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REVIEW ARTICLE

A Molecular Mucosal Adjuvant To Enhance Immunity Against Pneumococcal Infection In The Elderly

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Streptococcus pneumoniae (the pneumococcus) causes a major upper respiratory tract infection often leading to severe illness and death in the elderly. Thus, it is important to induce safe and effective mucosal immunity against this pathogen in order to prevent pnuemocaccal infection. However, this is a very difficult task to elicit protective mucosal IgA antibody responses in older individuals. A combind nasal adjuvant consisting of a plasmid encoding the Flt3 ligand cDNA (pFL) and CpG oligonucleotide (CpG ODN) successfully enhanced *S. pneumoniae*-specific mucosal immunity in aged mice. In particular, a pneumococcal surface protein A-based nasal vaccine given with pFL and CpG ODN induced complete protection from *S. pneumoniae* infection. These results show that nasal delivery of a combined DNA adjuvant offers an attractive potential for protection against the pneumococcus in the elderly.

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INTRODUCTION

In higher mammals, the mucosal immune system consists of an integrated network of tissues, lymphoid and mucous membrane-associated cells and effector antibody (Ab) molecules. Along with cytokines, chemokines and their receptors, these effector Ab molecules, which are primarily of the IgA isotype, are key players in mucosal immunity and appear to function in synergy with innate host factors (1,2). Thus, in order to induce antigen (Ag)-specific immune responses at these mucosal barriers, one must consider the common mucosal immune system (CMIS), which supports the concept of distinct mucosal IgA inductive and effector tissues (1,2). The Waldeyer's ring of tonsils and adenoids as nasopharyngeal-associated lymphoid tissues (NALT) serve as mucosal inductive sites. NALT is covered by a lymphoepithelium containing microfold (M) cells and well organized regions, the subepithelium with enriched Ag-presenting cells (APCs), a B cell zone with germinal centers (GCs), and adjacent T cell areas including an equal distribution of naïve and memory T cell phenotypes (1,2). Upon Ag-activation, memory B and T cell populations then emigrate from the mucosal inductive environment via lymphatic drainage, circulate through the bloodstream, and home to mucosal effector sites where abundant IgA producing plasma cells are present.

Effector sites for mucosal immune responses include the lymphoid cells in the lamina propria (LP) of the upper respiratory (UR), and reproductive tracts as well as secretory glan-

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Abbreviations: CMIS, Common Mucosal Immune System; CpG ODN, CpG oligodeoxynucleotide; GCs, germinal centers; LP, Lamina propria; M cells, Microfold cells; NALT, Nasopharyngeal-associated lymphoid tissues; PPs, Peter's patches; pFL, Plasmid encoding Flt3 ligand cDNA; PPV, Pneumococcal polysaccharide vaccine; PspA, Pneumococcal surface protein A; pIgR, Polymeric Ig receptor; SIgA, Secretory IgA; UR, Upper respiratory

dular tissues (1,2). Ag-specific mucosal effector cells include IgA-producing plasma cells as well as mature B and T lymphocytes. Secretory IgA (SIgA) is the primary Ig involved in protecting mucosal surfaces and is locally produced in effector tissues (1,2). In this regard, the majority of T and B cells in effector tissues are activated and express a memory phenotype (1,2). Although it has been shown that NALT shares common features with gastrointestinal (GI) inductive tissues such as Peyer's patches (PPs), their involution during immunosenescence, development during organogenesis and lymphocytes trafficking are distinctly regulated (3-20). Thus, the age-associated alteration including a reduction in PPs, intestinal Ag-specific SIgA Ab responses and lack of oral tolerance induction occur during the aging process (by 6∼12 months in mice) (6,7,11,12). In contrast, NALT function remains intact during aging with notable signs of immunosenescence seen only in the elderly (2-year-old mice) (6,7,9).

In this short review, we summarize the potential for the development of a safe and effective nasal vaccine to prevent pneumococcal infection in the elderly.

