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 West Nile virus, (3) vaccination against bacterial pathogens such as pneumococcus and pertussis, (4) vaccines against bacterial toxins such as tetanus

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and *Clostridium* difficile, and (5) vaccine approaches to mitigate effects of cytomegalovirus on immune senescence. New or improved vaccines developed over recent years demonstrate the considerable opportunity 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 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 · CD4 T cells · CD8 T cells · Antibodies · Cytokines · Granzyme B · Influenza · Herpes zoster · Pneumococcus · Cytomegalovirus

This chapter focuses 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 minority of the population aged 65 years and older; multiple chronic diseases, disability, and frailty contributing to further loss of 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 functional decline during an acute illness increases with chronic disease burden and frailty. From a public health perspective, usual aging older adults are the largest segment of the population aged 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 chapter 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 correlates of protection such as antibody titers to evaluate potential efficacy. The goal of vaccination in this population should be clinical protection against serious illness rather than sterilizing immunity; sterilizing immunity is predicted by the level of antibody titers needed to prevent infection as a surrogate of vaccine efficacy, whereas estimates of vaccine effectiveness and clinical protection requires an evaluation of both humoral and cell-mediated immune responses to a vaccine or the relevant pathogen. In addition, new vaccines should be tested in usual aging older adults who experience common medical conditions and mental and psychosocial health issues, all of which may interact with vaccine responsiveness. While studies of healthy older adults with no underlying chronic diseases or disability will help us to understand the effect of aging on the immune response, translating this research to improve health outcomes in the 65 and older population requires the identification of "modifiable risk" in both age-related and age-associated changes in immune function.

Immune Senescence: Stimulating a Previously Primed Response

Influenza Vaccination

Influenza, a 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 has been a cost-saving intervention 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 (Thompson et al. 2003, 2004). In spite of only 40–60% efficacy in older adults (Govaert et al. 1994), 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 (Nichol et al. 1994; Vu et al. 2002). Hospitalizations rates are dramatically increased in adults aged 65 years and older in years when influenza A/H3N2 is the predominant circulation strain, relative to the rates observed when H1N1pdm09 virus has been the predominant circulating strain. The fact that seasonal influenza 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 vaccine efficacy (DiazGranados et al. 2014). However, the immune mechanisms which underlie the increased risk for complicated illness, and the recent decline in seasonal vaccine effectiveness against A/H3N2 strains (Belongia et al. 2016), are poorly understood.

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 classically within the CD4 T cell subset and are further subtyped (according to the cytokines they produce) as T helper type 1 (Th1), T helper type 2 (Th2), T follicular helper (Tfh), T regulatory (Treg), and T helper type 17 (Th17) 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 (Virelizier et al. 1974). These specific antibodies bind to the surface glycoproteins (hemagglutinin [HA] and neuraminidase [NA]), to neutralize the viral particle. The peptide sequences on the outer surfaces of HA and NA, which are the antibody-binding sites, 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. These changes in the HA identify a new circulating strain of influenza virus requiring annual updates in the influenza vaccine strains 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.

