Published in final edited form as:
Expert Rev Vaccines. 2012 August ; 11(8): 985–994. doi:10.1586/erv.12.61.

Understanding the immune response to seasonal influenza vaccination in older adults: a systems biology approach

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Abstract

Annual vaccination against seasonal influenza is recommended to decrease disease-related mortality and morbidity. However, one population that responds suboptimally to influenza vaccine is adults over the age of 65 years. The natural aging process is associated with a complex deterioration of multiple components of the host immune system. Research into this phenomenon, known as immunosenescence, has shown that aging alters both the innate and adaptive branches of the immune system. The intricate mechanisms involved in immune response to influenza vaccine, and how these responses are altered with age, have led us to adopt a more encompassing systems biology approach to understand exactly why the response to vaccination diminishes with age. Here, the authors review what changes occur with immunosenescence, and some immunogenetic factors that influence response, and outline the systems biology approach to understand the immune response to seasonal influenza vaccination in older adults.

Keywords

bioinformatics; immunogenetics; immunosenescence; influenza; seasonal influenza vaccine; systems biology; vaccinomics; vaccine-induced immunity

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Financial & competing interests disclosure

GA Poland is the chair of a Safety Evaluation Committee for investigational vaccine trials being conducted by Merck Research Laboratories. GA Poland offers consultative advice on new vaccine development to Merck & Co., Inc., Avianax, Theraclone Sciences (formally Spaltudaq Corporation), MedImmune LLC, Liquidia Technologies, Inc., Emergent BioSolutions, Novavax, Dynavax, EMD Serono, Inc., Novartis Vaccines and Therapeutics and PAXVAX, Inc. He is also the co-inventor of intellectual property licensed to TapImmune Inc. IG Ovsyannikova is a co-inventor of intellectual property licensed to TapImmune Inc. R Jacobson is a member of a safety review committee for a postlicensure study funded by Merck & Co. concerning the safety of a human papillomavirus vaccine. He is also a member of a data monitoring committee for an investigational vaccine trial funded by Merck & Co. He also serves as a principal investigator for two studies, including one funded by Novartis International for its licensed meningococcal conjugate vaccine and one funded by Pfizer, Inc. for its licensed pneumococcal conjugate vaccine. The authors acknowledge support from NIH grant U01AI089859 for this work. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Influenza vaccinology is rapidly changing. From the point of view of vaccine recommendations, we have moved from a 'one size fits all', risk-based approach to a population approach that now calls for all Americans aged 6 months and older to be immunized annually. However, as far as which vaccine formulation to use, we have moved to a more individualized, directed approach [1]. This is evident in the recent licensure and availability of both high-dose trivalent influenza vaccines (HD-TIVs) and intradermal trivalent influenza vaccines, respectively, in the USA [201]. In Europe, an MF59-adjuvanted vaccine is available, and the pipeline of influenza vaccine development continues to grow.

Within this field is an area of special concern: immunosenescence and the resulting decreased immunogenicity and efficacy of influenza vaccines in older persons. This concern results from the reality that the older individual is more susceptible to morbid infection, may be unable to mount an effective vaccine-induced protective response, and is likely to have concomitant comorbidities that either contribute to the higher rates of morbidity and mortality if influenza infection results, and/or further impairs the development of an effective immune response to vaccine. While these are issues of considerable inquiry, to date, the understanding of immunosenescence remains limited. In turn, this impairs our ability to devise vaccines or adjuvants that can overcome such barriers. For this reason, an expanded research agenda and approaches to understand immunosenescence and its relationship to vaccine-induced immunity is essential.

In this review, the authors summarize data on the epidemiology of influenza in older persons, the current understanding of immunosenescence, the role of immunosenescence in reduced vaccine immunogenicity and finally, discuss a systems biology and vaccinomics approach to unraveling the impact of immunosenescence on decreased vaccine immunogenicity and the application of such knowledge to the development of improved influenza vaccines for older persons [2].

Epidemiology of influenza in older adults

While older adults suffer the highest rates of hospitalization and mortality, they neither have the highest rates of infection nor represent major contributors to local outbreaks. Local outbreaks begin suddenly and unaccountably, peak over a 2–3-week period and then persist for 2–3 months. The timing and nature of these outbreaks remain unpredictable, unexplained and a target for scientific speculation [3–6]. In an outbreak, the first cases of influenza appear in school-aged children and then spread to adults, including older adults, infants and younger children. Attack rates vary from 10 to 20% in the general population, reaching attack rates in the general population of more than 50% during a pandemic; attack rates can be extraordinarily high in institutional settings.

