

# Immunosenescence: implications for vaccination programs in the elderly

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**Abstract:** Worldwide, infectious disease is responsible for much of the morbidity and mortality in the elderly. As the number of individuals over the age of 65 increases, the economic and social costs of treating these infections will become a major challenge. Vaccination is the most effective and least costly preventative measure in our arsenal; however, vaccines that are effective in children and young adults are often ineffective in older adults. This is a result of the deterioration in immune function that occurs with age, referred to as immunosenescence. Age-associated changes in leukocyte phenotype and function impair primary vaccine responses and weaken long-lasting memory responses. In this review, we discuss current vaccination approaches in the elderly and strategies to improve responsiveness in older adults, which include increasing vaccine immunogenicity and overcoming the fundamental immune defects that prevent optimal immune responses.

**Keywords:** immunosenescence, vaccination, elderly, influenza, pneumonia, zoster

## Introduction

By the year 2050, more than 25% of the world's population will be 65 years of age or older.<sup>1</sup> Susceptibility to infectious disease increases with age and in addition to other age-related health issues, poses an enormous challenge to health care systems in the developed world. In the US, pneumonia and influenza were the eighth leading causes of death in 2005, and the elderly (aged  $\geq 65$  years) accounted for an estimated 90% of these deaths.<sup>2-4</sup> Combined, these diseases cost the US economy \$40.2 billion due to direct and indirect health care expenditures and mortality-related losses in productivity.<sup>5</sup> The economic and social costs of infection include acute treatment and long-term health outcomes. For example, having pneumonia in midlife to late life accelerates development of dementia, respiratory, and cardiac conditions, as well as fall-related injuries that require hospitalization. Consequently, calculations on the costs of acquiring pneumonia in midlife to late life must include the costs of long-term consequences of infection.<sup>6-8</sup>

Infectious diseases account for roughly 20% of hospitalizations in the elderly.<sup>9</sup> Vaccines are the most successful tools we have in preventing infectious disease. Four vaccines are currently recommended for use in the elderly: the seasonal influenza vaccine, the pneumococcal vaccine, the tetanus-diphtheria-pertussis vaccine, and the vaccine to prevent shingles, which is caused by reactivation of *Herpes zoster* virus (Table 1).<sup>10</sup> Despite the importance of preventing these infections and relatively high vaccination rates, protection is still suboptimal due to decreasing immune function with age.<sup>11</sup>

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**Table 1** Current vaccine recommendations in the elderly

Vaccine	Type	Adjuvant	Site	Schedule
Diphtheria-tetanus acellular pertussis (DTAP)	Subunit (Adacel <sup>®</sup> , Boostrix <sup>®</sup> )	Alum	IM	One dose administered if patient has not received in adulthood (age > 18 years) and boost every 10 years. Adults not previously vaccinated receive at least three doses at 0 (DTAP), 8 weeks (DT), and 6–12 months (DT).
Herpes zoster	Attenuated (Zostavax <sup>®</sup> )	N/A	SC	One dose administered regardless of prior history of shingles.
Influenza	Inactivated split virus (Fluviral <sup>®</sup> , Vaxigrip <sup>®</sup> , Fluzone <sup>®</sup> , Agriflu <sup>®</sup> , Influvac <sup>®</sup> , Intanza <sup>®</sup> )	N/A	IM or ID (Intanza <sup>®</sup> )	One high dose trivalent vaccine administered every flu season.
Pneumococcal	Subunit (Fluad <sup>®</sup> )	MF59	IM	
	Conjugate (Prevnar <sup>®</sup> 13) Polysaccharide subunit (Pneumovax <sup>®</sup> 23, Pneumo 23 <sup>®</sup> )	Alum N/A	IM IM/SC	Vaccine-naïve individuals receive one dose PCV13, followed by one dose PPSV23 6–12 months later. Individuals that previously received PPSV23 at age ≥65 years, receive one dose PCV13 ≥1 year later. Individuals that received PPSV23 before age 65 years and now are aged ≥65 years receive one dose PCV13 and one dose PPSV23 6–12 months later.

**Abbreviations:** N/A, not applicable; IM, intramuscular; SC, subcutaneous; ID, intradermal; DT, diphtheria-tetanus; PCV, pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.

Respiratory tract infections, particularly influenza and pneumonia, account for the majority of hospitalizations due to infectious disease in the elderly. Due to widespread use and careful monitoring of influenza and pneumococcal vaccines, data on the efficacy of these vaccines are well understood. Despite reasonably high vaccination rates in the elderly (61.3% influenza, 59.9% pneumococcal), influenza and pneumonia infections are still associated with serious adverse events leading to hospitalization, debilitating complications, and mortality in the elderly.<sup>9,12,13</sup>

