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Vaccine strategies to enhance immune responses in the aged

Julie S. Lefebvre and Laura Haynes

Trudeau Institute, 154 Algonquin ave, Saranac Lake, NY, 12983, USA

Abstract

The elderly population is more susceptible to infections with higher risks of morbidity and mortality. This is caused by the accumulation of immune defects with aging. The best way to protect people against infections is vaccination. Unfortunately, the same immune defects that render the elderly susceptible to infectious diseases also prevent the development of protective immunity following immunization. A good example of this is the influenza vaccine that only protects between 40–60% of the vaccinees over 65 years. In the past decade, tremendous efforts have been put toward improving the influenza vaccine for the elderly. We therefore use this example to present various strategies employed to overcome these age-associated immune defects and hence make vaccines more efficacious for the aged.

Introduction

Vaccination is incontestably one of the greatest achievements of modern medicine. The virtual eradication of diseases like polio and smallpox, and major progress in controlling infantile diseases gave much hope to our capacity to vanquish any disease. Unfortunately, there are some pathogens, such as HIV and *Plasmodium*, that still pose major challenges to vaccinologists. With increased longevity, the development of efficient vaccines for the elderly has become another challenge. Indeed, with the accumulation of various immune defects with aging, the elderly are poorly equipped to respond adequately to vaccines. This is evidenced by the mitigated results obtained with the influenza or pneumonia vaccines administered to people over 65 years of age.

To succeed in designing better vaccines for the elderly, it is essential to understand the defects that influence the aged immune response. Most vaccines, including inactivated influenza vaccines, aim to develop good neutralizing antibody. The generation of an efficient antibody response is a multistep process that involves: the activation of antigen-presenting cells (APCs); their migration to the draining lymph node where they activate CD4 T cells; the interaction of the activated CD4 T cells with their cognate B cells to form germinal centers; and finally, the maturation of the B cells into class-switched, affinity-matured plasma cells. Each of these steps are affected by aging. Indeed, defects in the migration [1], cytokine production [2;3], and antigen presentation [2;4;5] of aged dendritic cells has been reported in mice and humans. The impaired response of aged CD4 T cells is also well recognized and includes reduced proliferation and IL-2 production [6], reduced T follicular helper (Tfh) functions [7] and many others (reviewed in [8]). Finally, the effect of

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Corresponding author: Laura Haynes, Trudeau Institute, 154 Algonquin Ave, Saranac Lake, NY, 12983, USA, Tel: 1-518-891-3080 ext 374, Fax: 1-518-891-5126, lhaynes@trudeauistitute.org.

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aging on B cell differentiation and function has recently been reviewed [9]. Finding strategies that would circumvent all these defects, and hence improve vaccine efficacy for older people, is therefore a challenge. Focusing on the influenza vaccine, we review some of the strategies that can be used to enhance the immunogenicity of vaccines destined for the elderly population: the addition of adjuvants, altering the route of administration, increasing the dose of antigen delivered, and modifying the composition of the vaccine.

Adding adjuvants

Aluminium salts (alum) are the most commonly used adjuvants to date. Unfortunately, its use in the influenza vaccine have shown varying effects, some studies showing that the addition of alum was either beneficial [10;11], provided no benefits [12], or had a detrimental effect [13;14]. This supports the need for better adjuvants for the influenza vaccine that would also counter some of the immune defects present in the elderly. We have shown that the incubation of aged CD4 T cells with a cocktail of proinflammatory (PI) cytokines (IL-1/IL-6/TNF) could rescue their defect in proliferation, activation and B cell helper function [15;16]. Figure 1 shows an example of this where addition of PI cytokines, when compared to alum alone, enhanced the humoral response to vaccination in aged mice. Since the administration of PI cytokines is likely to cause unwanted side effects, the use of adjuvants that induce the production of these cytokines is a promising strategy.

Toll-like receptor (TLR) agonists are currently evaluated as potential adjuvants for various vaccines [17]. This is an interesting strategy since TLR agonists can induce the production of PI cytokines by stimulated APCs [18]. Moreover, TLR signalling plays an important role in germinal center formation (Reviewed in [19]). Unfortunately, there seems to be no consensus as to whether TLR expression or function is altered with aging. For example, in one study, splenic and peritoneal macrophages from aged mice were shown to express lower levels of TLR1 through TLR9 [20] which was associated with reduced cytokine production [20;21]. Conversely, another study showed no difference in either TLR expression or cytokine production from the same cells [22]. In humans, the impact of aging on TLR function was addressed in a comprehensive review [23]. In summary, whether cytokine production is intact, increased or reduced with aging in response to TLR triggering seems to depend on the cell type and the specific TLR evaluated [23]. This highlights the need for exhaustive testing when evaluating the usefulness of TLR agonists as adjuvant for the elderly.