Despite having no higher attack rate than in younger adults, influenza's effects are more significant in older adults. Barker's study focusing on the impact of influenza infection in the frail elderly showed a decline in functional status measurable 3–4 months after infection on at least one major function (e.g., bathing, dressing or mobility) for 25% of older patients residing in nursing homes as compared with 15.7% of controls – randomly selected residents living in the same facility not contracting influenza or influenza-like illness during the same outbreak [7].

As mentioned earlier, older adults also have higher rates of hospitalization and mortality. Thompson *et al.* used the CDC's influenza-infection surveillance data and the National Hospital Discharge Survey data to estimate the annual influenza-related hospitalization rates in the USA [8]. The results showed that hospitalization greatly increased with age in those aged 65 years and older; specifically, the rates increased with each 5-year block of age from 65 to 69, to 85 years and older. Where pneumonia or influenza was listed as the primary

diagnosis, the average hospitalization rate was 36.8 per 100,000 person-years, but this increased in older persons from 37.9 for those 50–64 years old, to 71.1 for those 65–69 years old, to 127.8 for those 70–74 years of age, to 302.2 for those 80–84 years old, and 628.6 for those aged 85 years and older. Furthermore, the length of hospital stay also increased with age from a median of 3 days for those less than 5 years of age, to 4 days for those 5–49 years of age, to 6 days for those 50–74 years of age, to 7 days for those 75 years and older.

Death rates from pneumonia and influenza in the USA have ranged from 5000 to 50,000 a year as a result of cardiovascular and respiratory pathology and depending upon the circulating influenza strain. While hospital rates in older adults approximate the hospital rates in infants and children younger than 2 years of age, the fatality rate associated with the elderly is much higher. Thompson *et al.* found in their study that mortality rates due to influenza have increased from the years 1976 to 1999, which they explained in part due to the aging of the US population [9]. While the mortality rates from underlying pneumonia or influenza for those younger than 50 years of age ranged from 0.3 per 100,000 person-lives, the rates increased at 50 years of age and above [9]. The rates were 1.3 per 100,000 person-lives for those 50–64 years of age and 22.1 person-lives for those 65 years and older [9]. The increased death rate found in this study matched findings of other investigations [9,10]. Increasing the risk of mortality are the presence of high-risk medical conditions; Nordin *et al.* found the lowest risk of mortality among those with no high-risk medical conditions who were 65–74 years of age, and the highest risk of mortality among those with high-risk medical conditions who were 75 years and older [10].

Using 2003 data, Molinari *et al.* estimate that the total financial burden of seasonal influenza infection in the USA amounts to $10.4 billion a year and that the older population bear 64% of the total economic burden [11]. Efforts to target the reduction of the disease burden in the older population therefore would have a substantial impact on the expense of seasonal influenza [11].

Vaccine efficacy & induced immune response in older adults

Vaccine efficacy against influenza illness in older adults is difficult to measure and reliable data are scarce. To date, there has been only one placebo-controlled trial of influenza vaccine efficacy against laboratory-confirmed illness in older adults [12]. The study estimated protection from influenza illness at approximately 50%. An alternative and widely accepted approach is the measurement of influenza-specific antibody titers as a correlate of protection. Titers are traditionally measured using a hemagluttination inhibition (HAI) assay, which quantifies the ability of hemagglutinin (HA)-specific antibodies to block *N*-acetylneuraminic acid-mediated viral agglutination of red blood cells [13,14]. Using the set guidelines of this assay, vaccine protection can be assessed based on patient seroconversion (fourfold increase in antibody titers postvaccination) and seroprotection (HAI antibody titers ≥1:40 postvaccination). Although some discrepancies exist in studies focusing on antibody response to influenza vaccine in older adults, a quantitative review concluded that HA-neutralizing antibodies are considerably lower in vaccinated older adults than in younger adults [15]. There is also a correlation between health status in older adults and HAI titers, with healthy older adults having statistically significant higher levels of HAI titers than those with chronic diseases [16].