Seasonal influenza causes moderate illness in healthy adults that is generally resolved within 2 weeks; however, children, those with comorbidities, and the elderly are at increased risk of complications (ie, pneumonia, bronchitis, and/or sinus infection) that may result in hospitalization and mortality. Consequently, in many countries influenza vaccination efforts focus on children (aged 6 months–17 years) and the elderly (aged ≥65 years). Vaccination rates are as high as 56.6% in children and 61.3% in the elderly, while coverage in adults (aged 1–64 years) is 35.7%.<sup>13</sup> Even though rates of coverage may be the same in the young and old, protection rates are very different. Studies measuring vaccine efficacy monitor “influenza-like illness”, which is used for a proxy of influenza infection without virology testing to confirm the infection. In studies that monitor the effectiveness of the trivalent inactivated vaccines, there were marked differences in protection from influenza-like illnesses between these two age groups. For example, a recent meta-analysis suggests that 58% of vaccinated children (aged <16 years) were protected

from influenza-like illness.<sup>14</sup> In a large meta-analysis of influenza vaccination in the nursing home or community-dwelling elderly, there was no significant protection from influenza despite the use of antigen-matched vaccines.<sup>15</sup> Age-related changes in immunity are believed to contribute to the disparity in protective efficacy of trivalent inactivated influenza vaccine (TIV) in these two populations.<sup>15</sup>

Bacterial pneumonia is a common consequence of seasonal and pandemic influenza infection, and is a major cause of morbidity and mortality in the elderly.<sup>16</sup> To prevent community-acquired pneumonia, nursing home-acquired pneumonia, and ventilator-acquired pneumonia, vaccines against the major causative agent, *Streptococcus pneumoniae*, have been developed. In the elderly, *S. pneumoniae* generally causes pneumonia, but more rarely, can cause invasive pneumococcal disease (IPD) (eg, meningitis or septicemia). The most recent Cochrane meta-analysis of the efficacy of the 23-valent pneumococcal polysaccharide vaccine (PPSV23) vaccine demonstrated that there was significant protection from IPD (odds ratio [OR], 0.26; 95% confidence interval [CI], 0.15–0.46), but there was no evidence for protection against pneumonia (including community-acquired pneumonia, nursing home-acquired pneumonia, and ventilator-acquired pneumonia) in the elderly.<sup>17</sup> With few exceptions, other studies have confirmed that there is some protection against IPD, but not pneumonia, in the elderly.<sup>18–21</sup> Current vaccines are clearly not sufficient to protect the elderly from the infectious diseases that they are most susceptible to, and this is likely due to waning immune function.<sup>22</sup> With age, there

is an increase in inflammatory mediators in serum and tissues, accompanied by phenotypic and functional changes to leukocytes, which affect all elements of the immune response necessary to mount a response to vaccination (Figure 1). This review will focus on age-related immune changes relevant to the vaccine response and will provide commentary, based on current data, as to how vaccines can be tailored to provide increased protection in the elderly.

