveness with aging is lacking, although our preliminary results indicate that depleting the Treg subset prior to immunization or infection with A/PR/8/34 virus enhanced both humoral and cellular immune responses in aged mice when compared to the control aged mice. An increased number of Tregs with age may aid in controlling the initiation of autoimmune disorders, but may come at the cost of reducing effective immune responses against infectious agents. The evolutionary significance of this finding is not clear. The functionality of CD4+ Th17 cells is beginning to be elucidated in mice, and very limited information is available on their role in humans and the impact of aging on the function of this subset (Bi et al. 2007; Nakae et al. 2007; Chen and O'Shea 2008).

CTL activity has been shown to be important for recovery from influenza virus infection in the absence of seroprotective antibodies to the infecting virus strain (McElhaney et al. 2006). CD8+ T lymphocytes recognize peptide fragments derived from viral proteins that are bound to class I MHC molecules and lyse the influenza virus-infected cells. The lysis of target cells can be mediated by perforin or by granule-mediated or Fas-mediated mechanisms (Apasov et al. 1993). CD8 T cell cytolytic activity is normally measured by labeling the MHC-compatible target cells (which are either pulsed with relevant peptides or infected with virus) with ^{51}Cr and determining the amount of ^{51}Cr released into the medium 4–5 h after the addition of CD8 T cells (Martz et al. 1974). Another assay to assess CTL activity is the measurement of granzyme B activity in lysates of influenza virus-stimulated PBMC; low levels of granzyme B have been correlated with risk for influenza illness in older adults (McElhaney et al. 1996, 2006).

3 Influenza Vaccine Efficacy in the Older Adult Population

Annually, influenza epidemics cause three to five million cases of severe illness with about 250,000–500,000 deaths worldwide (World Health Organization 2008). In an average year in the United States, complications from influenza infections result in about 250,000 hospitalizations and 36,000 deaths, with the majority of the fatalities occurring among the elderly population (Thompson et al. 2004; Simonsen et al. 2005). Complications from influenza viral infections resulting in hospitalizations and death are greatest among older adults, people with chronic medical conditions or immunological disorders, and infants and young children (i.e., ≤ 2 years of age) whose immune systems are still maturing (Fiore et al. 2007). Vaccination is the primary strategy for reducing the morbidity and mortality associated with

human influenza. An inactivated detergent-split trivalent influenza vaccine (TIV) containing two influenza A viruses (H1N1 and H3N2) and a type B virus as well as a live-attenuated nasal influenza vaccine containing all three components are marketed in the USA. While injectable vaccine is recommended for people at risk, including persons aged 50 years and older, live influenza vaccine is only recommended for persons 2–49 years of age (FDA 2007). Because older adults are a high-risk group for influenza-related deaths, the goal is to vaccinate 90% of this population (DHHS 2000). However, recent vaccination rates are stagnant and coverage still hovers around 65% (National Center for Health Statistics 2003). In healthy, younger adults, the vaccine may be 70–90% effective in preventing influenza-like illness if the vaccine antigen is antigenically closely matched with the circulating epidemic strain (Gross 2002). However, vaccine efficacy is substantially reduced to 30–50% in preventing complications from influenza infections among older adults (Nichol et al. 2007). The mortality benefits from influenza vaccination of older adults is a hotly debated topic (Simonsen et al. 2007). A meta-analysis of 18 cohorts of older adults in one HMO comprising data for ten seasons from 1990–1991 through 1999–2000 indicates that vaccination resulted in a 27% reduction in the risk for hospitalization due to influenza and a 48% reduction in the risk for death (Vu et al. 2002). However, the outcomes used for these studies included hospitalizations for pneumonia or influenza and death from any cause, which are not influenza-specific. Despite increased vaccination coverage of older adults since 1980, there was no decrease in influenza-related excess mortality rates among older adults in the USA (Thompson et al. 2003; Simonsen et al. 2005). Similarly, the results from studies of Netherlands and Italian groups suggest that vaccination did not result in a reduction in excess mortality due to influenza-like illnesses, although there was not enough statistical power to generalize those findings (Govaert et al. 1994; Rizzo et al. 2006). Ideally, a randomized placebo-controlled clinical trial with clearly defined clinical outcomes such as culture-positive influenza illnesses rather than influenza-like illness and pneumonia and all-cause mortality is required to evaluate the benefit of vaccination of older adults. However, policy decisions regarding the vaccination of all older adults make a placebo-controlled study ethically unacceptable to investigate the mortality benefits of influenza vaccination (Smith and Shay 2006). It has been shown previously that influenza vaccination is 49% and 32% effective in preventing hospitalizations from pneumonia or influenza and 55% and 64% effective in preventing death from any cause among older adults at low or intermediate risk, respectively. However, among older adults who are at high risk due to comorbid conditions, vaccination is 29% and 49% effective in preventing hospitalization and death, respectively. Furthermore, when efficacy and effectiveness of vaccination among older adults are stratified by age, a different picture emerges. The efficacy of vaccination in preventing illness and hospitalization decreases with advancing age and when associated with comorbid conditions, suggesting that old older adults do not mount optimal protective immune response to vaccination. Factors that impact vaccine efficacy are presented

