Immunosenescence Modulation by Vaccination

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Contents

Abstract: A decline in immune function is a hallmark of aging that leads to complicated illness from a variety of infectious diseases, cancer and other immune-mediated disorders, and may limit the ability to appropriately respond to vaccination. How vaccines might alter the senescent immune response and what are the immune correlates of protection will be addressed from the perspective of 1) stimulating a previously primed response as in the case of vaccines for seasonal influenza and herpes zoster, 2) priming the response to novel antigens such as pandemic influenza or other viruses, 3) vaccination against bacterial pathogens such as pneumococcus, and 4) altering the immune response to an endogenous protein as in the case of a vaccine against Alzheimer's disease. In spite of the often limited efficacy of vaccines for older adults, influenza vaccination remains the only cost-saving medical intervention in this population. Thus, considerable opportunity exists to improve current vaccines and develop new vaccines as a preventive approach to a variety of diseases in older adults. Strategies for selecting appropriate immunologic targets for new vaccine development and evaluating how vaccines may alter the senescent immune response

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in terms of potential benefits and risks in the preclinical and clinical trial phases of vaccine development will be discussed.

Keywords: Vaccination • Correlates of protection • Helper T-cells • Cytotoxic T-lymphocytes • Antibodies • Cytokines • Granzyme B • Influenza • Herpes zoster • Pneumococcus • Alzheimer's disease

1 Introduction

This review will focus on vaccine preventable diseases and the effect of vaccination on the senescent immune response to specific pathogens, observed in community-dwelling older adults and relevant experiments in animal models. It is important to distinguish these studies from those in older people in the nursing home setting who represent a small minority of the population age 65 years and older; multiple chronic diseases have already impacted on morbidity and disability and immune function may no longer be representative of the senescent phenotype. As a population, the majority of older adults experience "usual aging" where independence is maintained in the community but risk for complicated illness is associated with one or more underlying chronic diseases. From a public health perspective, usual aging older adults are the largest segment of the population age 65 years and older and should be the target population for new vaccine development; the goal is to compress morbidity to the extremes of life—"adding life to years".

A second focus of this review is to highlight the challenges in vaccine development for older adults and how vaccines may interact with the senescent immune response in ways that are not predictable using standard techniques such as antibody titres to evaluate potential efficacy. The goal of vaccination in this population should be clinical protection rather than sterilizing immunity; sterilizing immunity is predicted by antibody titres whereas estimates of vaccine efficacy with respect to clinical protection require an evaluation of both humoral and cell-mediated immune responses to a vaccine or the relevant pathogen. In addition, vaccines need to be tested in usual aging older adults who experience a variety of common medical conditions and related medications, and mental and psychosocial health issues, all of which may interact with functional independence. While studies of healthy older adults will help us to understand the effect of aging on the immune response, translating this research to vaccine preventable disease and improved health outcomes in the 65 and older population requires the identification of "modifiable risk" at all levels of innate and adaptive immune function.

2 Immune Senescence: Stimulating a Primed Response

2.1 Influenza Vaccination

2.1.1 Influenza, the Most Vaccine Preventable Disease in Older Adults

Influenza is foremost among all infectious diseases in terms of risk for serious complications and death in older adults and is the most vaccine preventable disease in this population. At least 36, 000 deaths and more than 100, 000 hospitalizations from respiratory and cardiovascular complications of influenza occur annually in the United States [1, 2]. In spite of only 40–60% efficacy in older adults [3], current influenza vaccination programs are cost-effective in older people and even cost saving in developed countries due to the 30–40% reduction in influenza-related hospitalizations [4, 5]. The fact that these vaccines also prevent complications of influenza (pneumonia, heart attacks, strokes and exacerbations of congestive heart failure) provides an even greater incentive to increase the use of existing vaccines and develop new vaccines that are targeted to improve the senescent immune response [6, 7]. However, a limited understanding of the immune mechanisms that underlie the increased risk for complicated illness and decline in the response to vaccination in this population, pose a significant challenge to new vaccine development.

2.1.2 Influenza Virus Stimulates both Humoral and Cell-mediated Immunity

The effect of influenza vaccination on the senescent immune response is best understood from the perspective of the adaptive immune response to influenza and how this may be altered through vaccination. Influenza vaccine is the most studied vaccine in older adults and well understood in terms of the potential immunologic determinants of clinical protection in this population. Thus, the response to this virus in the context of age-related changes in the adaptive immune system, will be discussed in significant detail as an example of what we might anticipate in terms of other potentially vaccine preventable diseases in older people.

Influenza virus stimulates an antiviral response in bone-marrow-derived lymphocytes (B-cells), monocytes and thymus-derived lymphocytes (T-cells) resulting in humoral and cell-mediated immunity, respectively. However, the effectiveness of this stimulus depends on the presentation of viral peptides to the T-lymphocytes There are two main cell types within the T-lymphocyte population, helper T-cells and cytotoxic T-cells. Helper T-cells are further sub-typed (according to the cytokines they produce) as T helper type 1 (T_h1), T helper type 2 (T_h2), T helper type 3 or regulatory T-cells (T_h3/Treg), and T helper type 17 (T_h17) cells. The response to influenza virus in adult populations is the result of restimulation of a previously primed response through exposure to natural infection or prior vaccination. Virus-activated T-cells, through a variety of cytokine mediators, stimulate B-cells to differentiate and

produce antibodies that are specific for the strains of virus contained in the vaccine [8]. These specific antibodies bind to the surface glycoproteins (haemagglutinin [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 high mutation rates in the influenza virus and selective pressure by the immune system against the native virus, a phenomenon known as antigenic drift. Influenza vaccines are updated annually to ensure that antibody-mediated immunity is stimulated to the relevant predicted strains of the H3N2 and H1N1 subtypes of influenza A, and influenza B.