## Influence of immunosenescence on vaccine-elicited immune responses

### Anatomy of a vaccine response

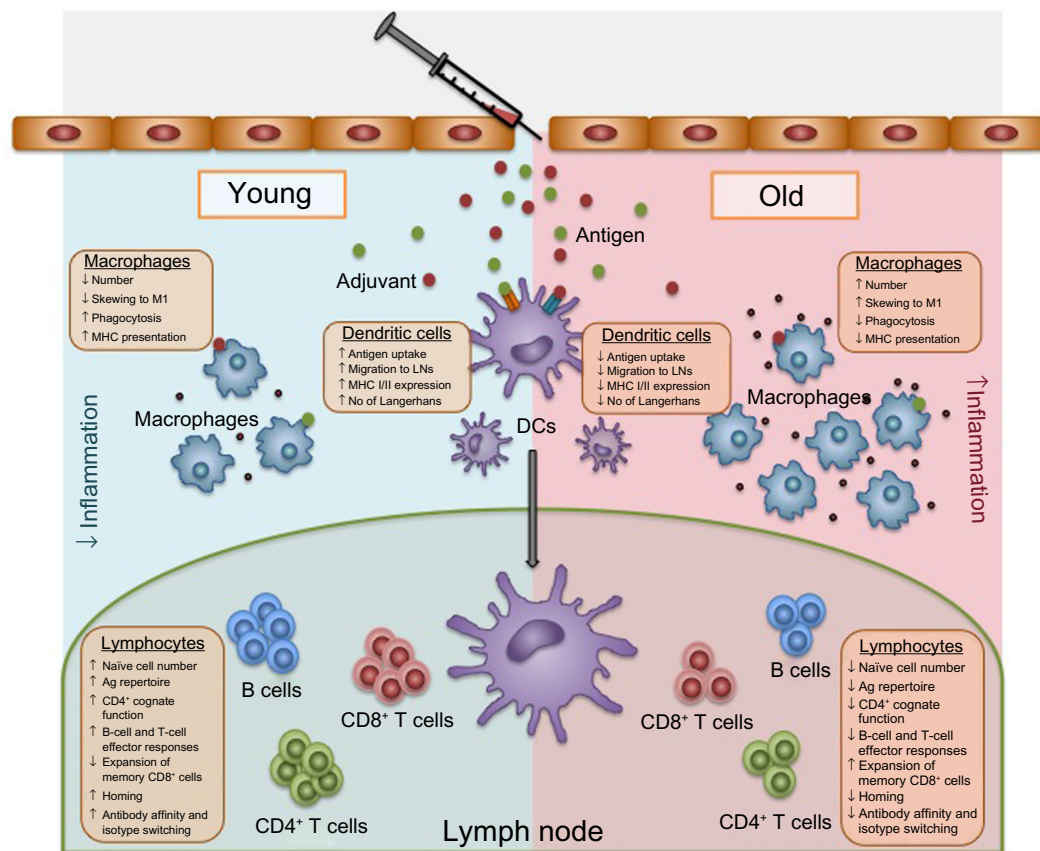
#### Vaccine effector responses

Vaccines prime the adaptive immune system to produce a rapid, robust, and protective immune response upon subsequent exposure to an infectious agent. This “memory” response is mediated by antigen-specific lymphocytes (ie, B and T cells). Antigen-specific antibodies produced by B cells bind and neutralize viruses and extracellular bacteria and also mediate their uptake and clearance by macrophages and neutrophils. T cell-mediated responses act to directly

or indirectly kill infected cells. Although vaccine effector mechanisms are executed by the adaptive immune system, their generation depends on the innate immune response.

#### Antigen uptake and antigen-presenting cell activation

Antigen-presenting cells (APCs), primarily dendritic cells (DCs), ingest the vaccine antigen at the site of administration, become activated, and later present the antigen to B and T cells. Activation of APCs is required to initiate production of pro-inflammatory cytokines that upregulate homing receptors, which are required for the DC to migrate to the draining lymph node and present antigens to T cells. An inflammatory response is also required to increase co-stimulatory molecule expression that is needed to activate T and B cells. The degree of APC activation is highly dependent on the type of vaccine that is administered (Table 2).<sup>23–25</sup> For example, live attenuated vaccines are potent immune activators because they are briefly able to replicate, leading to increased and prolonged exposure to antigens and immunostimulatory viral components such as nucleic acids. In contrast, immune responses to purified antigens (ie, protein, polysaccharide, glycoconjugate, and



**Figure 1** All elements of the immune response necessary to mount a response to vaccination.

**Abbreviations:** MHC, major histocompatibility complex; No, number; LNs, lymph nodes; DCs, dendritic cells.

**Table 2** Vaccine types and immune responses elicited

Vaccine type	Components	Response elicited
Non-live vaccines		
Subunit	Purified pathogen proteins, isolated toxins	T cell-dependent antibody production Minimal CTL responses
	Surface polysaccharides isolated from bacterial capsule	T cell-independent antibody production Minimal isotype class switching Poor recall response following booster
	Polysaccharide conjugate	Covalently attached polysaccharide to carrier protein T cell-dependent antibody production Minimal CTL responses
Inactivated	Whole pathogens inactivated by chemical or physical treatment	Antibody production No CTL responses Poor memory responses
Live vaccines		
Live, attenuated	Replicative pathogens with reduced virulence Retains some pathogenicity	T cell-dependent antibody production Long-lasting circulating antibodies Effective CTL responses Good memory responses
Live	Low doses of actual pathogen	Most effective protection T cell-dependent antibody production Long-lasting circulating antibodies Effective CTL responses Good memory responses

**Abbreviation:** CTL, cytotoxic T lymphocyte.

inactivated microbes) are more fleeting as they are rapidly cleared from the vaccination site. Purified protein (eg, viral capsid proteins) or carbohydrate (eg, the polysaccharide capsule of *S. pneumoniae*) antigens are poorly immunogenic on their own, except in very high doses. Adjuvants enhance immunogenicity of these proteins and carbohydrates by enhancing antigen presentation or co-stimulation by the APCs.<sup>26</sup> Adjuvants such as alum and oil-in-water emulsions (ie, MF59) trap antigens at the site of injection, creating a depot from which antigen is slowly released, providing longer antigen exposure and increasing recruitment of APCs. In unadjuvanted vaccines, antigen doses are increased considerably (ie, from 3.75 µg to 15 µg for influenza vaccine).<sup>27</sup>

### Protein subunit, conjugate, and inactivated vaccine responses

As mentioned in the “Antigen uptake and antigen-presenting cell activation” section, APCs play a crucial role in linking the innate and adaptive arms of immunity, because they present processed antigen to T cells.<sup>28</sup> Antigens are presented to T cells

in the context of major histocompatibility complex (MHC) molecules (human leukocyte antigen in humans). Proteins and inert particles are taken up and processed into peptides for presentation on MHC class II. Presentation by APCs activates CD4<sup>+</sup> T cells in secondary lymphoid tissue.<sup>29</sup> CD4<sup>+</sup> T cells support the differentiation of B cells, CD8<sup>+</sup> T cells, and macrophages that act to directly eliminate microbes. Naïve B cells can also take up antigen, which when presented in the context of co-stimulation from activated APCs and CD4<sup>+</sup> T cells, leads to their differentiation into low-affinity IgM-producing plasma cells. B cells also present antigen-to-antigen-specific CD4<sup>+</sup> T cells, which provide cognate help through co-stimulation. CD4<sup>+</sup> T cells help drive immunoglobulin class switching and affinity maturation, and result in plasma cells that produce high-affinity, antigen-specific antibodies. This T cell-dependent antibody response is slow and requires 10–14 days; however, it generates long-lasting, high-affinity antibodies and a memory B cell response.<sup>30</sup>

