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MINI-REVIEW

The effect of adjuvants on vaccine-induced antibody response against influenza in aged mice

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Abstract **While influenza remains a major threat to public health, researchers continue to search for a universal solution to improving the efficacy of the influenza vaccine. Even though influenza affects people of all different ages, it can be extremely hazardous to people of 65 years of age or older since that is the population that makes up the high majority of the death toll caused by influenza-related diseases. Elderly individuals suffer the effects of immunosenescence as they age, which is the diminishing of the overall immune response. Immunosenescence occurs by specifically affecting the adaptive immune response which controls the establishment of immunity after vaccination or infection. There are many studies under way that are trying to find a resolution to the problem of the influenza vaccine not providing enough protection in the elderly population. One of the possible strategies is to seek the use of an optimal adjuvant, an immunological agent that can enhance immune responses, with the current vaccine formulation. Here, we used the murine model to review the effects of adjuvants on the antibody response to influenza vaccines in aged mice. Since adjuvants can enhance the production of important inflammatory cytokines and activation of dendritic cells, the stimulation of these cells are boosted to increase the effectiveness of the influenza vaccine in aged mice which would hopefully translate to the elderly.**

Keywords adjuvant, influenza vaccine, aged mice

Introduction

Today, influenza continues to be a major threat to the health of the public. Between 1976 and 2007, it was shown that over 20000 people annually die in the United States as a result of influenza-related diseases with deaths ranging from 3300 to 49000 (CDC, 2013 (1)). There was an increase in influenza activity during the 2012-13 influenza season, which was considered moderately severe, in the United States. Results point to higher rates of hospitalization, a higher percentage of outpatient visits for influenza-like illnesses, and more reported deaths associated with pneumonia and influenza than in recent years (CDC, 2013 (2)). Although influenza affects people of all ages, it is especially dangerous to elderly individuals, people aged 65 years or older, as they account for 90% of the influenza annual death-toll despite making up only 15% of the population (Thompson et al., 2009). Since influenza ranks as one of the leading causes of death in the United States, it is essential to take the necessary steps to try to prevent the disease. In the elderly, it is especially worrisome because diagnosing infectious diseases is more demanding due to non-specific clinical signs and symptoms that are usually complicated by multidrug resistance (Rappuoli et al., 2011; Goronzy and Weyand, 2013). Influenza is very unpredictable and severe in how it varies from one season to the next due to a host of different factors which include the type of virus spreading, how much vaccine is available, and how many people are actually getting vaccinated. As a result, in order to contain influenza, the current public health strategy is to vaccinate annually (CDC, 2013 (1)). This is especially recommended for the elderly and those in risk factor categories that represent high morbidity and mortality since they are extremely susceptible to infections (Chen et al., 2009). In the 2009 H1N1 pandemic, young, healthy adults were affected more by the virus. However, the virus still caused more deaths in aged people (Chan et al., 2011). This is because young people have a higher capability of generating a more effective immune response than older people. Elderly individuals have weaker

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immune systems which reduce their overall immune response making them more susceptible to diseases such as influenza (Dorrington and Bowdish, 2013).

Just as it is important to recognize that elderly individuals usually suffer more due to weaker immune systems, it is equally crucial to dig deeper into why this occurs. The early findings of preliminary studies point to decreased antibody levels in the elderly after being immunized with influenza (Chen et al., 2009). Naturally, this leads to further studies that wanted to determine if the problem of low antibody levels in the elderly could be solved by increasing antigen strength. When an individual reaches 65–70 years of age, complex changes start to take place in their immune system which affects the way their bodies respond to illnesses or vaccination. As a result, the challenge for many doctors and researchers is to find a novel strategy or new vaccine formulation that will assist these aging changes in the immune system to benefit the elderly population as it becomes more susceptible to infectious diseases (Dorrington and Bowdish, 2013).