Hemagglutination inhibition assays are the current industry standard for measuring antibody responses to influenza vaccination as a proxy for vaccine efficacy. There is a significant literature reporting a decline in antibody responses to influenza vaccination with aging (Goodwin et al. 2005). However, recent studies suggest that this observed decline may not be related to aging but instead to the effect of prior exposure to influenza (Reber et al. 2015) and repeated annual vaccination (Mosterin Hopping et al. 2016), and the phenomenon of original antigenic sin, more recently identified as antigenic seniority (Kucharski et al. 2015). Even though the B cell response to influenza vaccination is maintained in older adults, the antibody response is not; this appears to be impaired differentiation of B cells to antibody-producing plasma cells due to a decline in the activity of activation-induced cytidine deaminase (AID) and the transcription factor E47. These results highlight the fact the changes in the immune response with aging cannot be attributed to immune senescence alone and are further complicated when one considers the health status of study participants, their vaccination status, or the setting in which they were living. Clearly, factors that contribute to increasing frailty are expected to cause a further decline in immune responsiveness. These results suggest that additional mechanisms beyond antibody responses are involved in the loss of protection particularly against A/H3N2 strains with aging. 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 in this population (Keitel et al. 1988; Ahmed et al. 1995; Voordouw et al. 2004) while annual repeated vaccination in children and adults has been associated with a loss of protection (McLean et al. 2014). Even so, the ability to mount a fourfold increase in antibody titers in response to influenza vaccination has been associated with a twofold reduction in influenza disease (Benoit et al. 2015). However, large influenza vaccine trials have not been statistically powered to determine if this protection, consistent with sterilizing immunity (antibody neutralization of the virus to prevent infection), translates to a reduced risk of hospitalization; thus, correlates of clinical protection against A/H3N2 disease (cell-mediated process to clear virus from the lungs) may be required. Vaccine-mediated protection may also be related to the duration of the antibody response to influenza vaccination, but a previous review of the literature found no difference between young and older adults in the rate of decay of antibody titers during the influenza season (Skowronski et al. 2008).

There has been a paradigm shift in understanding the limitations of antibody titers as a sole measure of influenza efficacy (Effros 2007). As a correlate of protection against influenza, our studies have shown that serum antibody titers do not distinguish between older adults who will go on to develop A/H3N2 influenza illness from those who do not, and may fail to predict vaccine failure (McElhaney et al. 2006, 2009; Shahid et al. 2010). Although antibodies are an important defense mechanism, both humoral and cell-mediated immunities are important for clinical protection in older adults (Murasko et al. 2002). A greater understanding of how T cell-mediated immunity contributes to increased protection against influenza is needed in the development of new influenza vaccines (reviewed in McElhaney et al. (2016)). Thus, the evaluation of the antibody response in clinical trials of new vaccines as a means to progress through the clinical trial phases to approve a new vaccine may fail to predict enhanced vaccine efficacy, particularly when efficacy is measured as a single endpoint of protection against all three vaccine strains (McElhaney et al. 2013). In this case, vaccine trials are more likely to give a true picture of enhanced efficacy when A/H3N2 is the predominant circulating strain (DiazGranados et al. 2014).

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 do not degrade with antigenic drift (Effros et al. 1977; Butchko et al. 1978; Russell and Liew 1979). 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) (Mitchell and Callard 1983). 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, and have been immunologic targets for the development of universal influenza vaccines.

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 (Nguyen et al. 1999). 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 (Nguyen et al. 1999). However, this T cell memory response to the internal proteins of the influenza virus, matrix (M1) and nucleoprotein (NP), can be recalled during infection and, in the absence of protective antibody titers, CD4+ and CD8+ T cells, respectively, correlate with protection against A/H3N2 (Wilkinson et al. 2012) and pdmH1N1 (Sridhar et al. 2013) in young adults. In older adults, the CTL response to split-virus influenza vaccines in older adults is enhanced when the vaccine has been exposed to influenza through natural infection in the previous influenza season (McElhaney et al. 2009).

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 histocompatibility complex to activate T cells. 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 (Hackett and Eisenlohr 1990). In contrast, $CD8^+$ CTLs recognize viral peptides in combination with MHC I; MHC I is expressed on most cells in the body (Ennis et al. 1981). Structural viral proteins and both live and inactivated viruses are phagocytozed 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 (Demotz et al. 1990). 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 is recognized as an important contribution in response to influenza vaccination (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 (Hackett et al. 1991; Lamb et al. 1991).