### Live attenuated vaccines

By infecting and replicating in host cells, antigens from live attenuated viruses will be presented on MHC class I molecules to CD8<sup>+</sup> T cells. Also referred to as cytotoxic T lymphocytes, CD8<sup>+</sup> T cells kill infected cells by destroying them with mediators such as perforins and granzymes. Peptide antigens from live attenuated vaccines may also be presented on MHC class II and may elicit CD4<sup>+</sup> T cell and antibody production from B cells.<sup>30</sup>

### Polysaccharide vaccines

Polysaccharide antigens act through T cell-independent pathways to generate an antibody response. Polysaccharides interact with marginal zone B cells in secondary lymphoid tissues. The polysaccharide cross-links the B cell receptor, causing activation and differentiation into a plasma cell-producing IgM. Some isotype switching from IgM to intermediate-affinity IgG occurs. In contrast to the T cell-dependent response, this response is rapid, transient (1 week long), produces low-affinity antibodies, and does not result in a memory response. In order to circumvent this short-lived response, polysaccharide vaccines are conjugated to immunogenic carrier proteins (ie, tetanus and diphtheria toxoids) to elicit a T cell-dependent B cell response.<sup>31,32</sup>

### Memory responses

The generation of memory responses is the ultimate goal of vaccination. Following the primary response to T cell-dependent antigens, antigen-specific B and T cell numbers decline. The efficacy of a memory response is dictated by



the primary immune response and the number of exposures to antigen. The primary immune response is highly dependent on antigen dose and persistence, which is why live attenuated vaccines generate long-lasting memory responses. Most other inactivated and subunit vaccines require booster vaccinations. A small fraction of B cells, now memory B cells, will migrate to long-term survival niches such as the bone marrow where they continue to undergo affinity maturation for 4–6 months, increasing their antibody affinity.<sup>33</sup> Thus, boosters are generally administered 4–6 months following primary responses, allowing time for the generation of memory B cells. Antigen-specific effector T cells are short-lived and also decline following primary response. Those that persist can become either an effector memory T cell ( $T_{em}$ ) or a central memory T cell ( $T_{cm}$ ).  $T_{em}$ s migrate through non-lymphoid organs, patrolling tissues for their antigens, and have high cytotoxic activity once reactivated. Conversely,  $T_{cm}$ s traffic through lymphoid organs and have high proliferative capacity, generating a large surge of effector cells.<sup>34</sup> The  $T_{em}$  response relies on the magnitude of T cell expansion following primary vaccination, which is increased with higher antigen load and persistence.<sup>30</sup> Increased numbers of  $T_{em}$ s will result in more  $T_{em}$ s following the contraction phase of the T cell response.

Reactivation of memory cells can occur through natural colonization by microbes with cross-reacting epitopes, infection, or by booster immunizations. This leads to activation of memory B cells, which do not require cognate CD4<sup>+</sup> T cell help, and leads to rapid proliferation and secretion of high-affinity antibodies. Memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells are also activated by cognate antigen and do not require costimulation. Generally, booster immunizations include higher antigen content than primary immunizations to increase the activation and proliferation of memory B cells. Upon multiple or prolonged exposures to antigens, activated memory B cells undergo further affinity maturation and produce higher affinity antibodies.<sup>30</sup>

## Changes in vaccine responses with age

Both humoral and cellular immune responses to primary immunization and boosters decrease with age.<sup>35–38</sup> These antibody- and T cell-mediated specific immune responses depend on priming by competent APCs, such as DCs and macrophages. APCs from aged individuals, however, are less able to take up antigens by the process of micropinocytosis, have decreased capacity to present antigens due to decreased MHC class I and MHC class II expression,<sup>39–43</sup> and are less responsive to the chemokine CCL19, which is required for migration to the lymph nodes.<sup>44</sup> The impairment of DC migration is due to both intrinsic age-associated defects in the DCs

themselves, in addition to the presence of changes in cytokine levels in the aging microenvironment.<sup>44</sup> Human DCs also have decreased expression of the co-stimulatory molecules, CD86 and CD80, which impairs T cell activation.<sup>44–47</sup>

With age, the number of naïve B and T cells produced by the bone marrow decreases,<sup>48</sup> due in part to changes in the aging microenvironment.<sup>49</sup> This results in a decreased ability to respond to new infections or vaccinations,<sup>50</sup> although a study shows that in some viral infections (eg, West Nile virus) the elderly are capable of mounting a *de novo* response.<sup>51</sup> Elevated levels of tumor necrosis factor (TNF) in the bone marrow decrease B cell lymphopoiesis and plasma cell survival.<sup>52</sup> Antibodies produced in aged individuals tend to be of lower affinity due to reduced isotype switching and somatic hypermutation, and consequently their neutralizing or opsonizing functions are decreased. This is due to impaired CD4<sup>+</sup> T cell help because of decreased germinal center formation in peripheral lymph nodes, which are necessary for an efficient, high-affinity humoral response.<sup>53–55</sup> The T cell compartment also undergoes significant changes with age. Levels of IL-7, a cytokine that promotes development of T cells in the thymus and thymic involution, result in decreased peripheral naïve T cells (CD45RA<sup>+</sup>CD28<sup>+</sup>).<sup>56,57</sup> Aged naïve T cells are increasingly difficult to prime.<sup>58</sup> Naïve CD4<sup>+</sup> T cells from aged animals show decreased effector cytokine production (ie, IL-2), less clonal expansion, and decreased expression of activation markers (CD25, CD62L, and CD154) following primary antigen presentation by APCs.<sup>58,59</sup> CD4<sup>+</sup> T cell function is impaired with age, leading to weaker humoral and CD8<sup>+</sup> T cell responses, which can contribute to vaccine failure in the elderly.<sup>53</sup>