Immune deterioration in the elderly

Immunosenescence, known as the degradation of the overall immune response that is associated with aging, is the main opponent of elderly individuals seeking to maintain good health (McElhaney and Effros, 2009). Despite the assumption that the immune system of elderly individuals loses its ability to generate a good immune response, it is inconsistent in its general decline. For example, some aspects such as the CD8 T cell polyfunctionality are maintained (Lelic et al., 2012) while others such as the pro-inflammatory cytokine production by macrophages are even heightened (Olivieri et al., 2013). Many point to the decreased efficacy of vaccination in the elderly and the enhanced susceptibility to infectious diseases as the result of the aging of the immune system starting at the level of the hematopoietic stem cell. As these cells age, their ability to engraft and proliferate starts to decrease (Van Zant and Liang, 2012). Coupled with a reduction in lymphoid precursors, this contributes to a sudden change of direction toward myeloid precursors. Despite the reason for this particular mechanism being unclear, it is widely believed to be due to a combination of exposure to chronic age-associated inflammation, DNA damage, telomere shortening, and epigenetic changes (Tollervey and Lunyak, 2012). In the end, this causes a reduction in circulating lymphocytes and a higher risk of pyogenic bacterial infections (Dorrington and Bowdish, 2013).

After vaccination, the adaptive immune response is accountable for reestablishing immunity in an aging individual. In order for the influenza vaccine to work, the formulation of the vaccine must consist of the induced antibodies against the antigens of the specific strains of that particular virus. Hemagglutination inhibition (HAI) assays allow for the assessment of antibody production, which can be counted in several ways. One of these methods is calculating the seroprotection rate by taking the percentage of people reaching a sufficient titer for protection which is typically around 40. Another instance is quantifying the geometric mean titers of antibodies to the antigen after the vaccination is performed. Also, the seroconversion rate can be used by showing the percentage of people that achieved a 4-fold increase in titer (Podda, 2001; Ruf et al., 2004; Camilloni et al., 2010). However, this is where we start to see critical differences when comparing the immune responses of young adults to those of elderly individuals. Recent studies have shown that even if the titers represented by the production of antibodies in elderly recipients reach levels that are protective in young adults, they can still sometimes be unsuccessful in affording protection from the influenza virus in these elderly adults. This shows the possibility of the role immunosenescence might play in the reduced capacity of antibodies to trigger protection for the aged population against infectious diseases (Fisher and Jiang, 2012).

The immune system contains an innate branch that allows for an individual to generate a rapid response, as well as a nonspecific response, to a conquering pathogen through the individual's pattern recognition receptors (Janeway and Medzhitov, 2002). It is known that the influenza virus likes to connect with innate RIG-I-like receptors, Nod-like receptors, Toll-like receptors, and innate signaling mediators (Diebold et al., 2004). Innate immunity is the reason for the origin of the adaptive response through the recruitment of immune effector cells in addition to initial pathogen clearance. Not surprisingly, this means that any deficiency that may be caused by aging in innate immunity can impact any correlating adaptive response negatively. Recent evidence continues to show that immunosenescence is directly responsible for numerous factors of innate immunity (Lambert et al., 2012).

Influenza vaccination in the elderly

The method of immunization for influenza consists of two different vaccines: a live attenuated influenza vaccine (LAIV) or a killed inactivated vaccine (TIV). While the live attenuated vaccine is administered intranasally, the killed inactivated vaccine is given intramuscularly. The LAIV can only administered to people ages 2 to 49 so it cannot be used on individuals 50 years of age or older. There is still relatively little evidence of which vaccine, the live attenuated or the killed inactivated, produces a more effective immune response to influenza. There was a slight trend of more fever among TIV receivers with influenza than those who contracted influenza after receiving LAIV, which was consistent with previous studies in elderly adults (Forrest et al., 2011). In another study, it was shown that when participants received a live attenuated virus vaccine in

addition to an inactivated trivalent influenza virus vaccine, they experienced an increase in anti-influenza A virus cytotoxic T cell activity than those participants who received a placebo along with the inactivated vaccine (Gorse et al., 1995). CD8 T cells brought about anti-influenza A virus cytotoxicity while remaining influenza A virus-specific as well as HLA-restricted. Another reason the inclusion of a live attenuated virus to participants was preferable was because they exhibited increased memory anti-influenza A virus cytotoxic T cell activity (Gorse et al., 1995).