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 inactive virus (contained in current parenteral influenza vaccines) is effectively presented on MHC II and not MHC I, helper T cells and B cells are stimulated to produce good antibody responses, but only weak CTL responses are seen in adults. This CTL response is not observed in influenza-naïve individuals, and results from restimulation of a previously primed response to influenza through natural infection (Hackett et al. 1991; Lamb et al. 1991; Hosaka et al. 1988). This is the presumed mechanism by which inactivated viruses including split-virus influenza vaccines can stimulate CTLs (Askonas et al. 1982). The relevance of antigen cross-presentation to new vaccine development is that toll-like receptor (TLR) ligands (Plowden et al. 2004; Behzad et al. 2012), virosomes (Huckriede et al. 2005), virus-like particles (Bright et al. 2007), and potentially adjuvants (McElhaney et al. 2013; Puig-Barbera et al. 2004) can be used to activate antigen-presenting cells and enhance expression of MHC I-viral peptide complexes and improve the poor CTL responses elicited by the current killed virus vaccines. Boosting T cell responses is an important priority for vaccine development, in general, due to broader protection against serologically distinct strains of virus (Ray et al. 2004). Because immunosenescence alters several aspects of cell-mediated immune function, vaccine design can include independent strategies for effectively stimulating helper T cell and CTL responses.

Effect of T Helper Cell Function on the Response to Influenza

The helper T cell (T_h)-mediated immune response to influenza virus plays a key role in the generation of both humoral and CTL responses to influenza vaccination. T_h1, T_h2, Tregulatory (Treg), T follicular (Tfh), and T_h17 all contribute to the immune response through interactions with T and B cells in the lymph nodes or bone marrow.

These T_h subsets are defined by their cytokine products and/or their location within lymphoid tissues. The T_h1 cytokine, interferon-γ (IFN-γ), provides help along with IL-2 in the development of both B cell and CD8 CTL responses to influenza, and is down-regulated by the production of IL-10 from Treg (Gardner and Murasko 2002). T_h2 also provide help to B cells but not CD8 CTL. The IFN-γ:IL-10 ratio declines with age (Skowronski et al. 2011) and is inversely correlated with risk for influenza illness in older adults (McElhaney et al. 2006), suggesting that a shift toward a Th1 response to influenza vaccination is important for protection in older adults.

The Role of Inflammatory Cytokines in Response to Influenza

"Inflammaging," the overproduction of inflammatory cytokines in older people, is associated with an increased risk of age-related frailty, morbidity, and disability, while the anti-inflammatory response involving IL-10 and other mediators that could potentially weaken resistance to infection contributes to healthy aging (Franceschi et al. 2007). This is in contrast to the known inflammatory responses to viral infections that trigger a variety of pathways involved in host defense. It has been shown in aged mice and humans that the addition of inflammatory cytokines (IL-1, IL-6, and TNF-α) or the combination of IL-2 and IL-6 can reverse age-related defects in T_h1 cytokine production, proliferation, and function (Haynes et al. 2004). Our recent study has shown that these combined effects of IL-2 and IL-6 can restore influenza-specific CD8 CTL function in older adults to that observed in young adults in both mice and humans (Zhou et al. 2016), and suggests a mechanism that can be targeted for developing more protective vaccines.

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 (McMichael et al. 1983a). 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 (Ennis et al. 1981). CTL activation leads to lysis of virus-infected cells by perforin- or granule-mediated killing (Pasternack and Eisen 1985; Bleackley et al. 1988; Jenne and Tschopp 1988), and is particularly important for the control of respiratory viral infections (Doherty et al. 1997).

Although self-renewing populations of virus-specific T cells are maintained for many years after influenza infection, protective cellular immunity is short-lived and disappears within 6 months (Nguyen et al. 1999; Ray et al. 2004; McMichael et al. 1983b). Even though current inactivated influenza vaccines stimulate a CTL response in older and even chronically ill older adults (Gorse and Belshe 1990), this response and degree of cross-reactivity is diminished compared to young adults (Powers 1993; Mbawuike et al. 1993; McElhaney 2005) and is not as robust as the response to vaccination following natural infection (McElhaney et al. 2009; Shahid et al. 2010).

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 (Darmon et al. 1995). Granzyme B (GrB) is a key element of the T cell response to influenza in the lung (Johnson et al. 2003; Lawrence et al. 2005; Jenkins et al. 2007). An assay of GrB activity in lysates of influenza virus-stimulated PBMC correlates with cytolytic activity(Ewen et al. 2003), and low ex vivo levels of GrB predict increased risk for influenza illness in older adults (McElhaney et al. 2006, 2009; Shahid et al. 2010). Other ex vivo studies have shown no difference in influenza-specific CTL frequencies in older compared to young adults (Boon et al. 2002) and susceptibility in older adults may be due to decreased cytolytic activity (and the amount of GrB produced) on a per cell basis (Ouyang et al. 2003).

