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REVIEW

Adjuvanted vaccines against influenza in the elderly

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Abstract **Influenza is an important public health issue, especially with the aging of the population, since the most serious consequences of the illness affect the elderly. Between 1979 and 2001, approximately 41000 annual deaths have been attributed to influenza in the United States (Dushoff, 2005). Annual vaccination is a key strategy employed to combat this illness, and while it is very effective in healthy young adults, it is much less successful in the elderly. The impaired immune system with aging may contribute to this diminished ability of the vaccine to afford protection. Strategies to improve vaccine efficacy, particularly for the aged population, are necessary. One potential strategy is the inclusion of adjuvants in the vaccine formulations to enhance the immune response. Adjuvants have been shown to improve antibody production, allow dose-sparing, and potentially increase cross-reactivity. These benefits are important in combating both seasonal influenza and pandemic influenza, as current seasonal vaccine effectiveness depends on close matching to the circulating virus, and fast production of pandemic vaccines are key to their effectiveness. While much is still unknown about adjuvants, especially their mechanisms of action, their potential at improving the efficacy of influenza vaccines has been well recognized, particularly in the elderly.**

Keywords influenza vaccine, elderly, adjuvant

Introduction

Influenza disease, though seen as an innocuous illness to many people, is the sixth leading cause of death in the United States when combined with pneumonia (Vajo, 2011). While the influenza virus infects people of all ages, infants, individuals with underlying health problems, and the elderly are most severely affected. Over 90% of deaths related to influenza occur in individuals aged 65 years or older (Thompson et al., 2009). The 2009 H1N1 pandemic, though primarily affecting young, healthy individuals, still caused more death in aged people. In people aged 5-14 years, the attack rate was 43%, leading to a hospitalization rate of 0.84% (Chan et al., 2011). However, even though the attack rate was only 4% in people aged 50–59 years, the hospitalization rate surpassed the young group with 0.87%; case-fatality rates were much higher among the older population, with 26.5 deaths per 100000 infections in 5–59 year olds, compared to 0.4 per 100000 in 5–14 year olds (Chan et al., 2011). A primary strategy to combat morbidity and mortality due to influenza is vaccination. Older adults infected with influenza have a much higher risk of death than young people, reinforcing the importance of vaccination in this population.

Vaccination is an appropriate and effective way of preventing a large portion of influenza illness. Since the 1960s, the Centers for Disease Control (CDC) has recommended annual influenza immunization of individuals > 65 and individuals with underlying medical conditions. These guidelines have been modified over time, reducing the recommended age for vaccination to 50 years old, and including children and care-providers of at-risk groups. In 2010, for the first time, the CDC expanded their recommendation for vaccination from high-risk subgroups and people spending time around these groups (i.e., the elderly, health care workers, nursing home employees, etc.) to include all individuals over the age of 6 months. Unfortunately, in elderly populations, where the highest risk of death exists, vaccine efficacy is much lower than in other populations (Ruf et al., 2004; Lee et al., 2009). While vaccine efficacy is strong in young adults, showing between 70% and 90% protection (Podda, 2001), diminished efficacy is consistently found in elderly recipients, with varying reports of 5%–60% reduction in illness and 47%–80% reduction in mortality (Govaert et al.,

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1994; Podda, 2001). Antigenic mismatch between strains recommended for vaccine formulation and circulating viruses can further significantly reduce vaccine efficacy in the aged population (Banzhoff et al., 2009).

Higher reductions in mortality indicate that although vaccination is not ideal in the elderly population, it can help prevent more serious outcomes of morbidity or mortality; therefore, it remains an important, if not optimal, strategy to combat this public health problem. With the aging of the population, improving influenza vaccine efficacy for the elderly becomes an important goal. Because of both antigenic drift and antigenic shift, influenza vaccination must be repeated annually, to afford protection to the new genetic variant of the virus (Deans et al., 2010). Particularly in cases of antigenic shift, a new virus strain may emerge that the human population is more susceptible to. This necessitates the quick production and disbursement of an influenza vaccine specific to the new strain. Limits in production speed of the vaccine suggest that reducing the dosage of vaccine, or antigen in the vaccine, necessary to induce immunity will be a key factor in controlling pandemic influenza outbreaks.