Novel vaccines and delivery systems for the prevention of pnuemonoccal infection

In order to prevent pneumococcucal infection, nasal immunization strategies have been employed using novel vaccines and delivery systems. It has been shown that PspA plus IL-12 as nasal adjuvant enhanced PspA-specific IgG and IgA Ab responses with increased protection from nasal carriage (21). In this regard, the safety of nasal application of IL-12 was evaluated by using *ex vivo* human bronchoalveolar lavage (BAL) cells (22). These studies suggest that IL-12 could be used in clinical trials for pneumococcal protein-based vaccines although additional safety evaluations may be required. In addition to classical admixtures of adjuvants and pneumococcal Ags, PspA-fusion proteins have also been used as nasal vaccines. Nasal immunization with recombinant fusion proteins consisting of PspA and FlaB (*flagellin of Vibrio vulnificus*) resulted in cross-protective mucosal immunity (23). Others showed that mice vaccinated with human Fcγ receptor type I (FcγRI)-specific humanized single-chain Ab linked to PspA showed complement- and lactoferrin-mediated anti-pneumococcal innate immunity (24).

In more recent developments, nano-technology has been introduced in order to improve pneumococcal vaccines. Thus, nasal immunization with chitosan-DNA nanoparticles expressing pneumococcal surface antigen A (PsaA) elicited protective immunity against nasal colonization by *S. pneumoniae* (25). More recently, nanogels containing pneumococcal surface protein A (PspA) alone induced PspA-specific mucosal IgA Ab and Th17 responses which contributed to protection from *S. pneumoniae* infection in the lower respiratory tract (26). Further, *Lactobacillus casei* and *Salmonella enterica* serovar Typhimurium-derived outer membrane vesicles were employed as a nasal delivery system for pneumococcal surface protein Ags and successfully elicited protective immunity against challenge with *S. pneumoniae* (27,28). Similarly, a bacteria-based, nasal delivery approach was performed using live attenuated *S. pneumoniae* (29-31). Nasal delivery of *S. pneumoniae* which possessed a mutation in pep27, an autolysis-inducing factor, or deletion of ftsY, the single recognition pathway component, resulted in serotype-independent protection (30,31). Most recently, the live attenuated SPY1 vaccine strain induced both specific Ab and Th17 responses for protection form *S. pneumoniae* infection (29). Despite these advanced vaccine strategies which provide significant protection from pneumococcal infection, their effectiveness and safety have never been evaluated for the elderly.

Secretory IgA Abs play an important role in immunity to *Streptococcus pneumoniae*

The highest incidence of influenza and pneumococcal diseases occur in humans over 65 years of age. Thus, severe illness and mortality caused by pneumococci sharply increases in the elderly (32). The development of effective vaccines for the elderly remains a largely unmet goal. The effectiveness of the currently licensed pneumococcal polysaccharide vaccine (PPV) for adults consists of capsular polysaccharides derived from 23 serotypes (PPV23); however, its effectiveness becomes markedly reduced at ages above 75 years (33,34). It has been previously reported that older adults have a less effective Ab response to PPV23 than do their younger counterparts (35-37). In this regard, one should strongly consider developing a new generation of vaccines that could induce pneumococcal-specific IgA immunity in the respiratory tract since it has been shown that IgA plays a central role in immune defense, controlling the spread of respiratory pathogens including *S. pneumoniae*. Indeed, nasal immunization with PspA and cholera toxin as adjuvant elicited PspA-specific mucosal IgA and systemic IgG Ab responses and provided protection against carriage of *S. pneumoniae* and pneumococcal infection (38-40). Further, it was reported that *S. pneumoniae* capsular polysaccharide-specific polymeric IgA

Abs more efficiently initiated complement-mediated killing of the organism (41). Others demonstrated that polymeric Ig receptor deficient (pIgR$^{-/-}$) mice which lack the ability to actively secrete polymeric SIgA Abs onto mucosal surfaces, failed to provide protection against nasal challenge with *S. pneumoniae* despite being given a mucosal vaccine which induced SIgA Abs in normal mice (42-44).

Our studies provide more direct evidence that PspA-specific SIgA Abs play a key role in the prevention of bacterial colonization of the nasal mucosa using IgA deficient (IgA$^{-/-}$) mice. Thus, although IgA$^{-/-}$ mice given nasal PspA plus plasmid encoding Flt3 ligand cDNA (pFL) revealed significantly higher levels of PspA-specific IgG Ab responses, and high numbers of *S. pneumoniae* CFUs were detected in the nasal mucosa. In contrast, vaccinated, wild type mice showed essentially no bacteria in the nasal cavity. Further, a nasal vaccine consisting of PspA plus pFL effectively reduced pre-existing *S. pneumoniae* in the nasal cavity (45). The importance of IL-17 synthesis for protection against pneumococcal infection (46-48). Although IgA$^{-/-}$ mice given nasal PspA plus pFL exhibited increased levels of IL-17 producing CD4^{+} T cells as did IgA$^{+/+}$ mice, this mutant mouse strain failed to prevent colonization by *S. pneumoniae*. These results show that a PspA-based vaccine induced specific SIgA Abs plays a necessary role in the regulation of *S. pneumoniae* colonization in the nasal cavity.