Interaction of Antibody and Cell-Mediated Immune Response to Influenza Vaccination

Inactivated influenza virus vaccines effectively stimulate T helper cells, but in older adults, dysregulated cytokine production leads to a decline in the IFNγ:IL-10 ratio in response to live virus challenge, and defects in the B cell response to vaccination (reviewed in Frasca and Blomberg (2014)) contribute to increased susceptibility to infection in this age group. A weakened CTL response to influenza that is poorly stimulated by inactivated vaccines contributes to the observed increased risk of serious complications. Recent developments including a high-dose influenza vaccine containing four times the amount of antigen in the standard dose vaccine has demonstrated a 24% improvement in efficacy against influenza illness and enhanced protection against hospitalizations for pneumonia during the influenza season. Live-attenuated intranasal vaccines were developed to provide more effective stimulation of CTL. However, these vaccines have shown minimal additional benefit in older adults probably due to neutralization of the vaccine virus by antibodies in the nasal mucosa (Gorse et al. 2003). Current efforts are focused on the use of adjuvants to improve vaccine-mediated protection.

The Effect of Cytomegalovirus 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. An age-related decline in IL-7 levels that support thymic rejuvenation corresponds to failure to maintain or expand the effector memory helper T cell response to influenza (Kang et al. 2004). More recently, this age-related change in the response to influenza has been associated with persistent cytomegalovirus (CMV) infection and the accumulation of late-differentiated T cells with a reversal in the normal CD4:CD8 T cell ratio, which declines to less than one. In CMV seropositive

compared to seronegative older adults, there is a decline in the CD4 T cell response to influenza peptides derived from the influenza internal proteins, matrix (M), and nucleoprotein (NP), which is not seen in young adults (Derhovanessian et al. 2013). Further, this effect of CMV appears to be a major driver of the shift of the naïve T cell pool to the central and effector memory subsets (reviewed in Pawelec et al. (2005)). This effect is more pronounced in CD8 T cells and yet there is no difference in their response to M- and NP-derived peptides in seropositive versus seronegative older adults (Derhovanessian et al. 2014). These results suggest that additional mechanisms underlie age-related changes in CD8 T cells.

Poor survival is predicted by this inversion of the CD4:CD8 T cell ratio (Franceschi et al. 1995; Ferguson et al. 1995), yet these cells in both seropositive and seronegative older show enhanced inflammatory responses to polyclonal stimulation (Wikby et al. 2006). 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 (Effros 1997). In CTLs, growth arrest associated with replicative senescence also shows increased production of several pro-inflammatory cytokines, resistance to apoptosis (Effros et al. 2003), and loss of the costimulatory molecule, CD28, required for optimal stimulation of CTLs (Effros et al. 1994; Engwerda et al. 1994). CTL that do not express CD28 (CD28-CTL) have little or no cytolytic activity (Effros 2003) and an increased proportion of CD28-CTLs is associated with a decline in antibody responses to influenza vaccination (Goronzy et al. 2001; Saurwein-Teissl et al. 2002) and a reduction in influenza-specific memory CTL (Xie and McElhaney 2007). These changes have been associated with chronic CMV infection driving the T cell response to terminal differentiation and expressing this senescent phenotype (Vasto et al. 2007; Vescovini et al. 2007). However, it remains uncertain the extent to which this change may affect the T_h and CTL responses to influenza and influenza vaccination.

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 and new more efficacious vaccines have recently been approved, a margin for further improvement still remains. 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, regulation of cytokine responses, and the addition of adjuvants. 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 titers 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.

New Vaccines for Herpes Zoster

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 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 30% (Oxman and Levin 2008). The risk of developing shingles dramatically increases with age.