Other steps have also been taken by investigators to seek an enhanced antibody response to influenza vaccination such as increasing the dosage of the trivalent inactivated vaccine (Deans et al., 2010). A high-dose influenza vaccine (Fluzone High-Dose) containing four times the antigen amount of the standard influenza vaccine was recently approved by the FDA (2010) for use by people over 65 to enhance their immune responses. Fluzone High-dose was approved in the United States via the accelerated-review pathway which allows for hastened availability for drugs that have advantages over existing treatments as long as further studies to demonstrate its efficiency are conducted (Sullivan et al., 2010). Promising results yielded that increased reactogenicity and higher antibody titers were correlated with increased doses. However, participants only received influenza vaccine formulations that contained the antigens from the previous year's vaccine, and the sample size tested was modest (Keitel et al., 2006). Reactions at the injection site and systemic adverse events are more frequent and severe compared with standard vaccinations. It has not been established, however, if this high-dose vaccine will significantly enhance protection against influenza in the aged population (Sullivan et al., 2010; Fiore et al., 2010).

Flu vaccine adjuvants

There is another option that is not included in any of the current influenza vaccines used in the United States- an adjuvant. An adjuvant is an agent that can help further enhance an immunological response when administered with the influenza vaccine. Many different adjuvants have been tested for efficacy in the quest to identify a successful adjuvant that would boost the immune response in aged mice (Rümke et al., 2013). Adjuvants perform through innate immune mechanisms and are responsible for increasing T cell-mediated and humoral responses to influenza vaccines, which leads to an improved adaptive response to the specific antigen(s) of the vaccine (McKee et al., 2007; Lee et al., 2009). Adjuvants can cause cell-mediated immune responses through the production of inflammatory cytokines as well as the activation and maturation of dendritic cells and increased antigen presentation. This is critical because the decline of the effectiveness of the influenza vaccine in the elderly correlates with a decrease in the excitation of cell-mediated and cytotoxic T-lymphocyte responses that are essential for providing protection against influenza. Adjuvants allow for the production of necessary inflammatory cytokines by acting as toll-like receptor ligands that initiate contact with receptors that are related to dendritic cells and other antigen presenting cells (McElhaney and Effros, 2009). There is an important need to develop an optimal formulation that will raise the immune response levels to influenza in the elderly since their immune system diminishes with aging (Franceschi et al., 2000). To search for these desired adjuvants, many researchers have turned to testing aged mice for experimentation.

Imject alum and poly I:C adjuvants

Currently, there are only a couple licensed adjuvants available for use in humans which includes aluminum salts (Alum), oil in water emulsions, and TLR4 ligand monophosphoryl lipid A (known under AS04). Two of these inflammatory agents, Imject alum and poly I:C, were tested alongside novel influenza virus-like particles (VLP) (Schneider-Ohrum et al., 2011). Imject alum originates from Nalp3 since its primary role is to stimulate for this Nod-like receptor. Poly I:C is a type 3 ligand that often acts as a toll-like receptor. Both of these adjuvants proved worthy of offering sufficient protection to aged mice when injected with a lethal dose of influenza virus challenge (Schneider-Ohrum et al., 2011). Only approximately 33% of the aged mice that were vaccinated with the novel influenza VLP survived the lethal dose challenge, while all aged mice vaccinated with the VLP plus adjuvants survived the challenge with a lethal dose (Schneider-Ohrum et al., 2011). Thus, the presence of adjuvants along with the novel influenza VLP allowed the immune system in aged mice to adapt and survive the influenza virus infection. Interestingly, the protection in aged mice is not associated with serum HAI antibody response, since both adjuvants boosted the VLP-induced HAI antibody titers in young adult mice, but not aged mice.