Haemagglutination inhibition assays are the current industry standard for measuring antibody responses to influenza vaccination as a proxy for vaccine efficacy. There is significant literature reporting a decline in antibody titres with aging summarized in a recent metanalysis of these studies [9]. However, many of these studies have not defined the health status of study participants, their vaccination status, or the setting in which they live. Studies of the antibody response to influenza vaccination over multiple influenza seasons comparing healthy young adults to relatively healthy adults (probably representing "usual aging") have shown no difference between these two groups [10, 11]. These results suggest that aging alone does not affect the antibody response to influenza vaccination as measured in hemagglutination inhibition assays, and thus do not explain the differences in vaccine efficacy between young and older adults. Furthermore, even though the antibody response to vaccination might be predicted to decrease with repeated vaccination in older adults, annual repeated vaccination, in fact, improves protection against influenza [12–14]. Another postulate for the differences in vaccine-mediated protection in young and older adults has been that the duration of the antibody response to influenza vaccination may be shortened in older persons and not provide protection through the influenza season. However, a recent review found no evidence in the published literature of a premature decline in antibody titres during the influenza season in community-dwelling older adults [15].

There has been a paradigm shift in understanding the limitations of antibody titres as a sole measure of influenza efficacy [16]. As a correlate of protection against influenza, our studies have shown that serum antibody titres are similar and do not distinguish between older adults who will go on to develop influenza illness from those who do not [17]; and (McElhaney, submitted for publication). This is not to say that antibodies are not an important defense mechanism, but emphasizes the point that both humoral and cell-mediated immunity are important for clinical protection in older adults [18]. Thus, the evaluation of antibody titres alone as a surrogate of vaccine efficacy may fail to correlate with estimates of vaccine effectiveness from epidemiologic studies.

2.1.3 T-Cell Responses to Influenza are Conserved Across Different Strains

In contrast to B-cells that mount a subtype and strain-specific response, the antigenic determinants of the T-cell response are more conserved across the different strains of influenza. Thus, T-cell recognition and the response to influenza does not

degrade with antigenic drift [19–21]. Internal peptide sequences of hemagglutinin and neuraminidase are similar within the subtypes of influenza A (e.g., A/H3N2 vs. A/H1N1). Internal viral proteins (matrix and nucleoproteins) are conserved within the types of influenza (e.g., influenza A vs. B) [22]. Thus, peptides derived from surface glycoproteins and internal viral proteins stimulate helper T-cell and CTL responses that are cross-reactive within the strains of influenza A or influenza B. In other words, antibody responses are relatively strain-specific, while T-cell responses are cross-reactive across strains within influenza A or B.

Previous studies have shown that exposure of the entire respiratory tract to live influenza virus is the most effective method of inducing cross-reactive T-cell responses to influenza virus infections [23]. A direct comparison between different routes of infection showed that protection correlated with the size of 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 in the lymphoid organs for many years after influenza and other respiratory virus infections, protective cellular immunity is short-lived and disappears within about 6 months [23]. However, this CTL memory response can be recalled by vaccination with split-virus influenza vaccines in older adults especially when the vaccine has been recently exposed to natural infection with influenza (McElhaney et al., submitted for publication).

2.1.4 Effective Stimulation of Helper T-cells and CTL

Virus is taken up and processed by antigen-presenting cells such as macrophages and dendritic cells, and the resulting peptides are presented with the major histocompatability complex to activate T-cells [24]. Helper T-cells (T_h) recognize antigens presented by the major histocompatibility complex Class II (MHC II); MHC II is expressed almost exclusively on antigen-presenting cells, B-cells and T-cells [25]. In contrast, CTLs recognize viral peptides in combination with MHC I; MHC I is expressed on most cells in the body [26]. Structural viral proteins and both live and inactivated viruses are phagocytosed by macrophages and dendritic cells. The virus is processed within the antigen-presenting cell and presented in combination with MHC II to helper T-cells [27]. In contrast, viral peptides presented in combination with MHC I, are generally the products of viral replication within the antigen-presenting cell, although antigen cross-presentation in dendritic cells does occur (discussed below). Thus, the form of the viral antigen, and the interaction with a specific MHC and its cellular location independently determine T_h and CTL responses to vaccination [28, 29].

Antigen cross-presentation is the process by which antigens including killed virus or viral proteins are taken up by the dendritic cells, undergo proteasomal degradation, and are processed for presentation on MHC I. Because killed virus (contained current parenteral influenza vaccines) is effectively presented on MHC II and not MHC I, T_h and B-cells are stimulated to produce good antibody responses, but only weak CTL responses are seen in adults; this CTL response

that is not seen in influenza-naïve individuals, results from restimulation of a previously primed response to influenza through natural infection [28–31]. This process is postulated to be the mechanism by which inactivated viruses including split-virus influenza vaccines can stimulate CTLs in populations primed by a previous influenza infection [30]. The relevance of antigen cross-presentation to new vaccine development is that Toll-like receptor (TLR) ligands [32], virosomes [33], virus-like particles [34], and potentially adjuvants [35] can be used to activate APC and enhance expression of MHC I-viral peptide complexes and improve the poor CTL responses elicited by the current killed virus vaccines in older adults. Boosting T-cell responses is an important priority for vaccine development, in general, due to broader protection against serologically distinct strains of virus [23, 36, 37]. Because immunosenescence alters several aspects of cell-mediated immune function, vaccine design can include independent strategies for effectively stimulating T_h and CTL responses.