Older persons bear the greatest burden of illness related to zoster (also called shingles), the clinical condition that results from reactivation of latent Varicella-zoster virus (VZV) within the dorsal root ganglia. 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 the disabling complication of zoster, post-herpetic neuralgia (PHN). Despite extensive epidemiologic studies of risk, the immunologic determinants for reactivating this exclusively human herpesvirus are poorly understood, as there are limited animal models for mechanistic studies. T cell-mediated immunity is essential for recovery from herpes zoster due to significant cell tropism of this virus. Varicella infection and "silent" reactivation of zoster appear to restimulate VZV-specific cellular immunity in adulthood but after age 50, this boost to the immune system becomes less effective, and thus the dramatic increase in the rates of zoster and PHN with aging.

Shingles Is an Important and Disabling Disease in Older Adults

VZV 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 aged 75 years and older and an astonishing prevalence of up to 50% of those over age 85 years (Hope-Simpson 1965; Schmader 2001; Brisson et al. 2001). Reactivation causes a painful dermatomal rash called shingles that is often followed by PHN, a chronic pain syndrome defined as pain lasting for >90 days following zoster and associated with significant disability in older people. The incidence of PHN is almost negligible before age 50, but at least 50% of patients older than 60 years and almost 75% beyond age 70 become affected following an attack of shingles. Health-related quality of life is significantly impacted by zoster with >90% of over 65 year olds reporting pain with a median duration of 1 month (Drolet et al. 2010). The functional consequences of pain are significantly diminished with the resolution of zoster, but in the context of PHN, the pain and impact on daily function often continues for more than 6 months (Drolet et al. 2010). This contrasts with shingles in children where the rash generally follows a mild case of chicken pox and is of little clinical significance (Feder and Hoss 2004). Antiviral therapy is available but older people often do not present within the 48–72 h 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 6 months after the

onset of the rash (Cunningham and Dworkin 2000). Particularly given the number of people who do not seek or receive timely and effective treatment of shingles, the prevalence of disability and poor quality of life 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 chapter, which focuses on prevention through vaccination. 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 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 (Thomas and Hall 2004).

The Link Between Risk for Shingles and Immunosenescence

Because VZV is exclusively a human herpes virus (Cohrs et al. 2004), there are limited animal models that study the immunological mechanisms of VZV infection and reactivation (Baiker et al. 2004). However, much is known about the virology of VZV infection from studies of the live-attenuated Oka vaccine strain of VZV showing rapid evolution of the virus within the first 13 days of infection with no further replication of the virus once latency is established (Weinert et al. 2015). From the limited 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 (Baiker et al. 2004; Arvin et al. 1996). At some point, reactivation of latent VZV is associated with marked inflammation of the dorsal root ganglion leading to nerve cell damage and the pain associated with PHN often precedes the onset of the dermatomal rash. The latent virus escapes to replicate in nerves and skin to cause a very painful condition that continues even after the rash resolves and appears to be related to both central and peripheral nerve damage. Although there is a general decline in circulating VZV-specific $CD4^+$ T cells with aging, these IFN-γ-secreting T cells demonstrate a robust response at the site of the shingles rash, with similar numbers and function to that seen in young adults. However, regulatory T cells ($Foxp3^+CD4^+$ T cells) also accumulate at the site of infection in older adults and may inhibit the activity of otherwise functionally competent VZV-specific T cells (Vukmanovic-Stejic et al. 2013; Vukmanovic-Stejic et al. 2015). Although there are no identified immunologic correlates of protection against zoster reactivation, VZV-specific CD4+ and CD8+ T cells are believed to play a central role in latency and reactivation of the virus within the dorsal root ganglion (Arvin 2008; Steain et al. 2014; Gowrishankar et al. 2010). These results highlight the importance of designing vaccines to stimulate cell-mediated immunity to prevent reactivation of VZV and protect against zoster and PHN, rather than the usual focus on antibody responses to prevent infection.