Immunosenescence and immunization in the elderly

Aging leads to a diminishing of the overall immune response, known as immunosenescence, and specifically affects the adaptive immune response, which is responsible for establishing immunity after vaccination (McElhaney and Effros, 2009). The influenza vaccine works mainly by inducing antibodies against the viral antigens of the specific strains that make up the vaccine formulation. Antibody production is usually assessed through hemagglutination inhibition (HI) assays, and quantified in several ways, including post-vaccination geometric mean titers (GMT) of antibodies to the antigen, seroprotection rate (percent of people achieving titer, usually considered to be 40, sufficient for protection), and seroconversion rate (percent of people showing a 4-fold increase in titer) (Podda, 2001; Ruf et al., 2004; Camilloni et al., 2010). However, there has been growing evidence that the antibodies produced in elderly recipients, even if reaching titers that are protective in young adults, sometimes fail to afford protection from influenza in these recipients (Bernstein et al., 1999; McElhaney and Dutz, 2008; Camilloni et al., 2010). This decreased ability of antibodies to facilitate protection from disease in the aged population may be due to immunosenescence.

Immunosenescence also causes a decline in the function of $CD8^+$ cytotoxic T cells, which are necessary for clearing primary viral infections, including influenza (McElhaney and Effros, 2009). Clonal expansions of $CD28^-$ memory $CD8^+$ T cells may impair vaccine efficacy by affecting antigen presentation by dendritic cells, and decreased CD4:CD8 ratios have been associated with poor antibody responses to influenza vaccination (McElhaney and Effros, 2009).

The diminished antibody response in the elderly may make the $CD8^+$ T cell response more important (McElhaney and Dutz, 2008). In a cohort study of seasonally vaccinated recipients over the age of 60, *ex vivo* cell-mediated responses (IFN-γ: IL-10 ratio and granzyme B), but not antibody responses, correlated with protection from influenza (Deans et al., 2010). Measurements of antibody titers and seroconversions as determined by HI assays should, therefore, not be regarded as evidence of clinical protection in the elderly.

Finding ways to improve vaccines in order to overcome this immunosenescence is necessary to prevent influenza-related illness and death in the vulnerable elderly population. Several potential vaccine modifications include: increasing the dose of antigen (i.e., hemagglutinin HA) in the vaccine (Deans et al., 2010), administering a booster dose (Cheong et al., 2011), application of an immunostimulatory patch on the injection site to stimulate Langerhans cells (Neafsey and Tyler, 2005), changing route of delivery from intramuscular to intranasal (Deans et al., 2010) or intradermal (McElhaney and Dutz, 2008), using virosomes as vehicles of antigen presentation (Deans et al., 2010), or including adjuvants in the formulation (Deans et al., 2010; Podda, 2001; Khurana et al., 2011). Encouraging immunization of the non-elderly population to increase herd immunity is also a strategy that can help protect the at-risk elderly (Neafsey and Tyler, 2005).

When considering the benefits of these strategies, it is important to take into account the characteristics of the influenza virus and its epidemiology. In pandemic situations, as occurred in 2009, when the circulating flu virus is more deadly and the general population does not have natural immunity from pre-exposure, the quick production and distribution of vaccine is important to prevent excess morbidity and death. Standard subunit vaccines against the highly pathogenic H5N1 virus exhibited low immunogenicity even when used at high doses or with two or more injections (Treanor et al., 2006; Beigel et al., 2009), necessitating formula alterations to enhance immunity. Strategies which reduce the amount of antigen needed in each vaccine dose are advantageous; adjuvants have been shown to allow dose-sparing (Fragapane et al., 2010; Khurana et al., 2011).

Adjuvants for flu vaccines in the elderly

Adjuvants are substances included in vaccines to enhance the immune response. The immune response is not targeted at the adjuvant itself, but instead the adjuvant stimulates the non-specific innate immune system, which leads to an improved adaptive response to the specific antigen(s) of the vaccine (McKee et al., 2007; Lee et al., 2009). Viruses, in their natural forms, contain molecular patterns which the human immune system recognizes as foreign, referred to as pathogen-associated molecular patterns (PAMPs). The recognition of

particular PAMPs, such as viral RNA, by the innate immune system directs the appropriate response by the adaptive immune system and amplifies the immune response (McKee et al., 2010). Vaccines that do not contain natural PAMPS or other co-stimulatory substances require the addition of adjuvants to stimulate and amplify an immune response (McKee et al., 2007). The influenza vaccine is available in several different formats: a live, attenuated vaccine in which the PAMPS of the virus are present; an unadjuvanted inactivated vaccine, in which the influenza antigen, HA, is purified (but with some natural stimulatory molecules remaining), and, in some countries outside the United States, an adjuvanted inactivated vaccine.