Thus far, the use of TLR agonists as adjuvants in aged-destined vaccines has provided promising results. The subcutaneous injection of CpG (TLR9), poly(I:C) (TLR3) or pam3CSK4 (TLR1/2) proved efficient in increasing the expression of co-stimulatory molecules (CD40, CD86 and MHCII) on the CD11b+CD11c+ APCs found in the draining lymph nodes of both young or aged mice [24]. This resulted in an improved CD4 T cell response that was IL-6-dependent [24]. We have also shown that the addition of poly(I:C) or CpG oligonucleotides to a haptenated protein vaccine restored the defects in B cell expansion and germinal center formation observed in aged mice [16], as shown in Figure 1. Moreover, the use of poly(I:C) as an adjuvant with an influenza virus-like particle (VLP) vaccine protected 100% of aged mice against a lethal challenge with the highly pathogenic avian influenza strain (H5N1) while 67% of those immunized with VLP alone succumbed to that same challenge [25]. Another study demonstrated that the addition of CpG to a pneumococcal conjugate vaccine improved the antibody response of aged mice to levels similar to those obtained in young mice in a CD4 T cell-dependent manner [26]. In humans, the incubation of peripheral blood mononuclear cells (PBMCs) with glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), a TLR4 agonist, in combination with the influenza split-virus vaccine for 5 days increased the granzyme B activity detected in PBMC lysates

20 hours post-influenza challenge for both young and aged subjects. It also significantly decreased the amount of IL-10 produced by the aged PBMCs to levels similar to those obtained in young cells [27]. Finally, a clinical trial aimed at evaluating the efficiency of flagellin, a TLR5 agonist, in a recombinant hemagglutinin influenza-flagellin fusion vaccine (VAX125) in the elderly showed a dose-dependent increase in hemagglutination-inhibition antibody titers that peaked at a dose of 5 µg [28]. Unfortunately, the formulation was not compared to one without flagellin leaving the question as to whether flagellin has or not a beneficial adjuvant effect.

Another type of adjuvant, the oil-in-water emulsion, has been tested in various vaccines including influenza vaccines. One representative of this category is MF59. MF59-adjuvanted influenza vaccines demonstrated good immunogenicity and safety in older individuals [29–34]. They also provide a significantly increased protective effect over the non-adjuvanted vaccines in the elderly. Indeed, older individuals who received the adjuvanted influenza vaccine had 25–50% less risks of hospitalization than those who received the non-adjuvanted one [35;36]. The adjuvanted vaccine also generated higher antibody titers and cross-protection against heterologous strains of influenza than the split or the virosomal vaccines in the elderly [37–40]. Similarly, an influenza vaccine adjuvanted with AS03, another oil-in-water emulsion, was recently shown to procure slightly increased protection (12%) over the non-adjuvanted form in a cohort of over 40,000 elderly in 15 countries [41]. Interestingly, oil-in-water emulsions were also shown to be good vehicles for the administration of E6020, a synthetic TLR4 agonist [42]. This suggests that combining the two approaches, an oil-in-water emulsion and a TLR agonist, could yield even better results in the elderly, a strategy used for a Meningococcal vaccine [43].

Changing the route of immunization

Another way of improving the immune response to an immunization is to change the route of administration. Because the type of APCs present at different locations can generate different kinds of T cell responses [44], using various routes of injection is likely to give diverse outcomes. The routes commonly used for the influenza vaccine administration are the intranasal and intramuscular routes. The use of live-attenuated vaccines given via the intranasal route has failed to demonstrate benefits in the elderly [45]. Intradermal administration is another route currently being evaluated for many vaccines, and its advantages have recently been reviewed [46]. When compared side-by-side, the intradermal route showed increased immunogenicity over the intramuscular route for the same seasonal influenza vaccine in a cohort of people over 60 years [47;48]. This was not the case, however, when comparing these two routes of immunization for a H5N1 vaccine tested in young (18–49 years) individuals [49]. A meta-analysis study of 13 trials comparing the immunogenicity and protectivity of the influenza vaccine administered intradermally or intramuscularly concluded that both routes generated equivalent responses in the overall population, but that the intradermal route was preferable over the intramuscular route for the elderly population when used at a higher dose [50]. These data further demonstrate the particularities of the aged immune system and the need to evaluate any new approach regardless of the results obtained in young individuals.