The Development of Zoster Vaccines for Older Adults

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 (Oxman et al. 2005). Furthermore, there was a reduction in the overall burden of illness in vaccinated subjects showing statistical significance for the primary end-point 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 measured by the IFN-γ enzyme-linked spot (ELISpot assay) (Levin et al. 2003; Smith et al. 2003). More recent studies have confirmed that the live attenuated zoster vaccine boosts both VZV-specific CD4^{+} and CD8^{+} T cells (Sei et al. 2015), which appear to be important in preventing zoster reactivation and peripheral control of the infection in the nerves and skin. It is also important to highlight that this live attenuated vaccine Oka zoster strain was not identified in any of the VZV isolates from the skin of shingles cases in the vaccine trial, and thus can be safely and effectively used to stimulate the senescent immune response.

More recently, a recombinant subunit vaccine (HZ/su) containing VZV glycoprotein E and the AS01B adjuvant system (containing monophosphoryl lipid A (MPL) and QS21) has been developed and includes a two-dose regimen administered 2 months apart. A randomized placebo-controlled trial enrolled over 15,000 adults ≥50 years old and demonstrated an estimate of overall vaccine efficacy of 97% against herpes zoster over approximately 3 years of follow-up with no significant age-related decline in vaccine efficacy. Although reactions to the vaccine that affected performance of daily living activities (Grade 3) were significantly higher in vaccine recipients (17%) compared to placebo recipients (3.2), there were no differences in the number of participants that went on to receive a second dose of vaccine or placebo in the study and no significant differences between the two groups in serious adverse events or potential immune-mediated diseases or deaths in the trial. Further, because there is no live virus in the vaccine, there is no concern about VZV infection of immunocompromised individuals. A parallel trial in adults ≥70 years old using the same study protocol was designed to address vaccine efficacy for the prevention of post-herpetic neuralgia (Cunningham et al. 2016).

The mechanism of protection related to the HZ/su vaccine appears to be mediated by the stimulation of CD4^{+} T cells specific for the glycoprotein E subunit of VZV. Glycoprotein E promotes cell-to-cell interactions while other viral proteins promote cell fusion in the pathogenesis of the virus. The enhanced adaptive immune response to the glycoprotein E is dependent on AS01-mediated activation of dendritic cells and increased antigen presentation to the CD4^{+} T cells (Didierlaurent et al. 2014). However, there were too few zoster cases in this study to evaluate this CD4+ T cell response as a correlate of protection and further studies are needed to determine how the immune response to glycoprotein E offers protection against herpes zoster with no age-related decline in vaccine efficacy.

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 post-herpetic neuralgia. 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 its 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 to select vaccines most likely to improve outcomes in the 65 and older population.

Implications of Effective Vaccines Against Respiratory Syncytial Virus

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 Falsey (2007)). The importance of this respiratory illness, particularly in older adults, is increasingly recognized; it was recently reported that RSV causes 10,000 excess deaths 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 (Thompson et al. 2003; Mullooly et al. 2007). Although the virus is genetically stable over time (in contrast to influenza), repeat infections throughout adult life are common suggesting the 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 immunocompromised, and those living in long-term care facilities (Falsey et al. 1992). Estimates of RSV disease in this setting range from 5 to 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 (Falsey et al. 2005). A recent analysis of RSV illness in the context of a large influenza vaccine trial showed that RSV causes a significant proportion of the moderate to severe influenza-like illness during the winter months (Falsey et al. 2014).

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 (Walsh et al. 2004; Walsh and Falsey 2004). More importantly, older adults have a greater rise in antibody titers post infection than do their younger counterparts (Walsh and Falsey 2004). Since the RSV virus does not undergo antigenic drift over time, one would predict from this result, better protection against recurrent RSV illness would be predicted in older adults and is inconsistent with the relative increased risk of RSV infection with aging. A shift from Th1 to a Th2 response to RSV causes significant pathology in humans and in the aged mouse model, diminished CD8+ CTL responses associated with decreased IFN-γ (Th1) and increased IL-4 (Th2), and higher RSV titers in lungs (Zhang et al. 2002). 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 anti-inflammatory (IL-10) cytokine levels may be altered (Looney et al. 2002).