A strain-specific robust humoral response to influenza is necessary to prevent primary infection, but eventual viral clearance is dependent on the presence of $CD8^+$ T cells directed toward conserved regions of the virus [17]. Influenza-specific $CD8^+$ T cells produce antiviral mediators and directly kill infected cells [18]. Another approach used to measure cellular-mediated efficacy of influenza vaccines against laboratory-confirmed disease is to

quantify the ratio of IFN-γ:IL-10 and the cytolytic enzyme granzyme B from T cells postvaccination [19]. Specifically, granzyme B production has been reported as a direct method of assessing vaccine failure and subsequent illness in older adults [20,21]. Furthermore, several studies have demonstrated a defect in the production of IFN-γ and granzyme B in $CD8^+$ T-cell subsets obtained from vaccinated older adults [22–24].

To overcome the diminished immune response observed in older adults, an 'increase the firepower' approach has been adopted. HA concentrations for each strain of 60 μg or more, as compared with 15 μg of HA in the standard trivalent inactivated vaccine (SD-TIV), result in increased immunogenicity for influenza A strains and noninferiority for influenza B in older adults [25,26]. This led to the formulation of an US FDA-licensed high-dose vaccine for adults 65 years or older [202]. Each HD-TIV contains 60 μg of HA antigen for each H1N1, H3N2 and B strain contained in the SD-TIV. The HD-TIV was more immunogenic for both influenza A virus strains in older adults than the SD-TIV in a Phase III trial [27]. However, both antibody and cell-mediated immune responses in older adults vaccinated with the HD-TIV never achieve the same levels observed in young adults vaccinated with a standard-dose vaccination [28]. Although the antibody titers achieved with the high-dose influenza vaccine in older adults may be effective against circulating influenza strains, the increasing emergence of deadlier strains demands the development of vaccines that focus on more than just increasing antigen dose. An aging immune system may not be able to mount a sufficiently protective response to current or novel strains regardless of the amount of antigen present without the addition of adjuvants or newer methods of antigen delivery.

Immunosenescence

A key factor driving vaccine failure in older adults is immunosenescence. Immunosenescence is a broad term used to describe complex alterations in the immune response attributed to aging. As the immune system ages, there is a significant increase in susceptibility to infection, autoimmunity and cancers, and a decrease in vaccine-induced immunity [29]. At a cellular level, immunosenescence is a combination of diminished immune cell numbers and function, coupled with an inappropriate/unregulated inflammatory response that results in less than ideal immunity. The following sections summarize published work that addresses the influence of immunosenescence on the innate and adaptive immune systems and how these properties may diminish vaccination response in older adults.

Immunosenescence & the innate response

The innate branch of the immune system affords the host ability to respond rapidly and nonspecifically to an invading pathogen by host pattern recognition receptors (PRRs) [30]. Specifically, influenza virus has been shown to interact with innate signaling mediators, Toll-like receptors (TLRs; e.g., TLR7), Nod-like receptors (e.g., NLRP3, NOD2) and RIG-I-like receptors [31–34]. Along with initial pathogen clearance, innate immunity is also responsible for the genesis of the adaptive response by recruiting immune effector cells [35]. An age-related deficiency in innate immunity can negatively influence any subsequent adaptive response. As described below, there is mounting evidence that the phenotypic responses of many components of innate immunity are influenced by immunosenescence.

Monocytes, dendritic cells, NK cells and other innate immunity cells express TLRs [36]. The interactions between conserved molecular patterns present on microbial pathogens and TLRs lead to a MyD88 or TRIF-dependent induction of proinflammatory cytokines and the upregulation of type I interferons [37]. There is increasing evidence that a combination of inappropriate activation of TLRs and diminished function in response to many ligands is present in an aged population. Peripheral blood mononuclear cells from older adults produce

decreased levels of IL-6 and TNF-α and TLR1 surface expression levels are reduced after stimulation with a TLR1/2 ligand [38]. In the context of viral infection, pro-inflammatory cytokine production and TLR3 expression levels are increased on West Nile virus-infected macrophages from older human donors, which may result in an inappropriate inflammatory response [39]. Plasmacytoid dendritic cells from aged donors secrete decreased amounts of both IFN-1 and IFN-III after stimulation with both the TLR7 ligand CpG and live influenza virus, which is due to impairment in IRF-7 phosphorylation [40]. These plasmacytoid dendritic cells also exhibit diminished induction and priming of CD4/CD8 T-cell immunity. Panda *et al.* demonstrated a correlation between defects in cytokine response from aged human dendritic cells stimulated with TLR ligands and diminished influenza vaccine-induced antibody production [41]. Taken together, impaired TLR response in immune cells from older adults directly affects both cellular and humoral immunity to influenza.