2.1.5 Effect of T-helper Cell Function on the Response to Influenza

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, down-regulated T_h2, and IL-10 downregulated T_h1 [38–40]. 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 (Treg or T_h3) and T_h17 subsets to cytokine regulation are only beginning to be understood [41]. Under a revised model, naïve 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, IL-5, IL-13; and IL-1, IL-6 and IL-23 stimulate T_h17 to produce IL-17, IL-22 and IL-26. These T_h subsets have counter-regulatory interactions between each other [42]. Our data showed that the IFN-γ:IL-10 ratio correlates with risk for influenza illness [17] but characteristics of the vaccine recipient and PBMC culture conditions may alter this relationship [43–46]. The apparent down-regulation of IFN-γ by IL-10 may be T_h3-mediated rather than a shift from a T_h1 to a T_h2 response [47], and the interaction with T_h17 has not been studied at all.

T_h17 appear to have developed as part of the adaptive immune response to combat extracellular pathogens not covered by T_h1 or T_h2 immunity based on studies in mice [48]. Studies in human PBMC sharply contrast with the results in mouse models. T_h17 promotes the recruitment of IFN-γ producing T-cells and as such, is regulated by the tissue level of IFN-γ [49]. Recent studies in human PBMC have shown that T_h17 can simultaneously produce IL-17 and IFN-γ suggesting that the two cytokines may work synergistically in the adaptive immune response[50]. Given the centrality of the T_h17 subset in immune regulation, T_h17 may have an important role in determining the cytokine response to influenza and responses to influenza vaccination.

2.1.6 Potential Cytokine-associated Correlates of Protection Against Influenza

Aging leads to a reduction in IL-2 synthesis [51, 52], an increase in IL-4 production [53], dysregulation of T_h1 and T_h2 cytokine responses and a decline in the CTL responses to influenza [54]. However, a recent review of the application of the T_h1/ T_h2 paradigm in older adults highlights the discrepancies of results across a number of studies in older people [47]. In light of an evolving understanding of interaction of multiple T-cell subsets in humans, the response to influenza and influenza vaccination may be more complicated than predicted by these earlier studies. A reduction in the ratio of IFN-γ to IL-10 levels in response to ex vivo challenge of PBMC with live influenza virus, is associated with increased risk for influenza illness [17]. However, the source of IL-10 may be from multiple different T-cell subsets in these cultures including T_h3/Treg. Also, absolute cytokine levels are less likely to predict risk for influenza illness suggesting that it is the regulation of the different T-cell subsets that determines the response to influenza and clinical protection from illness. It may be the balance and regulation of T_h1/ T_h2/ T_h3/ T_h17 responses that is important for a protective response to vaccination in older adults and recovery from influenza illness [55–57].

Recently, it has been shown in mice that with aging, antigen-presenting cells including monocyte/macrophages and dendritic cells produce lower levels of proinflammatory cytokines in response to ligation of Toll-like receptors [58]. The addition of these cytokines (IL-1, IL-6 and TNF-α) to spleen cells can reverse these age-related defects in T helper type 1 cytokine production [59]. The paradox is that IL-6 levels increase with age, chronic disease and stressors of the immune system and contribute to a proinflammatory state with increased production of IL-6 [60] and thus should stimulate rather than suppress T_h1. These results reflect conflicting postulates as to the determinants of influenza risk in older people based on cytokine levels. The recent identification of T_h17 cells and their regulation through TGF-β and IL-6 production and T_h3/Treg [61, 62], may shed further light on differences in cytokine regulation, susceptibility and health outcomes, and the relationship with acute illnesses versus chronic diseases.

2.1.7 Potential CTL-associated Correlates of Protection Against Influenza in Older Adults

Human studies have shown that CTL activity is important for recovery from influenza infection even in the absence of protective antibodies to the infecting virus strain [63]. 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 CTL [26]. Two mechanisms by which CTL activation leads to lysis of virus-infected cells include perforin- or granule-mediated killing [64–66], and fas-mediated killing [67, 68]. Granule-mediated killing is particularly

important for the control of respiratory viral infections although fas-mediated killing may provide an alternative but less specific mechanism [69].

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 6 months [23, 36, 70]. Even though current inactivated influenza vaccines stimulate a CTL response in older and even chronically ill older adults [71], this response is diminished compared to young adults [72–74] and is not as robust as the response to natural infection [75]. As well, the degree of cross-reactivity of CTL responses for different subtypes of influenza may decrease in chronically ill compared to healthy older adults [71, 72].

Virus-specific killing is mediated by granzymes contained in granules within CTL. Granules migrate to the "immune synapse" between the activated CTL 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 [76]. Granzyme B (GrzB) is a key element of the T-cell response to influenza in the lung [77–79]. An assay of GrzB activity in lysates of influenza virus-stimulated PBMC correlates with cytolytic activity by standard ^{51}Cr-release assays [80, 81] but has the advantage of being a more sensitive measure of cytolytic activity that is detectable in ex vivo virus-activated PBMC. Ex vivo levels of GrzB in lysates of influenza-stimulated PBMC correlate with risk for influenza illness in older adults [17]. Other ex vivo studies have shown no difference in influenza-specific CTL frequencies in older compared to young adults [82]. Taken together, these studies suggest that in influenza susceptible older persons, there is defect in the amount of GrzB produced on a per CTL basis.