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 completely overlap with those related to influenza illness (Wald et al. 1995). 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 a correlate protection in these trials. Recent efforts to develop an RSV vaccine for older adults have focused on combining RSV subunit proteins with adjuvants but these are in the early stages of development. The ongoing challenges to developing an RSV vaccine for older adults is that the correlates of protection are poorly understood, and the overlapping symptomatology and cocirculation with influenza over the winter months.

In summary, older adults experience significant complications of RSV illness but these complications are difficult to distinguish from the impact of 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.

Immune Senescence: Stimulating a Naïve Response

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 pre-pandemic and pandemic vaccines. Pre-pandemic vaccines if formulated to more potently stimulate T cells could offer cross-protective immunity that would be enhanced with strain-specific antibodies against the pandemic strain. Although this strategy may offer enhanced protection in older adults, pre-pandemic and pandemic vaccines need to be tested for their ability to stimulate adequate antibody responses but also 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.

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 (Chen et al. 2005). 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 (Nash et al. 2001). However, a recent study has shown that there is no loss with aging in the polyfunctionality of CD8+ T cells responding to WNV infection (Lelic et al. 2012). It remains to be determined whether changes in Foxp3+ CD4+ T cells may have a role in suppressing the CD8+ T cell response.

HIV prevalence is increasing and with aging of the population; HIV-infected patients aged 50 years and older now represent10–13% of the HIV-infected population

in the United States (Casau 2005). Both HIV and aging have been associated with the development of replicative senescence of T lymphocytes and increased risk of infection (Effros 2004). Replicative senescence results from chronic stimulation of the immune system and is associated with telomere shortening and loss of CD28 expression on CD8 T cells (van Baarle et al. 2005). These changes will need to be considered in the development of new therapies to improve the immune system and response to vaccination (Effros 2007). Recently, a population of memory T cells with a naïve phenotype has been identified and is a potential target for immunotherapy in the treatment of persistent infections such as HIV (Pulko et al. 2016).

Immune Senescence: Vaccines Against Bacterial Pathogens

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 aged 65 years and older. Although the current 23-valent vaccine containing pneumococcal capsular polysaccharide (PPV23) is cost-effective in this population (Fedson 1992; Sisk et al. 1997), 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 pneumococcal 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 (Rubins et al. 1999). In addition, opsonophagocytic activity, the major effector mechanism for clearing pneumococcus appears to decline with aging (Kolibab et al. 2005). Further, there is a significant decline in antibody titers to PPS at 6 years following pneumococcal vaccination (Musher et al. 1993). Recommendations for PPV23 vaccination at age 65 are currently under review to include the new PCV13, which is a protein-conjugated vaccine containing nontoxic diphtheria toxin conjugated to 13 serotypes of pneumococcal polysaccharide.

A large randomized placebo-controlled trial of PCV conducted in The Netherlands in almost 85,000 adults ≥ 65 years of age demonstrated an estimated 45.6% vaccine efficacy against both invasive (bacteremia) and noninvasive (pneumonia) pneumococcal disease for up to 4 years following vaccination (Bonten et al. 2015). This trial utilized a novel antigen detection assay of pneumococcal serotypes in the urine, in the diagnosis of both pneumococcal bacteremia and pneumonia. Prior to the development of this assay, vaccine efficacy and effectiveness studies have been limited the diagnosis of pneumococcal disease to the detection of pneumococcus in the sputum, blood, or cerebrospinal fluid, posing a significant challenge to studies in older adults. Studies have shown that the introduction of PCV7 into the childhood vaccination schedule has led to herd immunity in older adults whereby the

circulation of these serotypes contained in the vaccine begin to decline and thus are not transmitted to older adults. Previous studies were based on the detection of invasive pneumococcal disease. More recent studies utilizing urinary antigen detection have shown that this protection applies to both invasive and noninvasive pneumococcal disease in older adults (van Werkhoven et al. 2016). These observations support the emerging policies for pneumococcal vaccination in older adults to include both PCV13 and PPV23 in the vaccination schedule.