Although memory cells are generated from naïve T cells in the elderly and show persistence *in vivo*, they exhibit impaired cytokine secretion and proliferation upon recall responses.<sup>60</sup> Memory CD4<sup>+</sup> T cells generated in young mice were shown to be functional in their host as they aged, while those generated in aged mice were non-functional.<sup>60</sup> Moreover, young CD4<sup>+</sup> T cells transferred to aged, immunized hosts maintained their capacity to induce a robust humoral response.<sup>59</sup> Similar results were also shown with old CD8<sup>+</sup> T cells,<sup>61</sup> suggesting that changes to T cell function are intrinsic and dependent on the age of the host. This implies age at primary vaccination is a more important determinant of proper memory T cell function, rather than age at recall response. A recent study demonstrated that following primary exposure to West Nile virus, antigen-specific CD8<sup>+</sup> T cells from elderly donors maintained production of antiviral cytokines, granzyme B, and perforin for up to 2 years. These results were comparable to younger adults.<sup>51</sup> In contrast, older primates have weak antiviral

CD8<sup>+</sup> T cell responses to West Nile virus.<sup>62</sup> However instead of using live virus, the Lelic et al study<sup>51</sup> employed synthetic immunodominant peptides for re-stimulation that did not require further processing by APCs. Since antigen presentation is also compromised with age, employing peptide-based booster vaccines may be an alternative strategy to elicit a strong CD8<sup>+</sup> T cell response in the elderly.

Even when the elderly mount a robust primary immune response, they may be less able to maintain antigen-specific memory cells. Chronic infections (ie, Herpes simplex virus, cytomegalovirus [CMV], and Epstein Barr virus) provide constant antigenic stimulation and lead to an expansion of terminally differentiated effector CD8<sup>+</sup> T cells, reducing space in the T cell repertoire for other antigen-specific T cells, including those generated by vaccination. This has been demonstrated in the elderly who are chronically infected with CMV and whose peripheral memory T cell repertoires are dominated by CMV-specific effector T cells. This prevents the expansion of T cell clones with other specificities due to limited space in the T cell repertoire. Not only do CMV effector cells impair the response to co-resident Epstein Barr virus infection,<sup>63</sup> but their increased numbers in the elderly have been correlated with decreased humoral responses following influenza vaccination.<sup>64,65</sup> Additionally, CMV memory T cells produce increased levels of interferon gamma (IFN- $\gamma$ ), contributing to chronic age-associated inflammation.<sup>66,67</sup>

As we age, levels of pro-inflammatory cytokines in the circulation and tissues increase. This state of chronic, low-grade, systemic inflammation is often called “inflamm-aging”.<sup>22</sup> Although it is unclear why we become more inflamed with age, epidemiological data clearly demonstrate that the effects of age-associated inflammation are far-reaching. Age-associated inflammation seems to correlate with poor health in general, as higher than average levels of age-associated inflammation correlate with the development of chronic inflammatory disease, frailty, and general ill health.<sup>68,69</sup> Having higher than age-average levels of these cytokines increases susceptibility to infectious disease (eg, pulmonary pneumonia and influenza)<sup>70,71</sup> and is predictive of decreased vaccine responsiveness.<sup>67</sup>

Vaccine responsiveness does not decrease in a linear fashion with age, and often correlates more strongly with general health. Conversely, a robust vaccine response is a predictor of immune competence and good health.<sup>72</sup> In general, vaccine responsiveness correlates with frailty, defined as declining physical and mental function and reduced ability to resist environmental stressors.<sup>73,74</sup> Frailty is strongly associated with inflamm-aging, likely because immune competence is a

mandatory requirement for overall health (eg, TNF, C-reactive protein [CRP], and IL-6).<sup>75–79</sup> In order to disentangle which elements of decreasing vaccine responsiveness are due to age rather than ill health, protocols have been developed to study only the healthiest older adults. The most commonly used is the SENIEUR protocol,<sup>80</sup> which excludes anyone taking immune-modulating medication, those with chronic disease (ie, atherosclerosis, Crohn’s disease, etc), or abnormal values for common clinical measures (ie, leukocyte counts, urea, and glucose). Although this excludes the vast majority of older adults, it allows comparisons of the most immune-competent (“SENIEURs”) to those with the normal allotment of age-associated changes in health (“non-SENIEURs”). A recent study evaluated influenza vaccine responses in the elderly during an epidemic season. Serum levels of IL-6 were measured before and 1 and 6 months after immunization.<sup>67</sup> The healthy elderly, or SENIEURs, had consistently low levels of IL-6 throughout the study, while the frail elderly, or non-SENIEURs, had significantly higher levels. The serum IL-6 levels correlated inversely with a protective vaccine response, measured by anti-hemagglutinin (HA) titer. The SENIEURs, who had low levels of IL-6, responded following their first immunization, while the non-SENIEURs with high IL-6 levels were permanent non-responders.<sup>67</sup>