Cholera toxin B subunit (CTB) adjuvant

Some adjuvants are formulated to vaccines because of their ability to provide cross-protection against various strains of the virus infection. In this experiment, it was determined that the combination of the cholera toxin B subunit (CTB) and influenza HA vaccines generates serum IgG antibodies and anti-HA cross-reacting IgA antibodies (Asanuma et al., 2001). The latter antibodies are responsible for cross-protection in the upper respiratory tract against infecting viruses. Nasal-associated lymphoid tissue (NALT) is an interconnected network consisting of many different types of lymphoid and non-lymphoid cells that helps the upper respiratory tract to induce secretory IgA antibody responses. This is critical because adenoidectomy and human tonsillect-

omy reduce the secretory IgA in the nasopharynx. In addition, NALT is also involved in the systemic immune response based on the migration patterns of the lymphocytes since antiviral IgA antibodies have been shown to appear in the upper respiratory tract after the initial influenza virus infection. As a result, it was tested to see what the effects of the CTB adjuvant vaccine formulation would be on challenge virus protection, antibody responses, and primary antibody forming cells responses in the NALT in aged and young mice. It was shown that the CTB-combined influenza vaccine offered complete protection against infection in young mice while offering only partial protection in aged mice (Asanuma et al., 2001). A possible explanation is that the NALT-AFC responses may be a part of the mucosal immune responses. In certain aged mice, the downregulation of NALT-AFC responses and nasal IgA and serum IgG responses were evident. This implied that a vaccine formulation of a higher dose of what is given to young people should be administered to aged people in order to provide protection from influenza. Thus, this further shows the importance of finding an optimal adjuvant that could produce vaccine efficacy without relying on increasing dosage which would run the risk of running out of seasonal vaccine medication (Asanuma et al., 2001).

CRL1005 copolymer adjuvant

Another adjuvant used to boost the immune response generated by the influenza virus vaccine is a nonreactogenic adjuvant named CRL1005. The CRL1005 copolymer adjuvant is synthesized with propylene oxide and ethylene oxide since it is a surfactant-active nonionic block copolymer with a high molecular weight. Nonionic block copolymers are known to produce adjuvant activity depending on the factors of size and polyoxyethylene (POE). The amount of polyoxyethylene content that makes up the vaccination formulation is critical to the type of immune response produced as well as the actual amount of adjuvant activity. For example, copolymers with low content of POE increase the responses of mixed Type 1 and Type 2 helper T-lymphocytes whereas copolymers that have 10% POE typically enhance just Type 2 helper T-lymphocyte responses (Triozzi et al., 1997). Hence, CRL1005 is one of these copolymers with a 5% POE that has been evaluated as clinically safe and adjuvant-active in humans. After evaluating the results, it was shown that the aged mice failed to produce an optimal HAI antibody response either with the X-31 vaccine alone or with the copolymer-adjuvant included. Thus, the aged mice did not show any protection from influenza infection when challenged with a virus. However, the aged mice that received the CRL1005 copolymer adjuvant formulation did exhibit a significant decrease in lung virus titers after the challenge. After a second of the copolymer adjuvant vaccine formulation, the aged mice showed an increase in HAI antibody response suggesting that a single dose did no induce a detectable level of immunity by the HAI assay. As a result, it was found that there was increased protection from influenza infection due to the presence of an enhanced antibody response though it still did not reach levels of a young mice response. In addition to improving HAI antibody responses in aged mice, the CRL1005 copolymer adjuvant also improved IL-2 production and virus-specific IgG responses while reducing influenza infection in the lower and upper respiratory tracts of mice. This includes enhancing the effectiveness of the vaccine and the serum HAI antibody responses in aged mice that were infected with influenza prior to vaccination (Katz et al., 2000).