2.1.8 Interaction of Antibody and Cell-mediated Immune Response to Influenza Vaccination

Current killed virus vaccines effectively stimulate T helper cells and vaccination of healthy older people increases IL-2 to levels comparable to that of young adults [83–85]. Other studies have reported heterogeneous cytokine responses to influenza vaccination in healthy older people that are related to characteristics of the vaccine recipient and the vaccine [43–45]. The in vitro proliferative response to influenza vaccine is associated with protection from influenza illness in ambulatory older adults [18]while antibody titres, tested in different settings, have not been consistently associated with risk for influenza illness in older people [17, 86]. In institutionalized older adults, IL-2 and IFN-γ responses to influenza are significantly associated with the level of independence in activities of daily living but do not predict protection against influenza illness [46]. Current killed virus vaccines have been shown to decrease the IL-10 (T_h3/Treg) response to influenza for an overall increase in the T_h1 (IFN-γ) relative to the T_h3 response [87], but the short duration of the response is not effectively re-stimulated with a booster vaccination [88]. Further

the ratio of IFN-γ:IL-10 has been shown to predict a protective response to influenza vaccination [17].

Killed virus vaccines stimulate a weak cytotoxic T-cell response and have limited efficacy in older adults. New developments in vaccine technology that improve the regulation of T_h cytokines and potentially increase the CTL response look promising**.** Live-attenuated intranasal vaccines were developed to provide more effective stimulation of CTL. However, these vaccines have shown minimal additional benefit for preventing influenza in older adults when combined with the standard inactivated parenteral vaccines, although some improvements in symptoms [89] and immunogenicity [90] have been reported. Thus, the currently available killed virus vaccine given by injection is still the recommended vaccine for those aged 50 years and older.

2.1.9 The Effect of Replicative Senescence on Immune Function and Responses to Vaccination

With aging and the multiple immune responses that have been stimulated throughout one's lifetime, there is a gradual shift from predominantly naïve T-cells to increasing proportions of memory T-cells. Thymic involution and the loss of naïve T-cells with aging may thus exhaust the capacity to respond to new antigens. Recent studies have further delineated central and effector memory helper T-cells, and have shown that healthy older adults have T_h responses to influenza vaccination similar to young adults. However, an age-related decline in IL-7 levels corresponds to failure to maintain or expand the effector memory helper T-cell response to influenza [91]. The importance of memory T-cells in recalling the response to the many cross-reactive influenza epitopes may be a key element of both T_h and CTL responses to split-virus vaccines.

Features of 'successful aging' have been associated with well-preserved immune function while poor survival is predicted by high CTL counts, low helper T-cell counts, low numbers of B-cells and poor responses by T-cells to polyclonal stimulation [92, 93]. The phenomenon of replicative senescence has been associated with these changes and relates to the finite number of doublings (25–30 cycles) after which cell cycle arrest occurs [94]. In CTLs, this growth arrest is associated with increased production of several proinflammatory cytokines, resistance to apoptosis [95], and loss of the costimulatory molecule, CD28, required for optimal stimulation of CTLs [96, 97]. CTL that do not express CD28 (CD28- CTL) have little or no cytolytic activity [98] and an increased proportion of CD28- CTLs is associated with a decline in antibody responses to influenza vaccination [99, 100] and a reduction influenza-specific memory CTL [101]. These changes have been associated with chronic cytomegalovirus infection driving the T-cell response to terminal differentiation and expressing this senescent phenotype [102, 103]. However, it remains uncertain the extent to which this change may affect the T_h and CTL responses to influenza and influenza vaccination.

2.1.10 Summary

Influenza is a serious illness in older adults and largely accounts for rising hospitalization and death rates from acute cardiac and respiratory illnesses in older people despite widespread influenza vaccination programs. While current vaccines are cost-saving, new influenza vaccines will be needed to avoid the anticipated crisis in health care related to the aging of the population. Recent studies suggest that there is a significant opportunity to exploit the reserve capacity of T-cells to respond to influenza antigens through enhanced antigen presentation, appropriate costimulation, and regulation of cytokine responses. Targeting identified immunologic mediators that modulate influenza risk in older people, and screening candidate vaccines for clinical trials using appropriate correlates of protection in this population, is critical to development of more effective influenza vaccines for an aging population. Since the early phases of vaccine development often rely on antibody titres as a surrogate of protection, this measure may fail to detect a more robust T-cell response and thus, a more effective vaccine for the 65 and older population.

2.2 New Vaccines for Herpes Zoster

2.2.1 Risk for Herpes Zoster and Aging

Herpes zoster (or Shingles) is a painful blistering rash resulting from the reactivation of latent varicella-zoster virus (VZV), the agent that causes of chickenpox. Prior to routine vaccination for VZV, approximately 90% of people in the USA were infected with this virus and the chance of developing shingles during one's lifetime was 25–30%. The risk of developing shingles dramatically increases with age.