These findings emphasize the importance of assessing immune competence in the target vaccine population prior to immunization. Currently, the elderly are uniformly treated with regard to vaccination. By developing an indicative marker of immune competence and vaccine non-response (ie, elevated serum cytokines), we can more efficiently administer vaccines to those with a higher likelihood of responding, while pursuing alternative vaccination strategies in the remaining at-risk population.

## Adapting vaccination for the aging immune system

### Increasing dose

In pre-clinical studies, data suggest that increasing the dose of HA antigen in influenza vaccines would increase antibody titers in the elderly.<sup>81</sup> A double-blinded, randomized, multi-center trial comparing the standard dose (15  $\mu$ g HA per strain) to a high dose (60  $\mu$ g per strain) of Fluzone® (Sanofi, Bridgewater, NJ, USA) was conducted in adults 65 years or older. The group receiving the high dose had antibody levels that were 12%–25% higher than the standard dose group for the three viral strains (H1N1, H3N2, and B).<sup>82</sup> In another study where trivalent split influenza vaccine (Sanofi) dose was doubled (from 15  $\mu$ g to 30  $\mu$ g) in the frail elderly, antibody

responses were also increased.<sup>83</sup> Although increasing HA antigen dose induces more antibodies, which are presumed to be a correlate of protection, studies that definitively demonstrate protection against infection are lacking.<sup>14</sup> The relationship between increased dose and increased immunogenicity does not appear to be universal to all vaccines. Increasing the amount of live attenuated Varicella zoster virus (1×, 2.7×, and 13× standard dose) in the elderly did not increase the vaccine-specific antibody or cell-mediated response.<sup>84</sup> This may be because it may be necessary to increase the amount of viral antigen content per virion, rather than the total number of virions, to enhance immunogenicity and cell-mediated immunity.<sup>85</sup>

### Adjusting vaccine schedules

Older adults may mount efficient T cell responses in response to vaccination, but they are less able to maintain memory responses.<sup>86</sup> Following pneumococcal polysaccharide vaccine (PPV), not only do the elderly have decreased antibody potency against all serotypes, but there is a steady decline in serotype-specific antibody titers returning to pre-vaccination levels within 5–10 years.<sup>87,88</sup> In contrast, immune responses generated in youth are long-lasting and protective. An elegant example of this occurred during the H1N1 pandemic when influenza infections in even the oldest and most frail elderly were much lower than expected. Upon investigation, it was found that these individuals had protective antibodies that were generated many decades earlier in response to circulating strains that they were exposed to in youth.<sup>89</sup> This illustrates that memory responses that are generated in youth are long-lasting and protective well into old age. Consequently, one of the most effective ways to protect the elderly from infections may be to vaccinate them in youth. A robust primary immune response can also lead to more efficient responses to boosters, since it has been shown that pre-vaccination antibody titers dictate the magnitude of booster titers.<sup>90</sup> Live attenuated vaccines appear to be more efficient at providing increased protection over decades and following booster vaccinations, compared to inactivated vaccines.<sup>91</sup>

### Utilization of alternative routes of immunization

Historically, the preferred approach to vaccine administration has been via percutaneous injection, which includes subcutaneous and intramuscular methods of immunization. However, recent advances in vaccinology and immunotherapeutics have suggested that alternate routes of vaccination may provide superior immunogenicity and protection

in elderly populations.<sup>92</sup> In a large South African study by Holland et al, over 1,100 volunteers over the age of 60 received a trivalent, inactivated influenza vaccine via either intradermal microinjection or intramuscular administration. It was concluded that the intradermal route of vaccination elicited immune responses that were superior, as subjects had higher rates of seroconversion than those who received the conventional intramuscular administration.<sup>93</sup> Through exploitation of the skin immune system, intradermal vaccination directly delivers antigen to dermal DCs, which efficiently migrate and present antigen to T cells in draining lymph nodes, thereby naturally augmenting the primary immune response.<sup>93</sup> In theory, intradermal antigen delivery should allow for a reduction of the antigen dose required to obtain optimal protective responses in the elderly. Two separate studies have shown that using a 2.5-fold decrease in antigen dose, as compared to full-dose vaccines, achieves a suitable response via the intradermal route of vaccination.<sup>94,95</sup> Despite age-associated changes in skin integrity and physiology, and decreases in Langerhans and DCs, intradermal immunization can elicit protective immune responses in the elderly.<sup>96</sup>