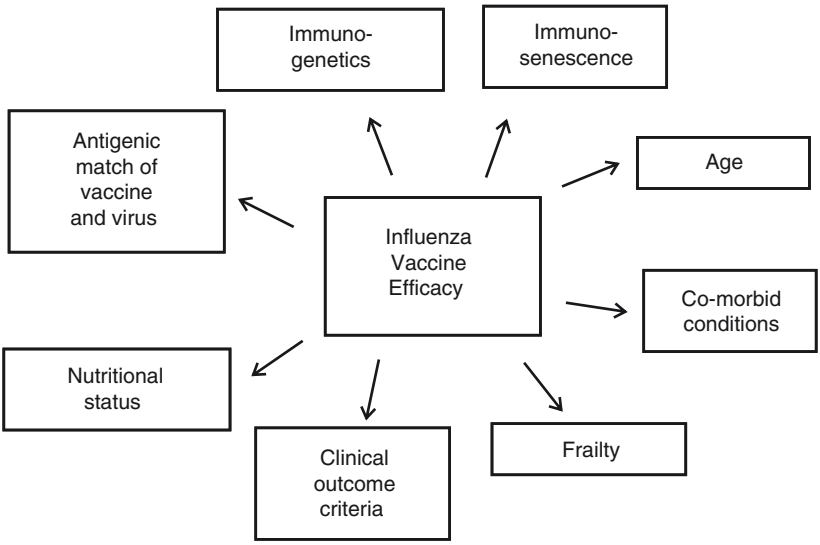

Fig. 3 Factors contributing to poor or suboptimal vaccine effectiveness in seasonal influenza vaccination of older adults

in Fig. 3, and immunosenescence is discussed above along with other factors that influence the outcome.

4 Active and Passive Immunization Strategies for Older Adults

A number of strategies to induce protective immune responses against influenza are presented in Fig. 4. Although passive antibody for influenza has not been considered a potential approach for both preventive and therapeutic needs, this approach has its own merits, especially when older adults who are at high risk or frail older adults who exhibit severe immune dysfunction are the target group. In addition, if the infection is caused by drug-resistant strains of influenza or a pandemic strain, passive therapy with human polyclonal antibodies offers a potential therapeutic benefit (Traggiai et al. 2004; Lanzavecchia et al. 2007; Simmons et al. 2007). Currently, transgenic animals that carry human immunoglobulin genes make human polyclonal immunoglobulins when immunized with antigens from infectious disease agents are available and these animals can serve as a potential tool to generate influenza strain-specific human antibodies for passive transfer (Fishwild et al. 1996; Tomizuka et al. 2000; Kuroiwa et al. 2002; Buelow and van Schooten 2006).