Older persons bear the greatest burden of illness related to shingles, the clinical condition that results from reactivation of latent varicella-zoster virus (VZV). Each year between 600, 000 and 1 million Americans develop shingles and the risk dramatically increases with age—50% of persons over age 85 will suffer from disabling post-herpetic neuralgia (PHN) as a complication of shingles. Despite extensive epidemiologic studies of risk, little is known about the immunologic determinants of risk for shingles. The age-related decline in T-cell function is well documented but there is only limited data on how T-cell responses to VZV change with aging. Further, as shingles is exclusively a human herpes virus, animal models are very limited and may not be helpful in identifying the mechanism by which aging precipitates shingles. Particularly due to the aging of the "Baby Boomers" the age 65 and older population will grow to represent 20% of the US population over the next three decades. Identifying the immunologic changes in the response to VZV that occur with aging, is the first step in a mechanistic approach to targeting vaccines for this important human disease.

2.2.2 Shingles is an Important and Disabling Disease in Older Adults

Varicella-zoster virus presents as chickenpox in childhood and becomes a latent infection in the dorsal root ganglion of the spinal cord. The increased risk of shingles with age has been well-documented with an annual incidence of 14/1000 in those age 75 years and older leads and an astonishing prevalence of up to 50% of those over age 85 years old [104–106]. Reactivation causes a painful dermatomal rash called shingles that is often followed by PHN, a chronic pain syndrome associated with significant disability in older people. The incidence of PHN is almost negligible before age 50, but 21% of patients older than 60 years and 29% beyond age 70 become affected following an attack of shingles. This contrasts with shingles in children where the rash generally follows a mild case of chicken pox and is of little clinical significance [107]. Antiviral therapy is available but older people often do not present within the 48–72 hour window of onset of the rash necessary for initiation of effective treatment. In addition, 20% of older people who receive therapy within the therapeutic window still experience pain six months after the onset of the rash [108]. Particularly given the number of people who do not seek or receive timely and effective treatment of shingles, the prevalence of disability related to PHN is a major public health concern.

There is a significant literature on the impact of shingles on the quality of life in older people and on various therapeutic strategies for the management of PHN, the review of which is beyond the scope of this review that focuses on the prevention of shingles. However, the importance of perceived quality of life and psychological conditions that have been identified from epidemiologic studies as risk factors for the development of shingles, are relevant due to their potential impact on immune function. A recent review of these studies suggests that in addition to age, poor self-perceived health, psychological stress and/or lack of social support and mechanical trauma may lead to loss of cell-mediated immunity to VZV and increased risk of shingles [109].

2.2.3 Studies of the Link Between Risk for Shingles and Immunosenescence are Limited

Because VZV is exclusively a human Herpes virus [110], there are limited animal models that study only some aspects of VZV infection and reactivation [111]. From the studies to date, it appears that with the resolution of chickenpox, VZV-specific CTL access the dorsal root ganglion where VZV lives, to keep viral replication in check [111, 112]. At some point the virus escapes to replicate in nerves and skin to cause a very painful condition that continues even after the rash resolves. Whether or not reactivation of VZV is due to a general decline in CTL-mediated immunity or due to changes in VZV-specific CTL is unknown. These findings have not been studied as a potential mechanism for reactivation of VZV in older people. Further, the mechanism that keeps virus restricted to the dorsal root ganglion is unknown and it may be postulated that reactivation of VZV in older people is due to an increased number

of CD8+CD28- VZV-specific CTL that produce IFN-γ but do not contain cytolytic mediators such as Grz B. This hypothesis would be consistent with the extensive literature on the age-associated loss of CD28 expression, telomerase activity and telomere length affecting both CD4+ and CD8+ T-cells that is also associated with repeated antigenic stimulation (Reviewed in [94]).

Reactivation of VZV is associated with marked inflammation of the dorsal root ganglion leading to nerve cell damage and the pain associated with PHN that often precedes the onset of the dermatomal rash. Inflammatory cytokines produced by a stressful event and in the early stages of VZV reactivation may further suppress CTL function. With aging, there is a loss of ability to downregulate the inflammatory response and probably leads to excess nerve damage in the dorsal root ganglion and increased pain that persists as PHN for greater than one year in more than 50% of adults age 70 years and older who experience PHN. Even appropriate antiviral treatment initiated within 72 hours of onset of the rash fails to prevent this complication. Clearly, re-establishing the normal immune response to this virus requires the stimulation of VZV-specific CTL and regulation of the appropriate cytokine response to suppress viral replication without causing inflammation.

2.2.4 The Loss of the Costimulatory Molecule, CD28, Affects Immune Function

Age-related changes in T-cell function have been associated with terminal differentiation of memory T cell and replicative senescence (previously discussed in Section 2.1.9 and Reference [93, 94, 96, 98]) and an overall age-related decline in VZV-specific T cells. These changes lead to an increased risk of reactivation of VZV and the development and probably severity of PHN in older adults. Although suppression of VZV is unlikely to drive the general process of terminal differentiation of T cells as is the case with CMV, the loss of CD28 on VZV-specific T cells and co-stimulatory function, is a likely contributor to the risk for shingles and PHN. Effective vaccines against shingles may therefore need to stimulate T-cell subsets that express CD28 costimulatory molecules [113] and respond to novel strategies for antigen presentation.