Mucosal vaccination may also be a viable alternative, especially for infections that originate in the upper respiratory tract, such as influenza and pneumonia.<sup>97</sup> The abundance of APCs in mucosal tissues such as the nasopharynx and gastrointestinal tract facilitate antigen responses.<sup>98</sup> The nasopharyngeal- and gut-associated lymphoid tissues are reservoirs of immune cells that induce effective antibody production, especially IgA, upon encountering antigen in the context of the appropriate adjuvant.<sup>99,100</sup> Unlike conventional immunization, antigenic exposure at mucosal sites activates antigen-specific T cells and IgA<sup>+</sup> B cells, which subsequently transit to the lymph, enter the circulation, and seed mucosal sites, primarily the mucosa of origin.<sup>99,101,102</sup> Upon arrival, mucosal lymphocytes differentiate into effector or memory cells. The anatomic affinity of such cells is determined by surface site-specific integrins (homing receptors) and complementary mucosal tissue-specific receptors.<sup>103,104</sup> Nasal administration generates both mucosal IgA and peripheral IgG responses. IgA antibodies are particularly effective at binding and neutralizing viruses; therefore, mucosal vaccinations should be particularly protective against respiratory infections such as influenza. Currently, only one commercially available mucosal vaccine exists. Intranasal administration of FluMist<sup>®</sup> has been shown to elicit robust protective responses in adults aged <49 years.<sup>105</sup> However, studies involving patients aged >50 years have yet to be conducted; therefore, safe and efficacious use of FluMist in the elderly has not been established. While

mucosal immunizations demonstrate great potential, there is currently limited research on the development of mucosal vaccines that specifically target the elderly population and overcome the age-associated immune barriers to successful and effective vaccination.

## Novel adjuvants that improve immunogenicity

In contrast to young people, the elderly often do not mount any detectable primary immune response to protein antigens, regardless of dose. In many cases, the commonly used alum adjuvant does not sufficiently increase the immune stimulatory activity of antigens in the elderly.<sup>106,107</sup> Use of more potent adjuvants may overcome this limitation. One promising candidate is the oil-in-water emulsion adjuvant, MF59<sup>TM</sup>.<sup>108</sup> Previously, oil emulsion-based adjuvants were associated with side effects such as inflammatory reactions, granulomas, and ulcers at the injection site.<sup>109</sup> Replacement of mineral oil used in other emulsions with squalene in MF59, however, has limited side effects.<sup>110</sup> A murine study demonstrated that old mice immunized with an MF59-adjuvanted vaccine produced antibody titers to levels equivalent in young mice.<sup>106</sup> A similar study demonstrated that MF59 reduced to dose of antigen required, and upon secondary challenge with a wild virus, decreased total viral load and provided significant protection in both young and old mice.<sup>107</sup> To evaluate MF59 efficacy in humans, multiple clinical studies involving several MF59-adjuvanted vaccines have been performed. Results have demonstrated enhanced immunogenicity in all age groups, while maintaining a high level of safety and tolerability. Being the first adjuvant licensed for human other than alum, MF59 is now part of an influenza vaccine (Fluad<sup>®</sup>) designed for the elderly and is readily available worldwide.<sup>108,110</sup> Though adjuvant activity of MF59 is only partially understood, studies have shown that it induces monocyte recruitment and macrophage trafficking, promotes differentiation of monocytes into DCs, and fosters enhanced antigen uptake by macrophages and DCs.<sup>111–113</sup> Increased utilization of MF59 in vaccine development, specifically for the elderly population, may serve as a practical solution to enhance immunogenicity.

Other potential immunostimulatory adjuvants, which may enhance immunogenicity in the elderly, include the lipopolysaccharide derivative 3-deacetylated monophosphoryl lipid A, the saponin-derived lipid, QS21, oligodeoxynucleotides containing CpG motifs, and cytokines.<sup>114</sup> QS21, which is a derivative of the lipid saponin from the bark of the *Quillaja saponaria* tree, is being tested as an adjuvant in a pneumococcal polysaccharide vaccine (Phase II).<sup>114</sup> Recently, utilization of toll-like

receptor (TLR) agonists as vaccine adjuvants in the elderly has delivered promising results in mouse studies.<sup>98,115</sup> By targeting evolutionary conserved receptors that recognize pathogens (eg, TLRs or nucleotide-binding oligomerization domain proteins), it is postulated that adjuvants might overcome the age-associated functional decline of innate immune response and induce production of pro-inflammatory cytokines.<sup>116</sup> Using TLR agonists as vaccine adjuvants is a method currently in the very early stages of clinical development. In older adults, TLR4 agonists have been shown to improve T cell response to influenza vaccination.<sup>98</sup> Additionally, HA-flagellin (TLR5 ligand) fusion proteins (VAX128) were shown to be well-tolerated and safe in aged individuals.<sup>117</sup> Recently, the use of cytokines in conjunction with vaccines has been explored. IL-7 is important to T cell survival, and therefore may be useful in maintaining a pool of naïve T cells in the elderly, thus allowing more efficient responses to novel antigens. While to date no studies have been performed in humans, experiments in aged macaques have had promising results, with 50% of animals demonstrating increased thymic output and restored influenza vaccination response.<sup>118</sup> Thus, in older adults, IL-7 could potentially be used to amplify vaccine responsiveness. Another potential cytokine candidate would be IL-2, which is well known to increase the number of peripheral T cells in addition to their responsiveness to antigen. Administration of a liposome-formulated vaccine and IL-2 induced significantly higher seroprotection and seroconversion rates against viral antigens as compared to other aged subjects receiving non-adjuvanted vaccine.<sup>119</sup> Furthermore, combination of coupled adjuvant systems (eg, microparticles which contain both antigen and DNA of a cytokine) may allow for a more targeted immune response in the elderly.<sup>120</sup>

