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B cell function and influenza vaccine responses in healthy aging and disease

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Abstract

Influenza vaccination is less effective in elderly as compared to young individuals. Several studies have addressed the identification of immune biomarkers able to monitor or predict a protective humoral immune response to the vaccine. In this review, we summarize these data, with emphasis on the effects of aging on influenza vaccine-specific B cell responses in healthy individuals and patients with Type-2 Diabetes, HIV and cardiovascular diseases.

Introduction

Vaccination has proven to be a very effective tool for preventing infectious disease [1]*. In general, vaccines elicit an immune response and consequently immunological memory that mediates protection from the infection. Influenza is associated with morbidity and mortality in very young and very old individuals as well as in individuals with immunodeficiency diseases, cardiovascular diseases, cerebrovascular disease, diabetes, chronic respiratory conditions and pregnancy [2]. The complications of influenza may include primary influenza pneumonia or secondary bacterial infections and exacerbations of pre-existing medical conditions. Hospitalization is the major contributor to the development of disability in elderly individuals [3,4]. The associated decline in physical activities and consequent disability represent a significant economic burden due to both direct (medical) and indirect costs (inability to work, reduction in productivity) [5]. Seasonal influenza epidemics are responsible for almost 200,000 estimated hospitalizations and 35,000 deaths each year in the United States and the elderly account for 90% of these 35,000 [6].

Vaccines against influenza require annual reformulation due to continuous viral evolution (antigenic drift and shift) which allows not only new human but also non-human influenza viruses to infect human beings. Annual influenza vaccinations help individuals to make protective antibodies specific for the currently circulating strains [7,8]. The influenza

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The authors regret that they could not include more of the relevant references to this field. We have tried to reference articles we thought were historically significant and/or recently published.

vaccine induces an antiviral response in B and T cells, resulting in humoral and cellular immunity, respectively [9]. The antibody response to the vaccine is the first line of protection from subsequent infection. An essential step in the generation of vaccine-induced antibody-secreting cells is the interaction of vaccine-specific B cells and T follicular helper cells (Tfh), to generate B cell proliferation, class switch recombination (CSR) and somatic hypermutation (SHM) [10].

It has been shown that some elderly individuals can still be infected with influenza even if they routinely receive the vaccine. This often leads to secondary complications, hospitalization, physical debilitation and ultimately death [11,12,13], likely due to a compromised immune system in these individuals. The fact that influenza vaccines also prevent complications from influenza (e.g. pneumonia) in most elderly strongly supports vaccination campaigns targeted to improve immune functions in these vulnerable individuals as will also be supported herein. Current influenza vaccination campaigns are able to reduce hospitalization to some extent [14], but rates of hospitalizations due to influenza-related disease are still very high [15].

The effects of influenza vaccination are different in individuals of different ages [16,17,18,19,20] and this depends on age-related differences in the innate and adaptive immune systems. These differences include a decrease in natural killer cell cytotoxicity on a per cell basis [21], a decrease in both numbers and function of dendritic cells in blood [22,23], a decrease in T cell function [24,25,26] and expression of CD28 [27], an increase in cytomegalovirus (CMV) seropositivity [28,29,30,31], and a decrease in B cell numbers and function [9,28,32,33,34,35], such as reduced CSR and SHM, leading to reduced generation of protective antibodies [35,36,37,38].

In this review we will summarize results on the effects of aging on influenza vaccine-specific B cell responses in healthy individuals as well as in individuals with Type-2 Diabetes (T2D), HIV and cardiovascular diseases (CVD).