2.2.5 The Development of a Shingles Vaccine

A large randomized double-blind placebo-controlled of a shingles vaccine enrolling over 38, 000 subjects showed in the vaccinated compared to the placebo group, a 61.1% reduction in burden of illness, a 51.3% reduction in shingles cases, and 66.5% reduction in those shingles cases complicated by post-herpetic neuralgia [114]. Futhermore, there was a reduction in the overall burden of illness in vaccinated subjects showing statistical significance for the primary endpoint in the trial. The vaccine strain of VZV is a previously attenuated live virus (Oka strain) that is predicted to stimulate humoral, T_h and CTL responses. Importantly for this disease, antibody titers do not predict protection from reactivating the virus to cause shingles. The postulated mechanism of protection is stimulation of the VZV-specific T-cell response to vaccination and meas-

ured by the IFN-γ enzyme-linked spot (ELISpot) assay [115, 116]. Given that this a live attenuated vaccine, safety testing included isolation of virus from all shingles cases following vaccination. Specimens collected from skin lesions in shingles cases in the post-vaccination period showed wild-type strains; the vaccine Oka zoster strain was not identified in any of the isolated specimens suggesting that this attenuated virus can be safely and effectively used to stimulate the senescent immune response. Since this clinical trial had relatively few exclusion criteria, the results of this clinical trial should be applicable to most adults age 60 or older who are not immunocompromised due to underlying diseases or medications.

2.2.6 Summary

Shingles is a major debilitating disease in the older adult population. Both age-related and age-associated changes in the cell-mediated immune response to VZV are clearly associated with increased risk of reactivating the virus to cause shingles and persist as PHN. The fact that antiviral therapy has limited effectiveness in the treatment of zoster in older adults points to the need for strategies to prevent the disease and it disabling complications. However, the development of a vaccine against Herpes zoster depended on a very large clinical trial to determine vaccine efficacy based on clinical outcomes. In the absence of reliable immunologic markers of vaccine efficacy, there was significant risk that the vaccine would fail to show an improvement. If the vaccine had failed in this trial, there may have been limited interest from industry in moving forward with alternate plans to develop an improved vaccine. This again points to the need for more reliable surrogates of vaccine efficacy to test new vaccines in the early phases of clinical development and select for subsequent clinical trials, the vaccines that are most likely to improve outcomes in the 65 and older population.

2.3 Implications of Effective Vaccines Against Respiratory Syncytial Virus

2.3.1 Respiratory Syncytial Virus Causes Serious Respiratorty Illness in Older Adults

RSV is a commonly circulating virus during the winter months and accounts for 2–5% of pneumonias in community-dwelling older adults (reviewed in [117]). The importance of this respiratory illness, particularly in older adults is increasingly recognized; it was recently reported that RSV causes 10,000 excess deaths in the United States and is second only to the A/H3N2 strains of influenza as a cause of death due to viral respiratory illness in the age 65 and older population [2, 118]. Although the virus is genetically stable over time (in contrast to influenza), repeat infections throughout adult life are common suggesting that immunity to this virus wanes over time. Those older adults with increased risk for severe disease are those with congestive heart failure and chronic lung disease, the severely immunocompro-

mised, and those living in long-term care facilities [119]. Estimates of RSV disease in this setting range from 5–10% of residents per year with pneumonia and death in 10–20% and 2–5% of cases, respectively. As with influenza illness in older adults, RSV results in prolonged lengths of hospital stay, significant disability and loss of independence in basic activities of daily living, and the need for a higher level of care at hospital discharge [120].

Studies of the immune response to RSV have shown that high levels of serum and/or nasal antibodies have been correlated with relative resistance to experimental challenge. Similarly, low serum and nasal antibody levels are risk factors for infection and disease severity in older adults but this is not an age-specific change [121, 122]. More importantly, older adults have a greater rise in antibody titres postinfection than do their younger counterparts [122]. Since the RSV virus does not undergo antigenic drift over time, one would predict better protection against recurrent RSV illness in older adults but instead there is a relative increased risk of RSV infection with aging. This observation may be explained by an age-related shift from a T_h1 to a T_h2 response to RSV, which has been shown to cause significant pathology in people. In the aged mouse model, diminished CD8+ CTL responses associated with decreased IFN-γ (T_h1) and increased IL-4 (T_h2), and higher RSV titres in lungs [123]. However, recent studies in human PBMC show no age-related changes in cytokine levels produced in response to RSV although the regulatory balance between inflammatory (IFN-γ and antiinflammatory (IL-10) cytokine levels may be altered [124].

2.3.2 The Development of a Respiratory Syncytial Virus (RSV) Vaccine

RSV circulates through much of the winter and often cocirculates with influenza during the mid-winter months. This presents a diagnostic challenge to clinicians as the symptoms of RSV illness completing overlap with those related to influenza illness [125]. Thus, treatment approaches would be particularly problematic as a strategy for limiting the complications of RSV and none are currently available for use in adults. The development of a vaccine against RSV has proven to be a significant challenge, perhaps due to the reliability of antibody titers as correlate protection in these trials. Deaths were observed in RSV-naïve children in whom RSV infection restimulated the immune response to vaccination and resulted in a significant inflammatory response to RSV infection. The challenge to developing an RSV vaccine for older adults is that RSV illness in older adults has not been well-studied, the virus circulates over a larger proportion of the winter months compared to influenza, and the symptomatology overlaps with influenza.

In summary, older adults experience significant complications of RSV illness but these complications are difficult to distinguish those related to influenza. Based on attack rates and impact of hospitalization in older adults, RSV is likely to cause significant disability in older adults. Immunologic correlates of clinical protection are not available and this presents a significant challenge to the development of the much needed vaccines against RSV for older adults.