## Reversing immunosenescence

In the near term, novel vaccination strategies will involve working within the confines of the aging immune system; however, in the long term, a number of ambitious strategies are being pursued to correct some of the underlying defects in the aging immune system. Nutritional interventions have been demonstrated to increase vaccine responses in older adults and experimental animals. For example, decreasing specific lipid intake (eg, conjugated linoleic acids) appears to increase vaccine success rates in the elderly.<sup>121</sup> Vitamin E supplementation improves signaling between antigen-presenting cells and T cells, especially in CD4<sup>+</sup>-naïve T cells in aged mice.<sup>122</sup> Caloric restriction seems to improve many aspects of immune function. It appears to delay T cell immunosenescence in non-human primates by maintaining both naïve T cell number and functionality, and reduces age-associated inflammation.<sup>123</sup>



Although caloric restriction is unlikely to ever be a viable strategy, it may be possible to target the major signaling molecule that is altered, mammalian target of rapamycin (mTOR). A recent study demonstrated that administration of an mTOR inhibitor in elderly volunteers increased their response to influenza vaccination by approximately 20%. Further, treatment reduced the percentage of CD8<sup>+</sup> and CD4<sup>+</sup> T cells that had low surface expression of co-stimulatory molecules. Therefore, mTOR inhibition during vaccination may be a potential strategy.<sup>124</sup> An alternate strategy would be cytokine intervention to improve thymic health in the elderly. There has been evidence that administration of IL-7 can reverse thymic atrophy and can rescue reduced naïve T cell population in old animals.<sup>125</sup> Other factors including IL-2, IL-10, and thymic stromal lymphopoietin have stimulatory effects on thymopoiesis.<sup>126,127</sup> Restoring thymic health and naïve T cell populations by modulating these cytokines may be a candidate therapy to increase primary vaccine-specific responses in the elderly.

## Optimizing herd immunity

Counterintuitively, one of the best ways to reduce vaccine-preventable infections in the elderly may be targeting vaccinations, not to the elderly themselves, but to those who live, work, and care for them. Selective vaccination of children, adolescents, and healthcare workers (HCWs) reduces transmission of infections and protects unimmunized and immunocompromised individuals, such as the elderly, through herd immunity.<sup>128–130</sup> The add-on effects of vaccinating children to protect older adults were apparent after the pneumococcal vaccine was introduced. Not only did the total hospital admissions for IPD in older adults decrease, but the “holiday spikes” that once occurred over the winter holiday season when children were presumed to come in contact with their grandparents, disappeared.<sup>131</sup> Children and adolescents are major vectors for transmission of infectious diseases because of their high infection rates, prolonged viral shedding with high viral load, and frequent association with other susceptible hosts.<sup>132</sup> A recent study demonstrated that mass influenza vaccination of children (ages 3–6 and ages 7–17 years) with inactivated influenza vaccine lessened influenza-associated morbidity by 2- to 3.4-fold in unvaccinated, community-dwelling elderly.<sup>133</sup>

Establishing herd immunity is best achieved through vaccination of the youth and of HCWs, especially those working in close contact with individuals aged  $\geq 65$  years.<sup>134</sup> In a systematic review inclusive of 18 trials assessing the impact of HCW immunization on vulnerable populations, it was concluded that vaccination of HCWs against influenza provides significant

indirect protection to the high-risk individuals.<sup>130</sup> Additionally, further evidence suggests that HCW vaccination is associated with substantial decreases in patient mortality.<sup>129,130</sup> Immunity through further implementation of vaccination programs that preferentially immunize HCWs and children shows promise in protecting our vulnerable elderly.

## Conclusion

Approximately one-third of deaths in the elderly (aged  $>65$  years) occur due to infectious disease.<sup>135</sup> Acquiring infections such as bacterial pneumonia in midlife or late in life often exacerbate or accelerate subclinical or existing chronic inflammatory conditions and can be the harbinger of declining health and decreased quality of life.<sup>7,8</sup> Therefore, the economic and social costs of infectious disease do not only include the cost of acute care, but also long-term health consequences. Prevention through vaccination would have an enormous impact on reducing the cost of care and improving the quality of life of the elderly. In the immediate term, we need to pursue the use of high-dose vaccines, optimized vaccine schedules, alternate routes of immunization, and novel adjuvants. In the longer term, we may be able to reverse some of the fundamental defects in the aging immune response, which would both increase vaccination responsiveness but also leave the elderly less vulnerable to infectious disease, should they become infected. It is imperative that we expand our understanding of the biological and molecular mechanisms underlying immunosenescence in order to provide older adults with the many years of healthy, independent living that they deserve.

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