CD80 and CD86 are costimulatory molecules expressed on antigen-presenting cells and help activate T cells after interaction with CD28 [42,43]. Costimulatory molecule expression on TLR-activated monocytes can predict influenza vaccine immune response in both young and older adults; in one study, TLR-induced CD80 levels were approximately 68% less in older adults ($p = 0.0002$) compared with young adults [44]. A decreased ability to interact with and activate effector T cells would ultimately result in both a deficient cellular and humoral response to vaccine.

NK cells are vital to the clearance of viral infection by the production of IFN-γ and lysis of infected cells [45]. Multiple studies have highlighted the importance of NK cells during influenza infection in both humans and mice [46–49]. NK cell activity in human subjects is augmented by influenza vaccination [50]. Interestingly, the overall numbers of NK cells are increased in healthy older adults [51]. However, the function and number of NK cells decrease with diminished health status, and NK activity correlates with health status and HAI titers in vaccinated older adults [16,52]. Any perturbation in NK cell function would be detrimental to the development of a protective immune response to infection.

In contrast to the many diminished responses associated with immunosenescence is the subclinical hyperinflammatory state known as ‘inflamm-aging’ [53]. Immune cells isolated from older adults produce higher concentrations of inflammatory cytokines, such as IL-1β, IL-6 and TNF-α after stimulation. Serum IL-6 levels increase with age in humans and are associated with disability and geriatric frailty [54–56]. Constant inflammation could leave a host susceptible to infection by not having the ability to recognize a true inflammatory response to a pathogen. This is true in a mouse model of systemic herpes viral infection, where an elevated state of inflammation increases susceptibility [57]. Vaccination failure and susceptibility to influenza illness may be a result of too much inflammation and not enough regulation.

Immunosenescence & the adaptive response

Bone marrow-derived T-cell progenitors undergo development and selection in the thymus and emerge as mature naive T cells [58]. One of the more dramatic observations associated with aging is thymic involution, which results in a measurable decrease in circulating levels of new naive T cells [59]. Surprisingly, there is no change in overall circulating T-cell numbers with age [60]. Research postulates that T-cell homeostasis and the production of new T cells is maintained through clonal expansion of peripheral, antigen-specific T cells [61].

An adverse effect of new T cells produced from existing T cells is a decrease in the diversity of T-cell receptors (TCRs) [62]. A robust immune response to influenza infection is dependent on TCR diversity and there is evidence of a decrease in influenza-specific $CD8^+$

T-cell repertoire in older adults [22,63]. T-cell population diversity is also diminished in older adults after lifelong exposure to certain antigens and the accumulation of memory T cells [64].

A decline in T-cell diversity and massive expansion of memory T-cell clones has also been linked to persistent viral infections. For example, chronic infection with CMV in the older population results in extensive accumulation of exhaustive, high-affinity, CMV-specific memory T cells [65]. CMV-specific $CD8^+$ T cells also produce higher levels of IFN-γ, which could partly explain age-associated 'inflamm-aging' [66]. The abundant numbers of CMV-specific memory T cells alone can alter homeostasis and decrease the amount of circulating naive T cells.

The expression of the costimulatory molecule CD28, which is needed for differentiation of naive T cells after initial antigen exposure, on $CD8^+$ T cells decreases with age [67]. There is also a direct link between a decrease in CD28 expression ($CD8^+$ $CD28^-$ T cells) and a poor immune response to influenza vaccine. In one study, a 10% proportional increase in $CD8^+$ $CD28^-$ cells correlated with a 24% decrease in humoral response to influenza [68]. The presence of other late effector T-cell subsets ($CD8^+$ $KLRG1^{hi}$ $CD57^{hi}$) is also inversely correlated with influenza vaccine immunogenicity [69]. The identification of specific cellular subsets in older adults that successfully predict immune outcome could be a powerful tool in developing the next generation of vaccines.

A portion of decreased humoral response in older adults can also be attributed to a deficiency in extrinsic cellular signaling between $CD4^+$ T cells and B cells [70]. Senescent $CD4^+$ T cells express lower levels of CD154 (CD40L) and this molecule is crucial for stimulation of B cells. Antibody response in older adults is also altered by a shift in B-cell homeostasis from naive to effector cells similar to that observed in T cells [71]. B-cell class switching, recombination and somatic hypermutation are also defective in older populations [72]. This defect would result in an inability to produce high-affinity antibodies against influenza.