Adjuvants have been considered as a possible strategy for enhancing the protection afforded by influenza vaccines in the elderly, by increasing antibody production (Podda, 2001). However, if the antibody titer produced during vaccination is not sufficient to neutralize the virus, the virus can enter cells, now requiring cytotoxic $CD8^+$ T cells to function. Vaccination with an adjuvant can help generate a $CD8^+$ T cell memory response, so that despite limited antibody function, $CD8^+$ T cells are capable of responding (McElhaney and Effros, 2009). Stimulating CTL memory with a vaccine could afford protection against other variations of influenza viruses, since CTLs respond to more conserved portions of the virus than antibodies. One adjuvant that is currently used with influenza vaccines, MF59, has been shown to increase cellular immune responses when used in clinical trials for HSV and HIV immunization (Podda, 2001); if this response can be replicated in the context of the influenza vaccine, it could increase cross-reactivity to other strains of influenza virus.

While the precise mechanisms of adjuvants are not entirely understood, possibilities include alterations in antigen presenting cells such as: increased APC recruitment to the vaccination site and uptake of antigen (McKee et al., 2010), improved maturation and migration to lymph nodes (Cheong et al., 2011) or enhanced cytokine production (McKee et al., 2010). Also, dendritic cells, activated by the adjuvant, can provide costimulatory signals to the CTLs. Stimulation of the immune system by the inclusion of poly I:C or inflammatory cytokines (TNF-α, IL-1, and IL-6) in an influenza inoculation in mice has also been shown to improve antibody affinity and maturation (McElhaney and Effros, 2009). Analysis of human serum pre- and post-vaccination with adjuvanted vaccines has shown improved antibody diversity, affinity, and magnitude of response. A possible mechanism for this action of the adjuvant is through its effects on naïve B cells by enhancing somatic hypermutation (Khurana et al., 2011).

Several different adjuvants including aluminum salts (alum), monophosphoryl lipid A (MPL), and AS04 (the combination of alum and MPL) are currently used in a variety of vaccines in the United States, though none are approved for use in influenza vaccines. Elsewhere including Europe, Australian, Canada, and Hong Kong, MF59 and AS03 are currently approved for use in seasonal or pandemic influenza vaccines (McKee et al., 2010). Other adjuvants are being tested in clinical trials. Alum had been be included in influenza vaccines in the 1960s and 1970s, but was shown to increase adverse effects while only minimally increasing immunogenicity of the vaccine, so was removed from the formulation. MF59 and AS03 are both squalene oil-in-water emulsions and have been shown to increase and sustain antibody titers to influenza vaccines (McKee et al., 2010). Squalene is an oil naturally produced in plants and animals, including humans, and is purified from shark liver for inclusion in many substances, including cosmetics, supplements, and food. Fears about squalene developed after a study indicated anti-squalene antibodies were the cause of autoimmune reactions in Gulf War veterans who had received an anthrax vaccine. However, a recent study demonstrated no association between squalene antibody status and the chronic multisymptom illness referred to as Gulf War Syndrome (Phillips et al., 2009). Now, it has been demonstrated that MF59 is a safe and potent vaccine adjuvant, and has been licensed for more than 13 years in Europe for use in an influenza vaccine for the elderly (Fluad®, Novartis, Cambridge, MA, USA) (O'Hagan et al., 2011; Vesikari et al., 2009).

In laboratory analyses of serum samples of immunized older adults, MF59-adjuvanted trivalent seasonal influenza vaccines have been shown to generate sufficient antibody titers, seroconversion rates, and seropositive rates to meet the established criteria for vaccines (Ruf et al., 2004; de Bruijn et al., 2006). The European criteria for influenza vaccines, as defined in the Committee for Medicinal Products for Human Use (CHMP) requires that 60% of recipients over age 60 must reach HI titers of 40 (seroprotection), seroconversion rate must exceed 30%, and mean geometric increase (fold increase of post-vaccination titers from pre-vaccination titers) must be greater than 2 (Ruf et al., 2004).