PPV23 vaccine containing only purified pneumococcal polysaccharide stimulates antibody production through a T-independent type 2 (TI-2) response (one not requiring T cell help and lacking memory) (Bruyn 1992). A Finnish study of older adults showed serotype-related differences in the serum antibody response to PPV23, suggesting that some serotypes are weak immunogens in older adults (Sankilampi et al. 1996) and these T-independent responses are more short-lived and may not be adequately boosted with revaccination. Protein-conjugated pneumococcal vaccines (PCV) were developed to facilitate T cell cooperation (Perry and Catterall 1994) and thus may be more effective in targeting T cell responses that are known to decline with aging. Although fewer serotypes are represented in the current PCV13 vaccine, it appears to be more effective than PPV23, albeit for the serotypes contained in the vaccine as there is no known cross-reactivity in the antibody responses between the different serotype polysaccharides. In light of the enhanced efficacy of PCV13 and the anticipated increase in the circulation of non-PCV13 serotypes due to the PCV13 effects on herd immunity, pneumococcal vaccine schedules for older adults are being modified to include PCV13 followed by PPV23 to provide protection against the serotypes not contained in PCV13.

Cytokine responses to pneumococcal antigens have been shown to regulate responses to protein- and polysaccharide-specific antibody responses (Khan et al. 2004) and may be important in the pathogenesis of pneumococcal diseases (Kemp et al. 2002). Cytokine responses to the whole pneumococcus could explain changes in the virulence of different serotypes in older adults (Robinson et al. 2001) and in comparison to cytokine responses in younger adults (Arva and Andersson 1999). TNF-α produced by macrophages is associated with an initial inflammatory response to pneumococcal invasion (Perry and Catterall 1994; van der Poll et al. 1996) and has been found to be important in the development of antibodies to pneumococcal surface proteins (Khan et al. 2004; Khan et al. 2002). Increased levels of IL-10, an anti-inflammatory cytokine product of both macrophages and regulatory T cells, have been associated with increased risk for pneumococcal infection (van der Poll et al. 1996), but this cytokine becomes important for immunomodulation of the inflammatory response in the recovery from pneumococcal infection (Williams et al. 2015). Moreover, the gut microbiota has been shown in the mouse model to have role in the regulation of TNF-α and IL-10 in defense mechanisms against pneumococcal pneumonia (Schuijt et al. 2016) suggesting that the dysregulation of cytokine responses with aging may be more complicated than what can be ascribed to immune senescence alone.

Immune Senescence: Altering Responses to Endogenous Proteins

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 $Aβ_{1-42}$ 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 titer to $Aβ_{1-42} \geq 1:2200$) but 22% of "responder" compared to 2% of "nonresponders" developed aseptic meningoencephilitis (Patton et al. 2006). These results suggested that adverse effects of the vaccine were related to the immune response to the vaccine rather than toxicity of the $Aβ_{1-42}$. Post-mortem 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 Th1 cytokines, 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 (Monsonego et al. 2003). 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 $Aβ_{1-42}$ effectively stimulated a proliferative response in human peripheral blood mononuclear cells (PBMC). This response was increased in older compared to young adult subjects and a further significant increase was observed in older adult subjects with AD (Monsonego et al. 2003). It had been shown that $Aβ_{1-15}$ was responsible for B cell stimulation and the production of antibodies to Aβ, and $Aβ_{15-42}$ 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 producing Th1 cytokines. Although the antibody response appeared to effectively clear Aβ, the inflammatory cytokine response of the T cell infiltrate leads to meningoencephalitis. Since the $Aβ_{15-42}$ 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 (Monsonego and Weiner 2003). While efforts continue to develop immunologic-based therapies for AD, the results of this clinical trial continue to have significant consequences for future vaccine development and a recent review cites a critical need for new development strategies (Marciani 2016).

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) and the suppressive effects of regulatory T cells. This complex set of interactions in the activation of a protective immune response highlights the importance of developing correlates of protection and identifies novel targets for vaccine development to improve protection against a variety of infectious diseases in older adults. In spite of our limited understanding, a number of available vaccines have been shown to be 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 and prevent disability in older adults will make a significant contribution to "adding life to years."

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