In summary, immunosenescence and its contribution to suboptimal vaccine response in older adults is a complex and multi-faceted process. The majority of research has focused on pinpointing singular components of the immune system responsible for a diminished response. In reality, many key systems contribute to immunosenescence. A successful model to predict and define vaccine outcome in older adults must therefore take into account not only individual aspects of the aging immune system, but also other systems, such as epigenomic, genomic, proteomic and transcriptomic factors.

Immunogenetic factors associated with host responses to seasonal influenza vaccine

Relationships between genetic polymorphisms (and nongenetic factors) and immune response to influenza vaccine in the human population have been reported [73–76]. With regard to human influenza infection, evidence was found for a heritable predisposition to the development of severe influenza virus infection and death, strongly suggesting genetic associations with the immune response to influenza infection [77,78]. It is also thought that the predisposition to a fatal outcome of influenza illness also depends on environmental, nutritional, demographic and virologic factors [79]. The authors of these reports comment that "… it is important to identify those genes associated with the ability to respond (to influenza) with protective immunity after natural or vaccine challenge" [77]. One specific gene responsible for the anti-inflammatory response to severe influenza infection is the inducible heat shock protein gene, heme oxygenase-1 (*HO-1*) [80]. Recent studies have

demonstrated the lungs of mice that were infected with highly pathogenic strains of influenza virus exhibited increased levels of *HO-1* gene expression and a decrease in the expression levels of antioxidants *Gpx3* and *Prdx5* [81]. Furthermore, impaired antibody production in response to influenza vaccination was observed in aged HO-1-deficient mice [82]. Importantly, a recent study suggested that decreased influenza vaccine response in humans is associated with polymorphisms in the *HO-1* gene [82]. Also, in a genome-wide association study of 147 influenza-vaccinated individuals, promoter SNP rs743811 and intronic SNP rs2160567 in the *HO-1* and constitutively expressed isoform *HO-2* genes, respectively, were found to be associated with decreased H1-specific HAI titers following influenza vaccine [82]. Thus, the *HO-1* and other gene polymorphisms should be investigated to better understand possible genetic determinants for influenza disease and vaccine effectiveness.

Host genetic polymorphisms probably play a significant role in immunity against influenza vaccine. There is limited immunogenetic information available to explain significant interindividual variations observed in immune response to influenza vaccines. Population-based association studies revealed the importance of HLA and other immunity-related gene polymorphisms in influenza vaccine-induced humoral immunity [73,76]. HLA class I and class II molecules present antigenic epitopes to $CD8^+$ and $CD4^+$ T cells, respectively, and initiate adaptive immune responses. Influenza-derived peptide presentation by HLA class I and class II molecules induces T-cell populations with diverse specificities and functions [83,84]. Various HLA class I (A*2, A*11, B*27 and B*35) and class II (DRB1*07, DRB1*13 and DQB1*06) alleles have been reported to correlate with the serologic response to influenza vaccination [73,76,85]. These differences in HLA class I and class II pathway presentations of immunodominant epitopes are likely the source for some proportion of the interindividual variation in influenza vaccine-induced immune responses.

Preliminary data from the candidate gene studies demonstrate significant correlations between influenza H1-specific HAI antibody levels and single nucleotide polymorphisms (SNPs) in cytokine (*IFNG*, *IL6*, *IL12A*, *IL12B* and *IL18*), and cytokine receptor (*IFNAR2*, *TNFRSF1A*, *IL1R*, *IL2RG*, *IL4R*, *IL10RB* and *IL12RB*) genes (range of p values 0.005–0.045) [76]. Associations were also discovered between polymorphisms in genes regulating vitamin A receptor retinoic acid receptor γ and innate immunity (TLR4) and variations in influenza H1-specific antibody levels [Poland GA, Ovsyannikova IG, Jacobson RM, Unpublished Data]. For example, in the pilot studies, an increased frequency of the minor allele of the 5′UTR SNP (rs7398676; p = 0.08) in the retinoic acid receptor γ gene was associated with protective serum H1 antibody titers (median HAI titer of 1:320) after influenza vaccine. Similarly, an intronic SNP (rs1927907; p = 0.1) in the *TLR4* gene was marginally correlated with higher H1 antibody levels (median HAI titer of 1:320); however, a larger sample size is needed in order to improve statistical power and confidence. These preliminary data provide evidence that the immune-related gene polymorphism is associated with influenza H1-specific antibody titers after vaccination.