The antibody production requirements for vaccine efficacy have also been met by MF59-adjuvanted H1N1 2009 pandemic vaccines (Gasparini et al., 2010; Cheong et al., 2011) and H5N1 pandemic vaccines (Banzhoff et al., 2009; Fragapane et al., 2010). Studies of vaccines to pandemic influenza strains have shown decreased immunogenicity in humans, compared to seasonal vaccines, necessitating the inclusion of adjuvants to stimulate a sufficient immune response (Madhun et al., 2010). Numerous studies have been conducted to test the uses of adjuvants with the pandemic H5N1 and H1N1 vaccines (Fragapane et al., 2010; Madhun et al., 2010; Cheong et al., 2011; Khurana et al., 2011). Stronger immune responses can be stimulated with adjuvants using lower doses of antigen, enabling dose-sparing. This would enable more people to receive vaccinations to these viruses in a shorter time-span (Madhun et al., 2010; Khurana et al., 2011). Both of these results are especially important in

pandemic situations, where a large proportion of people are without prior protection to the new virus strain. The oil-in-water adjuvants AS03, MF59, and AF03 have all been shown to elicit antibody production to H5 when vaccinated with lower antigen concentrations than unadjuvanted vaccines (Madhun et al., 2010). Additionally, increased adjuvant-induced antibody affinity has been observed in young recipients and those with less previous exposure to the antigen, which has positive implications for the usage of adjuvants for emerging strains (Khurana et al., 2011). An important feature of MF59 is the ability of the emulsion to induce fast priming of influenza antigen-specific CD4 T cell responses, stimulate strong and long-lasting memory T and B cell responses, and broaden the immune response beyond the influenza strains included in the vaccine (O'Hagan et al., 2011). MF59 can quantitatively and qualitatively enhance functional antibody responses to HA-based vaccines by improving both epitope breadth and binding affinity, reflecting the value of adjuvanted influenza vaccines (Khurana et al., 2011).

Though antibody production is the measure used for approval of vaccines, it does not always correlate with protection, particularly in the elderly. Data directly showing protection in the elderly is a stronger indicator of the effectiveness of MF59-adjuvatanted vaccines. A case-control study was conducted on people over the age of 64 in Spain to examine how emergency hospital admissions for pneumonia related to seasonal influenza vaccination. Almost 95% of the vaccine recipients had received MF59-adjuvanted influenza. Influenza immunization showed a 48% effectiveness in preventing admissions for pneumonia, reducing the incidence from 3.3 admissions per 1000 unvaccinated elderly, to 1.71 per 1000 recipients (Puig-Barberà et al., 2004).

In direct comparisons with unadjuvanted influenza vaccines, formulations containing MF59 have repeatedly shown superior immunogenicity, with lower HA antigen concentrations. In a meta-analysis of 20 clinical trials conducted in elderly subjects, which compared the MF59-adjuvanted seasonal flu vaccine, FLUAD™, to conventional vaccines, significantly higher antibody responses were observed with the inclusion of MF59 (Podda, 2001). These results correlated with prior studies done in mice, in which the immune response of aged mice given the MF59-adjuvanted vaccine compared with that of young mice given non-adjuvanted vaccine (Podda, 2001). In a 2-dose administration of MF59-adjuvanted 2009 pandemic H1N1 vaccine testing lower doses of antigen, it was found that a single vaccination with 7.5 μg HA fulfilled criteria for licensing in recipients aged 65 and up, but a booster dosage enhanced the antibody response (seroconversion and seroprotection rates about 55% after prime, 90% after boost). A 3.75 μg vaccine was also tested, and though it did not meet criteria after one dose, its seroconversion and protections rates of about 78% after boosting surpassed that of the 7.5 μg vaccine after one dose. A direct comparison of these two adjuvanted formulations and a 15 μg HA-containing unadjuvanted vaccine was performed in subjects 50–64 years old, with only the 7.5 μg HA MF59-adjuvanted vaccine achieving vaccine acceptance criteria after one dose (all three did so after boosting) (Cheong et al., 2011).