3 Immune Senescence: Stimulating a Naïve Response

3.1 Pandemic Influenza Vaccines for Older Adults

The threat of pandemic influenza has increased with the direct transmission of highly pathogenic avian H5N1 viruses to humans and many countries are in the process of or have completed plans to manage an anticipated influenza pandemic. While animals have transmitted H5N1 influenza to people in close contact with livestock, additional mutations or reassortment events will be required for widespread human-to-human transmission. Current research is focused on predicting the strains that are likely to evolve so that new influenza vaccines can be developed to protect against these new strains. The development of effective pandemic influenza vaccines is likely but continued reliance on killed virus or subunit vaccine technology will leave older adults at significantly higher risk of illness, disability and death in the event of an influenza pandemic.

Targeting improvements in T-cell responses and thus protection against a number of strains will be particularly helpful for stimulating the senescent immune system against both seasonal and pandemic strains. In the case of H5N1, vaccines will not only have to stimulate an antibody response to the new vaccine strain but will also have to prime the T-cell response to influenza peptides derived from H5; age-related changes in naïve T-cells would result in decreased production of IL-2 and hence, the proliferative response to the vaccine in both B- and T-cells. This has implications for both prepandemic and pandemic vaccines. Pre-pandemic vaccines if formulated to more potently stimulate T-cells could offer cross-protective immunity and would enhance the production of strain-specific antibodies against the pandemic strain. Although this strategy may offer enhanced protection in older adults, prepandemic and pandemic vaccines will need to be tested for their ability to stimulate adequate antibody responses and cross-protective cell-mediated immunity. In the absence of improvements in the current split-virus vaccine technology, an influenza pandemic could have a significant impact on older people with overwhelming consequences for the health care system.

3.2 Other Viruses

As individuals age, infectious diseases cause increasing morbidity and mortality. This is especially evident when older adults contract newly emerging diseases such as severe acute respiratory syndrome (SARS), which killed 50% of infected individuals over the age of 50 [126]. The rapid human-to-human transmission of SARS exposed the entire age spectrum to a novel virus and highlighted the changes in the immune system that lead to increased morbidity and mortality rates with aging. Fortunately the outbreak was controlled without a vaccine and before it could reach epidemic proportions. Older adults may also be naïve to viruses such as West Nile Virus

(WNV) and Human Immunodeficiency Virus (HIV) and appear to be at increased risk of serious complications. When these viruses are contracted by an aged host, the senescent immune system may produce a less effective response compared to young adults. Evidence for this decline is from epidemiologic studies showing much higher mortality rates in older compared to young adults with WNV [127].

HIV prevalence is increasing and with aging of the population, HIV-infected patients age 50 years and older now represent10–13% of the HIV-infected population in the United States [128]. Both HIV and aging have been associated with the development of replicative senescence of T-lymphocytes and increased risk of infection [129]. Replicative senescence results from chronic stimulation of the immune system by the HIV virus and is associated with telomere shortening and loss of CD28 expression on CD8 T-cells [130]. These changes will need to be considered in the development of new therapies to improve the immune response to viral infections and vaccination [131].

4 Immune Senescence: Vaccines Against Bacterial Pathogens

4.1 Pneumococcal Vaccination

Streptococcus pneumoniae is an important cause of morbidity and mortality as a leading cause of community acquired infections including bacterial pneumonia, meningitis, and bacteremia. Amongst the highest risk groups and who bear the greatest burden of disease in the developed world, are adults age 65 years and older. The current 23-valent vaccine containing pneumococcal capsular polysaccharide (PPS) is cost-effective in this population [132, 133], but its efficacy may be limited by age-associated changes in the immune response to these vaccines. Although there is no age-related decline in the antibody response to pneumococal vaccination when healthy young and older adults are compared, consistent antibody responses to all 23-serotypes contained in vaccine may not be achieved in older adults [134]. In addition, opsonophagocytic activity, the major effector mechanism for clearing pneumococcus appears to decline with aging [135]. Further, there is a significant decline in antibody titres to PPS at six years following pneumococcal vaccination [136]. Repeat vaccination at least every 6 years in older adults may be needed to maintain protection against pneumococcal disease and can be safely administered in older adults.

Current vaccines containing PPS stimulate antibody production through a T-independent type 2 (TI-2) response (one not requiring T-cell help and lacking memory) [137]. Given that it is primarily cell-mediated, rather than humoral immunity that declines with the normal aging process, the efficacy of pneumococcal vaccines in older adults may also depend on how the cell-mediated immune response to the whole pneumococcus is stimulated. A Finnish study of older adults, showed that serotype-related differences in the serum antibody response following vaccination with 23-valent capsular polysaccharide vaccine, suggesting that some serotypes are weak immunogens in older adults [138]. Protein-conjugated PPS vaccines have

been developed to facilitate T-cell cooperation [139] and are effective in children. Fewer serotypes are represented in these vaccines and their benefit over traditional polysaccharide vaccines, have not been demonstrated in older adults.