Influenza vaccine-specific antibody responses in individuals of different age

Healthy individuals

Aging significantly decreases the influenza vaccine-specific antibody response in healthy individuals as we [36,37,38] and others have shown [9,17,39,40]. Most of the studies conducted so far have shown that this correlates with the well characterized age-dependent decrease in T cell [26,41,42] and dendritic cell [23] function. For T cells in particular, a shift with aging toward an anti-inflammatory response characterized by IL-10 production and decreased IFN- γ :IL-10 ratio in influenza-stimulated lymphocytes has been shown to be associated with reduced cytolytic capacity of CD8+ T cells which clear influenza virus from infected lungs [43]. However, we have shown that age-related intrinsic B cell defects also occur in blood and these contribute to decreased vaccine response. These include decreases in class switch recombination (CSR), the process that generates protective antibodies and memory B cells; decreases in the expression of the enzyme, activation-induced cytidine deaminase (AID), the transcription factor E47, which contributes to AID regulation; and

decreased percentages of switched memory B cells (CD19+CD27+IgD-) before and after vaccination as compared with younger individuals.

We have measured the antibody response to the influenza vaccine in sera (in vivo response) and have associated this with the B cell response after vaccination to the vaccine in vitro. In vivo and in vitro B cell responses have been measured respectively by hemagglutination inhibition assay (HAI) and by AID mRNA expression by qPCR after B cell restimulation. AID is a measure of CSR and of B cell function which we have previously established to reflect the generation of specific IgG and associate with other mechanistic markers such as E47 [44]. Our results have shown that the specific response of B cells to vaccination in vivo and in vitro are both decreased with age and are significantly correlated [36,37,38]. These results support our hypothesis that the in vitro AID response recapitulates what has occurred in vivo in the germinal center in the generation of memory B cells. We have also shown that switched memory B cell percentages are good B cell biomarkers that are reduced in the blood of elderly individuals and are significantly correlated with the in vivo antibody response to the vaccine [36,37].

We have also demonstrated that CpG-induced AID at t0 and the percentage/number of switched memory B cells at t0 predict the ability to respond well to the influenza vaccine. Therefore, we suggest that AID in stimulated B cells and switched memory B cells at t0 are good predictive biomarkers for response to vaccines and infectious agents in humans [36,37]. More recently, we have demonstrated that unstimulated B cells make intracellular TNF- α and the levels of this pro-inflammatory cytokine are negatively correlated with the antibody response to the vaccine [45]. These 3 predictive biomarkers we have identified correlate with optimal antibody responses to the influenza vaccine in the majority of individuals (Figure 1). We have confirmed and extended these results showing for the first time a negative association between CMV-seropositivity and these B cell predictive biomarkers of optimal vaccine responses (Frasca et al., submitted). CMV seropositivity had also been shown to have a negative effect on influenza vaccine-specific antibody responses [39], due to accumulation of late-differentiated T cells (T_{EMRA}).

The role of AID in CSR was well characterized previously [46,47] but its role in SHM and polyclonal antibody affinity maturation was only associated in those with genetic deficiency in mice [46] and humans [48] or weakly associated [49,50]. More recently, we have shown that AID correlates with high affinity antibodies specific for the pandemic (p)H1N1 influenza vaccine [38]. Our results showed that AID mRNA expression before vaccination in stimulated B cells as well as the fold-increase of AID mRNA expression after vaccination directly correlated with the increase in polyclonal antibody affinity to the HA1 globular domain of pH1N1 which is the domain most associated with protection to infection. In young individuals, significant affinity maturation to the HA1 globular domain was observed, which associated with initial levels of AID and fold-increase in AID after vaccination. In most of the elderly individuals, high affinity to the HA1 domain was observed before vaccination which resulted in minimal change in antibody affinity and correlated with low AID induction in this age group. In the elderly this likely reflects previous vaccination or infection with highly conserved pH1N1 determinants which generated an optimal memory

response. These findings have demonstrated for the first time a strong correlation between AID induction and in vivo antibody affinity maturation in humans.

Others have measured the quality of antibodies secreted from single plasmablasts in young and elderly individuals 7 days after vaccination [40]. Results showed that not only the number of vaccine-specific plasmablasts decreased in elderly individuals, but also the amount of antibodies made by these cells is decreased. Conversely, the avidity of these vaccine-specific antibodies and the affinity of recombinant monoclonal antibodies obtained from single cell plasmablasts were similar in the two age groups. The authors reported that results support the conclusion that the lower efficacy of the influenza vaccine in the elderly is primarily due to a quantitative difference in the number of antibody-secreting plasmablasts which would be consistent with our data above showing optimal affinity towards cross-reactive or previously encountered antigens in the elderly already at t0 as well as after vaccination [38].