Increasing the dose

Some of the immune defects reported with aging include reduced immunological synapse formation, leading to reduced antigen-presentation, and a higher activation threshold for TCR signalling. A way to overcome these defects is the use of higher doses of antigens in the vaccine preparation to increase antigen-presentation. This strategy has been tested and showed that a four-fold higher dose (60 µg instead of a standard 15 µg of HA) could

improve the response of the elderly to the vaccine [51–54], but did not reach the level of protection achieved in young individuals receiving the regular dose [52]. These data suggest that increasing the dose of antigen in a vaccine is a valid but insufficient approach to adequately protect the elderly.

Changing the composition

Currently, the influenza vaccines most commonly used are the trivalent inactivated vaccine and the cold-adapted live attenuated vaccine. These two vaccines are composed of intact influenza virus particles that generate immunity principally against the surface proteins of the virus, hemagglutinin (HA) and neuraminidase. Although this allows for the generation of neutralizing antibodies, the high variability of the virus prevents the establishment of a lasting immunity. Hence, the vaccine composition should aim to also induce antibodies against conserved antigens such as the matrix (M) proteins, the stalk portion of HA, or the nucleoprotein (NP). A vaccine against NP is particularly appealing since we have recently shown that this also protects against secondary pneumonia elicited by *Streptococcus pneumoniae*, a major cause of morbidity and mortality associated to influenza infection [55].

Another issue raised with the current vaccines is that the antibodies generated in older people do not always correlate with protection, which is more closely associated with the T cell response [56]. While the inactivated vaccines induce a good CD4 T cell response, CD8 T cell activation, however, usually requires a productive infection. A study performed in older individuals showed that fewer than 50% of the subjects generated a cytotoxic T lymphocyte (CTL) response after immunization with the present live-attenuated vaccine [57]. Interestingly, the influenza nucleoprotein (NP) contains immunodominant epitopes for both CD4 and CD8 T cells [58]. This suggests that, in addition to generating antibodies efficacious against multiple strains of influenza, a vaccine eliciting a response against NP could also generate good T cell immunity. To this end, a new influenza vaccine using the modified Vaccinia virus Ankara (MVA), that can infect cells but not replicate, expressing both the NP and M1 influenza proteins has been developed [59]. This vaccine was safe and successfully stimulated T cell responses in people 50–85 years old similar to that of younger individuals [60]. Whether this vaccine also generates an antibody response, however, was not determined.

Conclusion

Considering the multifunctional defects reported with aging, the best strategy for improving vaccines for the elderly would likely include a combination of the approaches presented herein. These approaches and their intended goals are summarized in Table 1. The ideal vaccine would induce good humoral and cellular responses to efficiently reduce morbidity and mortality associated with influenza. This would also apply when developing other vaccines aimed at the elderly population. In the perfect world, the best strategy would definitely be to create a universal influenza (or else) vaccine that generates a life-long immunity so it could be administered young and last into old age. Until we reach this ultimate goal, we have to use everything available to us to improve the current vaccines for the elderly.

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Highlights

- The elderly population responds poorly to vaccination that hence needs improvement.
- We cover strategies including changing adjuvant, route, dose, and composition.
- A combination of multiple strategies is likely to provide the best results.

Figure 1. Adjuvants can enhance the response to vaccination in aged mice

Young (2–4 month) and aged (20–24 months) B10. Br mice were vaccinated with 200 ug nitrophenyl conjugated to pigeon cytochrome c (NP-PCC) in alum alone, alum with proinflammatory (PI) cytokines TNF , IL-1 and IL-6, poly (I:C) or CpG oligonucleotides. On day 14, the number (mean $\pm$ SD) of NP-specific B cells (left graph) and the number of germinal center (PNA^{hi}CD38^{lo}) NP-specific B cells (right graph) was determined by flow cytometry. The only significant difference between young and aged cell numbers was observed when alum alone was used as an adjuvant. *p<0.05 by Student's *t* test.

Summary of available approaches and associated goals to improve vaccines targeting the elderly population

Table 1

Approaches	Goals
Adding adjuvants (e.g. TLR agonists, oil-in-water emulsions)	<ul style="list-style-type: none">• Increase APC activation and PI cytokines production• Stimulate B cells for germinal center formation• Improve T cell responses
Changing the route of administration	Target APCs with optimal activation and antigen-presentation capacities
Increasing the dose of immunogen	Improve antigen-presentation, and thus, T cell activation
Changing the composition (e.g. select conserved epitopes such as influenza NP)	<ul style="list-style-type: none">• Generate immunity that withstand antigen drifts and shifts• Generate T cell and humoral immunity