Cytokine responses to pneumococcal antigens have been shown to regulate responses to protein and polysaccharide-specific antibody responses [140] and may be important in the pathogenesis of pneumococcal diseases [141]. Cytokine responses to the whole pneumococcus could explain changes in the virulence of different serotypes in older adults [142] and in comparison to cytokine responses in younger adults [143]. TNF-α, a macrophage product, is associated with an initial inflammatory response to pneumococcal invasion [139, 143] and has been found to be important in the development of antibodies to pneumococcal surface proteins [140, 145]. T_h2 cytokines including IL-4, although classically involved in stimulating B-cells to produce antigen-specific antibodies, may downregulate antibody responses to pneumococcus due to inhibitory effects on antigen-presenting cells [140]. IL-10, a product of both macrophages and T_h3 lymphocytes, is an antiinflammatory cytokine that also skews the in vivo immune response toward a T_h2 (humoral) by inhibiting a T_h1 (cell-mediated) response [146]. Increased IL-10 levels in animal models have been associated with increased risk for pneumococcal infection [142]. IL-12, a product of both phagocytic and antigen presenting cells, is a potent proinflammatory cytokine with a key role in resistance to bacterial infections. IL-12 upregulates the T_h1-mediated responses (IFN-γ) which recruits neutrophils to the lungs and thus has a protective role in the response to pneumococcus [147, 148]. Dysregulation of T_h-mediated cytokine responses with aging may thus contribute to the increased risk for pneumococcal infection in older adults. A greater understanding of the interactions between cytokine and antibody responses to pneumococcus and the immunologic determinants of risk for pneumococcal diseases are needed if improved vaccines are to be developed in older adults.

5 Immune Senescence: Altering Responses to Endogenous Proteins

5.1 Vaccines for Alzheimer's Disease

Alzheimer's Disease (AD) is caused by the deposition of β-amyloid protein (Aβ) in the brain with toxic effects leading to neuronal cell death, amyloid plaque formation and the development of neurofibrillary tangles. Based upon studies in mice, a vaccine containing the $A\beta_{1\text{-}42}$ peptide was shown to stimulate antibody production and improved cognitive function in mouse models of AD. This vaccine was advanced to a Phase II clinical trial based on the demonstration of no significant adverse effects of vaccination in a Phase I trial that included 200 subjects. The Phase II trial was halted due to the development of aseptic meningoencephilitis in 6% of the 300 vaccinated subjects. Analysis of the antibody response to Aβ showed a trend toward

cognitive enhancement in the "responders" to vaccination (antibody titre to $A\beta_{1\text{-}42}$ ≥ 1:2200) but 22% of "responders" compared to 2% of "nonresponders" developed aseptic meningoencephilitis [149]. These results suggested that adverse effects of the vaccine were related to the immune response to the vaccine rather than toxicity of the $A\beta_{1\text{-}42}$. Postmortem studies of the meningoencephilitis cases showed substantial clearing of Aβ from the brain but with marked T-cell infiltration in brain tissue.

The postulated age-related defect that leads to the pathology of AD related to APC uptake of Aβ and stimulation of T_h1 cytokines, is an inflammatory response to Aβ. This defect is associated with inefficient phagocytosis of Aβ and the production of inflammatory cytokines (IL-1β, TNF-α) and chemokines, and nitric oxide leading to complement activation and T-cell apoptosis [150]. The immune mechanism being targeted by the vaccine was T-cell-dependent antibody production against Aβ to form Aβ-antibody complexes for more efficient clearing of the Aβ. Earlier studies had shown that $A\beta_{1\text{-}42}$ effectively stimulated a proliferative response in human peripheral blood mononuclear cells. This response was increased in older compared to young adult subjects and a further significant increase was observed in older adult subjects with AD [151]. It had been shown that $A\beta_{1\text{-}15}$ was responsible for B-cell stimulation and the production of antibodies to Aβ, and $A\beta_{15\text{-}42}$ most effectively stimulated T-cell proliferation and the production of both T_h1 (IFN-γ) and T_h2 (IL-13) cytokines. Because a T_h1 (vs. T_h2) response in the AD mouse model was associated with more effective clearance of Aβ in the mouse model, QS21 adjuvant was added to the Aβ vaccine used in human trials to stimulate a Th1 response to the vaccine. It is postulated that the adjuvanted vaccine activated Aβ-specific memory T-cells that migrated to the sites of Aβ deposition in the brain and produced Th1 cytokines. Although the antibody response appeared to effectively clear Aβ, the inflammatory cytokine response of the T-cell infiltrate lead to meningoencephalitis. Since the $A\beta_{15\text{-}42}$ stimulated both Th1 and Th2 cytokines, it appears that the addition of the QS21 adjuvant may have been responsible for the serious adverse effects of the Aβ vaccine [150]. While efforts continue to develop immunologic-based therapies for AD, the results of this clinical trial will have significant consequences for future vaccine development. Lessons learned from the Alzheimer vaccine trial suggest that targeting the aged immune system through vaccination to produce a more effective response may be a "double-edged vaccine"[152].

6 Summary

Age-related changes in the immune system have been associated with increased risk for infectious diseases. These are largely attributed age-related changes in T-cell-mediated immunity and defects in defense mechanisms mediated by Th1 (IFN-γ) and CTL. This would suggest that more potent vaccines for older adults should stimulate Th1 and CTL to a particular pathogen. However, the underlying mechanism for defective immune responses in older people remains poorly understood including the potential negative impact of elevated levels of inflammatory cytokines (including IFN-γ). In

spite of our limited understanding, a number of available vaccines have been shown to cost-effective and even cost-saving in older adults. Future research to better understand the immunologic targets for the prevention or treatment of a variety of acute and chronic diseases will make a significant contributions to "adding life to years".

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