Also consistent with the above results, analyses of the clonal structure and mutation distribution of B cell repertoires in individuals of different ages vaccinated with the seasonal influenza vaccine have shown that elderly individuals had increased mutations before vaccination in their repertoires, suggesting that a priming by previous infections or vaccinations may have occurred [51]. Moreover, most of these elderly individuals show a reduced B cell clonal diversity as compared to young individuals. Their overall conclusion that the “higher numbers of somatic mutations observed earlier in elderly individuals arise from clonal expansions that draw upon a pool of B cells having more somatic mutations to begin with” agrees with the other results above [38,40].

Because the 3 antigens in the influenza vaccine were repeated in the 2010/11, 2011/12 seasons, possible defects in the antibody response in the elderly due to the deficits we have seen in AID would have been underestimated. We are currently measuring HAI responses and avidity to the new antigens present in the vaccine in the last 2 seasons.

Patients with T2D

T2D patients are at elevated risk for infections due to influenza or for complications related to it [52] and therefore annual vaccination is highly recommended in these patients. Viral and bacterial infections and consequent diseases in T2D patients have been shown to cause loss of metabolic control and consequent increase of glycosylated serum proteins, ketoacidosis which may result in an increased hospitalization, increased mortality rates and prolonged complications [53]. Not much is known about the effects of influenza vaccination on lymphocyte populations of T2D patients. A few studies have measured T cell function in vaccinated young [54] and elderly [55] T2D patients as compared to healthy controls and have shown reduced [54] or similar [55] responses in patients versus controls.

We have evaluated in vivo and in vitro B cell responses to the seasonal influenza vaccine in normal healthy controls and in T2D patients [56]. Briefly, we demonstrated that both in vivo and in vitro responses decrease by age in healthy individuals but not in T2D patients, despite high levels of serum and B cell-intrinsic inflammation (TNF- α) in T2D patients. This was surprising as we had previously demonstrated these negatively impact B cell function in

mice [57] and humans [45]. We hypothesized that this may be due to the fact that the innate immune system of T2D patients is beneficially hyperactivated, as we found elevated serum levels of bacterial lipopolysaccharide (LPS) and soluble (s)CD14, and we suggest that these may not only counteract the negative effects of inflammation from increased TNF- α , IL-6 and CRP, but also to induce a direct stimulation of B cells. Another explanation for these data is that the T2D patients in our study were taking anti-inflammatory agents, such as Metformin, which is known to block TNF- α signaling in all cells, including B cells.

Patients with HIV

HIV viral infection has been shown to be associated with an aberrant activation of cells of the immune system, including B cells, which show phenotypic and functional alterations. B cells from HIV-infected patients show impaired reactivity to in vivo and in vitro activation [58,59]. Seasonal influenza vaccination is recommended for HIV-infected individuals to reduce influenza-related morbidity and mortality. HIV-infected people are at a significantly higher risk than the general population at all ages for influenza infection, despite vaccination and virologic control with combined antiretroviral therapy (cART) [60,61]. However, the response to influenza vaccination is frequently impaired in these individuals [62,63]. Intrinsic defects in B cells have been described in chronically HIV-positive individuals [64]. In addition, HIV infection has been shown to have a crucial role in the cellular senescence and immunodeficiency of both T and B cells [65,66].

In order to investigate the cumulative effects of HIV infection and aging on the response to the seasonal influenza vaccine, we recently performed a study in which we enrolled HIV-infected post-menopausal women undergoing cART and healthy controls [59]. We found that the antibody responses to the influenza vaccine were lower in HIV-infected as compared to uninfected controls. Moreover, HIV-infected women had higher frequencies of activated, proliferating CD4 and Tfh cells (CD38+HLADR-Ki67+) and of activated, senescent CD8 T cells (CD8+CD28-). These were negatively correlated with antibody titers after vaccination (also in the HIV-uninfected group). High pre-vaccination levels of TNF- α in plasma were found to be associated with Tfh activation and low antibody responses to the influenza vaccine.