Despite these optimistic results, local post-immunization reactions occur more frequently with the inclusion of MF59 in the influenza vaccines (Cheong et al., 2011; de Bruijn et al., 2006; Podda, 2001; Ruf et al., 2004). These adverse reactions are generally mild though, and occur less frequently in older recipients (Gasparini et al., 2010; Banzhoff et al., 2009). Systemic adverse reactions have not been found to be increased with MF59 (Cheong et al., 2011). In addition, subjects with chronic diseases receive greater benefit by using the adjuvanted vaccine, compared to healthy subjects, as measured by a higher pre-vaccination to post-vaccination fold increase in GMT with the adjuvanted vaccine (Podda, 2001). Having underlying health conditions is more common in the elderly and is a risk factor for more serious influenza illness and related complications. The increased immunogenicity that the adjuvant elicits in the elderly therefore increases the benefit:risk ratio, supporting the inclusion of the adjuvant.

In one study, however, an unadjuvanted seasonal vaccine showed non-inferiority when compared to the adjuvanted vaccine, both containing 15 μg HA (Ruf et al., 2004). This study agreed with the increased frequencies of overall and local adverse events with MF59, but interestingly indicated that systemic side effects were less prevalent (statistics not reported) in recipients of the adjuvanted form.

In addition to MF59, AS03 has been studied in clinical trials of pandemic influenza vaccines and has been shown to have similar results. A placebo-controlled, randomized trial tested the effectiveness of a two-dose AS03-H5N1 vaccination course, each dose containing 3.75 μg HA, given three weeks apart (Langley et al., 2011). Three weeks following the boost, licensing criteria for both US and European standards were met. In participants aged 65 + , the seroconversion and seroprotection rates were both about 74%. These exceeded criteria for seroconversion of $> 30\%$ and seroprotection of $> 60\%$ for 65 + age groups (Langley et al., 2011). Antibodies persisted when re-tested at 6 months after immunization. In another trial testing an AS03-adjuvanted vaccine to pandemic 2009 H1N1, 60 + year olds were administered one or two doses of vaccine containing 3.75 μg HA (Roman et al., 2010). Three weeks after the first dose, seroprotection was 87% and persisted at day 42 after immunization. Among the recipients given a second dose at day 21, seroprotection increased to 98% at day 42. Adults aged 18–60 years old were also included in the study, and had even higher immunogenicity rates. The 60 + age group reported lower frequencies of local (68% vs. 87%) and general (44% vs. 64%) adverse events than the 18–60 age group. These results demonstrate that AS03-adjuvanted flu

vaccines can enhance the antibody response to influenza virus in the elderly.

In a case-control study conducted in England, but only including patients with underlying chronic medical conditions, an AS03-H1N1 vaccine was found to have an overall effectiveness of 62%, when vaccination was at least two weeks earlier (Andrews et al., 2011). Substantial differences in effectiveness were found when stratified by age: 77% in children < 10 years (who only received half the vaccine dosage, containing 1.875 μg antigen), 100% in young adults (10–24 years), 22% in adults aged 25–49 years, and 41% in adults of 50 + years of age. Similar efficacies were seen when including subjects who had been vaccinated within one week, suggesting immunity may develop as early as 7 days post-immunization with an adjuvanted vaccine. The low protection rates in older adults with underlying conditions warrant more investigation, as these are the individuals most at risk for influenza and its complications.

Conclusions and future directions

Improving the efficacy of the influenza vaccine in the elderly is crucial with the current aging of the population. Since most deaths and severe illnesses affect the elderly, more excess deaths can be expected as the number of elderly increase. Vaccination is highly effective in young to middle-aged people; the immunosenescence that occurs with aging may inhibit its effectiveness. One potential strategy to improve protection in the elderly is to include adjuvants in the vaccine formulations. Adjuvants have been shown to improve antibody production, allow dose-sparing so more people could receive the vaccine, and potentially increase cross-reactivity and cellular immunity. These benefits are important in combating both seasonal influenza and pandemic influenza, as current seasonal vaccine effectiveness depends on close matching to the circulating virus, and fast production of pandemic vaccines is key to their effectiveness.

Further research is necessary to elucidate the mechanism of adjuvants and improve their ability to stimulate cytotoxic T cells, as these may be important in establishing immunity in the elderly. Aging causes a shift from Th1 responses, which help stimulate cytotoxic T cells in addition to antibody response, to Th2 responses, which only stimulate antibody response; since antibody titers do not seem to equal protection in the elderly, using adjuvants to reverse this shift in T helper responses may be advantageous. Also, since antibody titers, as measured by HI assays, are not good indicators of protection in the aged population, alternative assays for measuring effectiveness should be developed or used when vaccines are being developed and approved.

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