In addition to the findings associated with TLR4, it has been demonstrated that gene polymorphisms in TLR4 (that recognizes lipopolysaccharide) may influence innate immune responses to respiratory syncytia virus and influence the predisposition to severe respiratory syncytia virus disease [86,87]. Another study of the transcriptional targets of immune responses to influenza virus in human peripheral blood mononuclear cells following influenza vaccination demonstrated a high expression of interferon-induced and -regulated genes, including IFN-γ-induced protein precursor 10 (*IP-10*) gene, suggesting their function in immune response to influenza antigens [78]. In addition, the *RIG-I* gene is involved in the influenza virus-specific production of IFN-β. Also, influenza virus non-structural protein-1 has been demonstrated to interact with RIG-I and inhibit the RIG-I pathway, thereby

inhibiting the generation of IFN-β [88]. By understanding genetic influences on the generation of immunity due to vaccination, it is feasible to develop new vaccines against influenza [1,89–92]. By applying knowledge on the interactions of various pathways of key gene families critical to developing protective immune responses, it is feasible to gain an understanding of the host response to influenza vaccine antigens.

Systems biology approach

Each of the aforementioned components is an important individual contributor to the ability of an older adult, or indeed any individual, to mount an effective immune response following vaccination against influenza; evidence supporting their roles has been well established. While this understanding has come through extensive studies, these investigations have primarily focused on relatively small components of the immune system. In order to fully understand the way in which vaccines induce protection against foreign antigens, it is important to take a broader view of the components of the system that together give rise to immunity. In the study of biological processes, approaches are being developed that address this more expansive view and use more comprehensive modeling techniques that are integrated with existing biologic knowledge bases [93–96]. These approaches comprehensively integrate data gathered from a variety of often high-throughput, high-dimensional assays with human-collated models of biological function. These approaches, which have come to be known as systems biology, have not coalesced into a single defined entity, but rather encompass a broad class of methods that all seek to arrive at a deeper and global understanding of biological processes and the complex inter-relationships of systems that compose an organism [97–100].

The general idea guiding the study of systems biology is that to understand the full process by which specified biologic systems function, one needs both empirical data and structured models of existing knowledge. In the current era, the availability of technology to extract data measuring a wide variety of both inputs and outputs of molecular systems is greater than ever. Thus, it is relatively simple to obtain simultaneous detailed information about genomic, transcriptomic, proteomic and other measures. There is also an ever-increasing knowledge base consisting of models of genetic and protein networks, as well as other models of immune function. The key to effective systems biology research is to effectively apply robust multivariate statistical analyses of these data in the context of the existing biological knowledge base. These analyses make it possible to either modify known models or to confirm and refine already-described models. Such approaches carry the promise of making it possible to more deeply understand the initiation and maintenance of immune responses [2,96,100].

The current research group has initiated a series of studies within the context of a systems biology approach in order to more deeply understand the mechanistic underpinnings and complexities of diminished vaccine response in older adults. The authors organize information from a wide variety of sources, including genetic polymorphisms, gene transcripts, epigenetics, genetic pathways and protein–protein interactions. Using these data sources as inputs, the authors employ a variety of state-of-the-art statistical models and approaches to determine the extent to which the interplay of these data clarify existing models or bring new understandings that may lead to the development of novel biological models.

Specifically, a systems biology generated immune profile will be constructed around time points associated with distinct temporal stages of influenza vaccine response. The baseline data, or day 0, correspond with prevaccination immunity. Days 3, 28 and 75 will be associated with innate, adaptive and the immune system’s return to homeostastis,

respectively. The authors will then have a distinct set of data points for each individual over a broad duration of immune response to seasonal influenza vaccination. The innovation of this study lies in pairing traditional influenza vaccination outcomes, such as cellular and humoral measures, with flow cytometric markers for adaptive and innate immunity, proteomics and cutting-edge technologies such as next-generation mRNA sequencing (Figure 1). The authors also incorporate assays to quantify and compare the contribution of immunosenescence markers to vaccine response by measuring TCR diversity, CD28 expression and TCR excision circles analysis.