Patients with CVD

Immunization against seasonal influenza has a critical role in preventing morbidity and mortality in patients with CVD. The American Heart Association and the American College of Cardiology recommend influenza vaccination as part of comprehensive secondary prevention in children, adults and elderly individuals with coronary and other atherosclerotic vascular diseases [67]. Nevertheless, influenza vaccination coverage levels among persons with CVD remain below national goals and influenza-related death is more common among individuals with CVD than among patients with any other chronic condition [68,69]. This situation is exacerbated in the elderly in which the dysregulation of the inflammatory network due to various age-associated chronic stresses also occur, increasing the susceptibility of these individuals to develop CVD [70].

There are limited references on the effects of influenza vaccination on lymphocyte subpopulations in CVD patients and the few studies performed have evaluated in vivo antibody production to the vaccine and associated this with T cell function. In one of these studies, the relationship among the development of influenza, serum antibody titers and ex vivo cellular immune responses to influenza vaccination in community dwelling elderly (60–90 years of age) including those with congestive heart failure (CHF) has been evaluated [71]. Results indicate that both healthy and CHF elderly showed seroconversion after vaccination, although the CHF patients had a lower titer as compared to healthy young adults. In addition, in healthy but not in CHF elderly individuals, T cell cellular responses (granzyme B levels) increased after in vitro stimulation of PBMC with the influenza virus. Another study also showed that patients with CHF have decreased T cell responses to the influenza virus, as compared to healthy controls, despite similar rates of seroprotection and seroconversion [72]. These apparently discrepant results may be due to different individuals and/or different degrees of pre-immunization.

Conclusions

Vaccination, and in particular the influenza vaccine, protects the majority from severe health complications associated with infection. Those populations especially susceptible include the elderly and those with co-morbidities such as T2D, HIV, CVD, as well as cancer and autoimmunity. Autonomous B lymphocyte deficiencies have been found which contribute to lower vaccine response in the elderly as well as in some young individuals and these should also be predictive in other risk groups. These include lower switched memory B cells, higher intracellular TNF- α in unstimulated B cells, and lower AID in stimulated B cells before vaccination. The basic science and clinical communities are increasingly aware and have contributed important new information to identify deficits in particular lymphocytic (T, B, NK) and myelocytic (monocytes, dendritic cells) subpopulations. Improvement in future vaccines will depend upon identifying and correcting deficits in multiple cell types to achieve optimal protective immune responses.

Acknowledgements

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Highlights

- B cell defects increase with age and contribute to lower influenza vaccine response
- Defects include lower Ig class switch recombination (CSR), lower activation-induced cytidine deaminase (AID), and higher serum/B cell TNF- α
- T2D patients have high inflammation but do not have decreased vaccine response
- HIV patients have high serum TNF- α , activated Tfh and lower vaccine response
- Elderly CVD patients have decreased vaccine response

Figure 1. B cell predictive biomarkers of in vivo influenza vaccine responses

Left. AID mRNA expression (measured by qPCR) in CpG-stimulated B cells at t0 (before vaccination) predicts the in vivo response (as measured by fold-increase after vaccination) in 92% young (<60) and 74% elderly (≥ 60) individuals. Results are from 62 young and 39 elderly recruited during the 2011–2012 influenza vaccine season. **Center.** The percentages of switched memory (SM) B cells (CD27+IgD-) at t0 predict the in vivo response in 87% young and 79% elderly individuals (same in A). **Right.** Intracellular TNF-α (measured by flow cytometry) in unstimulated B cells at t0 predict a low in vivo response in 85% young and 73% elderly individuals. Results are from 33 young and 30 elderly recruited during the 2011–2012 influenza vaccine season.