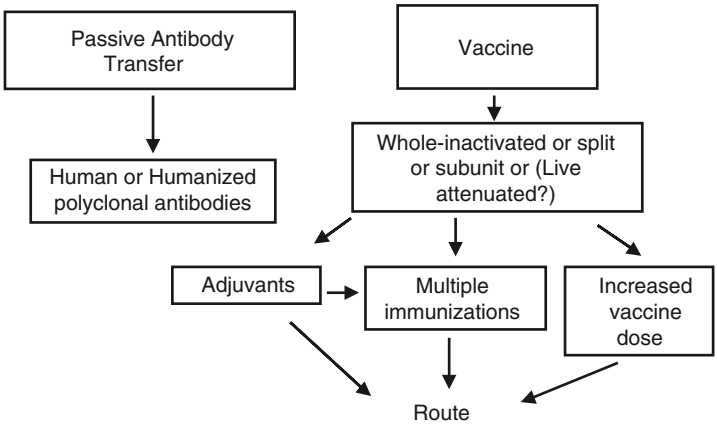

Fig. 4 Passive and active immunization strategies for older adults against influenza

It is clear that influenza vaccine needs to be formulated differently for older adults to overcome the age-related decline in immune function and enhance the immunogenicity and protective levels of both humoral and cellular immune responses. Although MF59-adjuvanted TIV vaccine is marketed in Europe for older adults and has been shown to be safe and immunogenic, it is not yet approved in the USA (Podda and Del Giudice 2003). Newer adjuvant systems such as ASO3 have been shown to enhance the immunogenicity of H5N1 virus vaccines, and may provide a potential benefit to the older adult population if formulated with seasonal vaccines. (Treanor et al. 2006, 2007; Leroux-Roels et al. 2007; Sambhara and Poland 2007). Increasing the vaccine dose from 15 μg of HA of each vaccine component of a TIV vaccine is a potential option for enhancing the levels of protective antibodies. In a recent multisite, phase II, randomized, double-blind clinical study, older adults who received 60 μg of HA of each component were shown to generate higher levels of HAI and neutralizing antibodies when compared to those who received the standard vaccine dose of 15 μg of HA of each of the components. However, the vaccinees who received the higher dose of HA experienced more local and systemic reactions than those who received the standard vaccine dose (Keitel et al. 2006; Couch et al. 2007). This result is consistent with earlier studies and the concern that increasing the vaccine dose produces unacceptable local reactions, an effect that may be overcome with an adjuvanted vaccine to improve responses in older adults. Another possibility is to vaccinate older individuals more than once during the influenza season in order to boost antibody responses. In a small clinical study, revaccination of older adults twelve weeks later did not enhance HI titers, suggesting that such an approach may not be a viable alternative (Buxton et al. 2001). However, one of the caveats of this study was the lack of baseline titers of the vaccinees who received the second dose of the vaccine. Hence, additional

studies are needed to evaluate if such an approach is a viable strategy to enhance the levels of protective antibodies in the older adult population.

5 Summary and Conclusions

Influenza is a vaccine-preventable disease, and the benefits of vaccination in preventing infection and complications arising from infection among adults are clearly documented. It is clear that the immune response declines with age due to alterations in innate and adaptive immune functions and that the vaccine does not provide adequate protection in this population. Hence, for older adults—a major target population for annual influenza vaccination, vaccine efficacy at preventing infection is low and the risk of serious complications from these infections is compounded by increasing age and comorbid conditions. Thus, efforts should be directed at formulating vaccines with adjuvants specifically for older adults to overcome immunosenescence, and passive immunization strategies with human polyclonal antibodies should be considered. In addition to conventional serological assays in the selection of vaccine candidates, other parameters namely, induction of cellular immune responses as well as activation of the innate immune system to facilitate an optimal microenvironment for the mobilization, activation, differentiation, maturation, and migration of antigen-presenting cells should be considered.

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