Once all data have been generated and analyzed in the context of the current biological knowledge base, the authors will utilize the findings to examine the entire spectrum of biological responses and compare and contrast them across a range of ages and immunization strategies. This will enable the authors to comprehensively understand interactions among the components of the aging immune system and their impact on the development and maintenance of immunity to influenza vaccine. Ultimately, this will lead to a method of more directed development of vaccines against influenza, perhaps by ‘reverse engineering’ around identified genetic or cellular elements.

Similar approaches have already been applied and these have led to novel information relative to processes by which immunogenicity might be induced. The earliest example of this is the case of yellow fever vaccine, where a large collection of data gathered across multiple time points were analyzed with multivariate statistical techniques to identify a collection of gene signatures that predicted the immunogenicity of the YF-17D vaccine [96]. Importantly, these gene signatures were validated in an independent sample set; an important step in the research process when complex statistical approaches are applied with the goal of integrating information across a number of high-throughput technologies and existing knowledge bases. The advantage offered by this approach to studying immune responses is in its focus on simultaneously studying a large number of input and output data; something that more closely approximates the reality of the complex interactions that take place within living organisms mounting an immune response against an antigen. Classical approaches that are typically used to study correlates of immunity tend to focus on simple associations between a single input and a single output, perhaps while adjusting for a small number of potentially contributing factors, and are therefore not able to provide insight into the full cellular and immunologic milieu. Because of this, it is essential that research be extended into the realm of systems biology, where information across a wide range of data sources can be integrated to provide insight into the immunologic processes.

Moving forward

This review has briefly outlined the epidemiology of influenza in older persons, acknowledging the high rates of morbidity and mortality that older adults experience as a result of influenza infection. In addition, the huge economic costs associated with influenza resulting in increased medical care, lost work and lost time in school, in tandem with annual epidemics of influenza (and periodic pandemics), combine to make prevention of influenza a major public health concern. An additional and pertinent temporal trend must also be recognized, and that is the rapid increase in the aging of populations throughout the world. For example, in the USA, the fastest growing segment of the population is individuals over the age of 85 years. The implications are considerable. Older persons are increasing in number, have increased rates of illness, hospitalization, medical care use and death from increasing virulent strains of influenza, in the context of yearly epidemics, and respond poorly to current influenza vaccines. It therefore becomes imperative that the research agenda be expanded to both understand the mechanisms that result in poor immunity in

older persons, and use such information to devise more immunogenic influenza vaccine candidates.

Critical to our work, and to progress in the field, is to 'unravel' the complexity of the immune response in older persons, and to understand how it differs from younger persons. The task is daunting, although made easier by the plethora of high-throughput, high-dimensional technologies rapidly becoming available at an affordable price. A more serious obstacle, however, are the bioinformatics personnel and processes needed to analyze and make sense of such data. Consider that the combination of transcriptomic, other immunophenotyping and sequencing data can result in a terabyte of data in just one experiment involving a single subject. Analyzing such data in the context of models built on the current understanding of the immune response network theory and a vaccinomics approach requires a significant investment in devising and testing bioinformatics models [1,89–92,101]. In many cases, the current models are simply insufficient and reflect the difficulty in reducing extremely complex systems to more simple models.

As the authors have reviewed, immunosenescence has far-reaching implications in terms of generating immune responses on innate, adaptive, T-cell and B-cell function. Further research is needed on the critical changes and impairments that together result in immunosenescence, and possibilities for reversing adverse changes associated with the aging immune system. Important findings have been published, and progress made – but there is a long way to go to meet the challenge of protecting an aging population against infectious diseases for which they are particularly susceptible.

Expert commentary

With the approval of an HD-TIV for older adults, novel vaccines are being developed to address the issue of immunosenescence. However, as stated previously, both the humoral and cellular responses in HD-TIV-vaccinated older adults do not reach the same level as those in SD-TIV-vaccinated younger adults [28]. With the emergence of highly pathogenic influenza strains and a decrease in vaccine response in older adults, we have to consider a different and more directed approach to vaccine research and development. To truly understand why vaccine efficacy decreases with age, we will have to decrease our dependence on reductionist-based science [102]. Although the immune system can be thought of as the summation of multiple smaller parts (innate and adaptive), aging causes too many complex alterations to these systems to attempt to understand the whole of immunosenscence by focusing on a single component.

Our own work, and that of others, is directed at just such issues. Importantly, the NIH has developed and funded a research program that seeks to uncover drivers of immune response to viral and other vaccines. The Human Immunology Project Consortium is currently funding seven centers throughout the USA to perform exactly the systems-level research work described above [203]. In addition, through this program funds are available to finance preliminary human-based studies that are consistent with the priorities of the consortium, and that are performed in collaboration with one of the funded primary centers.