r*Ov*-ASP-1

There is another adjuvant that has shown promising results in our preliminary studies that gives hope toward finding an optimal adjuvant to formulate with the influenza vaccine. One protein with adjuvant potential is the *Onchocerca volvulus* activation-associated secreted protein (*Ov*-ASP-1) (Tawe et al., 2000). Recent studies have demonstrated that recombinant Ov-ASP-1 (r*Ov*-ASP-1) is a powerful immunostimulatory adjuvant which promotes a balanced Th1/Th2 antibody response and a Th1-biased cellular response to several vaccine antigens (MacDonald et al., 2005; Xiao et al., 2008). r*Ov*-ASP-1 has shown an ability to increase the influenza specific IgG response to the influenza vaccine in aged mice. When immunizing with TIV alone, only a marginal level of the antibody was found in aged mice as compared to the specific IgG that was detected in young adult mice. Nevertheless, when vaccinated with the TIV + r*Ov*-ASP-1 formulation, both young adult and aged mice significantly enhanced IgG levels. One of the critical discoveries of this experiment was that the level of IgG in aged mice after the adjuvant formulation (TIV + r*Ov*-ASP-1) was similar to the levels of IgG produced by young adult mice that were vaccinated with TIV alone. These preliminary results show that the adjuvant r*Ov*-ASP-1 can significantly increase the specific antibody response to influenza vaccine in young adult and, most importantly, aged mice (Jiang et al., 2014 unpublished data).

Another interesting result we found has shown that r*Ov*-ASP-1 performs better as an adjuvant than Alum when it comes to inducing the influenza specific IgG response in aged mice. While both r*Ov*-ASP-1 and Alum significantly increased specific IgG response to influenza vaccine, r*Ov*-ASP-1 showed a significantly higher enhancement of the titer of IgG when compared with Alum. There was also evidence of no significant difference in IgG levels between low and high doses of TIV given alone. This further backs the argument that increasing dosage of the vaccine will not increase the antibody response to the influenza vaccine in the elderly (Jiang et al., 2014 unpublished data).

Conclusion and future directions

As long as the influenza virus continues to exist and drastically affect the health of individuals everywhere, especially the elderly, it is critical to search for a universal solution that will provide consistent success when facing infectious diseases. As evidenced earlier in this review paper, improving the efficacy of the influenza vaccine should remain paramount among medical experts and researchers everywhere. It is increasingly important when considering that the era known as the "baby boomers" continues to age and enter the elderly stages of their lives where they are more susceptible to the influenza virus and its correlating diseases. One thing that we know for sure is that immunosenescence is an ongoing threat to the health and lifestyle of elderly individuals, and steps must be taken in order to combat the deficiencies it presents with an aging immune system.

A potential solution that could possibly prevent lethal aspects of the influenza virus and offer sufficient protection against it is the inclusion of an adjuvant with the influenza vaccine. Since adjuvants allow for the capability to produce enhanced antibody generation, they offer the aging immune system an opportunity to trigger a sufficient response against the influenza virus and its related diseases. Along with the obvious health benefits, adjuvants would be advantageous economically since the volume of doses would not need to be as high, thus preventing the unnecessary waste of resources. One strategy that should be further investigated is the search for an optimal, universally used adjuvant to combine with vaccine formulations for the elderly population. Several different adjuvants should be tested among similar aged mice populations in order to determine which adjuvant consistently produces an efficient, effective immune response. Future studies could also entail a full review of the specific mechanisms of action of corresponding adjuvants to wholly understand their potential to benefit the prevention of influenza viruses. This would go a long way toward fulfilling the quest of finding a suitable vaccine formulation that would successfully protect elderly individuals from the harsh realities of immunosenescence and influenza infectious diseases.

Compliance with ethics guidelines

Mark A. Conannon and Jiu Jiang declare that they have no conflict of interest.

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