Five-year view

Recent innovative work demonstrates that a systems biology approach can be successful in elucidating predicative markers of immune response [96]. We believe that our approach, and others like it, will be adopted to not only gain a thorough understanding of host interactions with vaccines, but will also be applied to the interactions between host and specific pathogens.

Our model is unique in that we focus on the influence of immunosenescence on seasonal influenza vaccination, but immunosenescence is a contributing factor in host response to other viral vaccines as well. Interestingly, the elderly exhibit a delayed antibody response to yellow fever vaccination (YF-17D) and an increase in adverse events [103]. In addition, both cell-mediated immunity and antibody response against herpes zoster vaccine declines with age [104]. If we are successful in developing a holistic predictive immune profile to seasonal influenza vaccination, this model can be applied to research focusing on other vaccine systems and the contribution of immunosenescence. Such work will be accelerated by increasing complex bioinformatc models that will allow us to understand the simultaneous contributions of genetic, proteomic, epigenetic and cellular systems; and ever-expanding, high-dimensional, high-throughput, whole systems-level assays becoming available.

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Key issues

- Older adults have a significantly higher rate of influenza-related morbidity and mortality.
- Vaccine efficacy is decreased in older adults, and compromises efforts to protect the elderly.
- Immunosenescence is associated with complex and multifaceted changes in both the innate and adaptive response to influenza.
- Our previous work has demonstrated that immunogenetic factors contribute to immune response variations to seasonal influenza vaccination.
- A systems biology approach incorporates assays aimed at measuring complex interactions between the aging host and immune responses to seasonal influenza vaccine, and complex statistical models that aid in understanding these interactions.

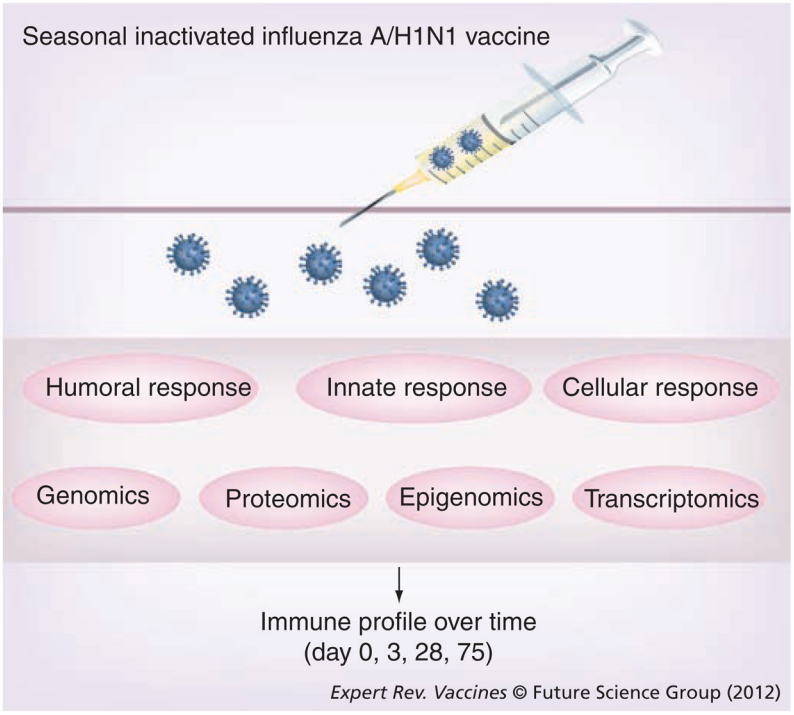

Figure 1. Systems biology approach to developing an influenza A/H1N1 vaccine-induced immune profile

Multifunction immune and systems analysis over the duration of vaccine response will be used to determine individual immune outcomes, functional pathways and longitudinal immune profiles that will lead to the explanation and prediction of immune response to influenza A/H1N1 vaccine. This will be accomplished using a fusion of traditional measures of humoral, cellular and innate immunity, paired with measures of gene regulation and large-scale analysis of protein response. Immune response to seasonal influenza vaccination will be measured after *in vitro* stimulation of subject peripheral blood mononuclear cells with live influenza A/California/H1N1 virus. Assays specific to markers of immunosenescence will also be used to measure the influence of age on immune response to vaccine.