Novel vaccine adjuvant development for use in the elderly

The results obtained in IgA$^{-/-}$ mice were very similar to the immune responses seen in aged mice which failed to elicit Ag-specific SIgA Ab responses in the mucosal lymphoid tissues despite significant IgG Ab induction (9). Thus, it is essential that we continue to explore new avenues for effective pneumococcal vaccine development for the elderly which include the induction of specific SIgA Ab responses. Adjuvant systems have provided significant improvement in the development of vaccines for another respiratory pathogen, i.e., the influenza virus vaccines for the elderly (49-51). Thus, a combination of alum and poly I:C as an adjuvant enhanced the effectiveness of an influenza virus-like particle vaccine in aged mice (51). Further, an H5N1 vaccine with MF59 adjuvant induced a rapid rise in broadly cross-reactive Abs as well as long-lived human memory B cells (49). More recently, the AS03 adjuvant system (Squalene, DL-α-tocopherol and polysorbate 80, GlaxoSmithKline) improved the immune response to inactivated 2009 H1N1 influenza vaccine in both healthy (18～64 years) and older adults (> 65 years) (50).

CpG oligodeoxynucleotide (CpG ODN) as vaccine adjuvant has been shown to restore Ag-specific immune responses to ovalbumin (OVA), diphtheria toxoid, hepatitis B, polysaccharide of *S. pneumoniae*, amyloid β and tumor cells in aged mice and rats (52-58). When 3 month old (young adult) and 18 month-old (moderately aged) mice were orally immunized with OVA plus CpG ODN as adjuvant, both groups of mice showed high and equivalent levels of OVA-specific systemic IgG and mucosal IgA Ab responses (59). Furthermore, we have shown that a combination of nasal pFL and CpG ODN selectively increased the frequencies of CD11c^{+} dendritic cells (DCs) in the nasal mucosa of young adult mice. Thus, absolute cell numbers of CD8^{-} B220^{+} plasmacytoid DC (pDC) and CD8^{+} B220^{-} non-pDC populations were preferentially increased by use of the combined adjuvant regimen (60). Although native cholera toxin (the most potent nasal adjuvant) failed to induce OVA-specific SIgA Ab responses in 2-year-old mice (9), nasal immunization with 100 μg of OVA and a combination of 50 μg of pFL and 10 μg of CpG ODN in aged mice resulted in significant SIgA Ab responses in external secretions, which were essentially equivalent to those observed in young adult mice (60). These results clearly indicate that a combination of pFL and CpG ODN can be used as a potent nasal adjuvant system for the induction of mucosal Ab responses in aged mice (60,61). In addition, it has been shown that the effect of aging on IL-2 production was abrogated by exogenous IL-2 delivery (62). Thus, mucosal IL-2 treatment reversed age-impaired mucosal immune responses by enhancing mucosal immunity or abrogating unresponsiveness in aged mice (63). Furthermore, recent studies showed that keratinocyte growth factor or IL-7 treatment prevented thymic atrophy (64,65). IL-15 treatment also restored impaired DC function in mesenteric lymph nodes of aged mice (66). Taken together, we hypothesize that mucosal delivery of DC-targeting pFL and CpG ODN as a mucosal adjuvant, would elicit Ag-specific SIgA Ab responses in the elderly against *S. pneumonia* infection.

A nasal combined adjuvant elicits protective siga immunity to *S. pneumoniae* in aged mice

Since a combination of pFL and CpG ODN was a potent nasal adjuvant in aged mice, we next examined whether this double adjuvant system could successfully induce bacterial antigen i.e., PspA-specific SIgA Ab responses in the UR tract mu-

cosa for prevention of both *S. pneumoniae* carriage and infection. Nasal immunization with PspA plus a combination of pFL and CpG ODN elicited elevated levels of PspA-specific SIgA Ab responses in external secretions and plasma in both young adult and aged mice (61). Significant levels of PspA-specific $CD4^+$ T cell proliferative and PspA-induced Th1- and Th2- but not Th17-type cytokine responses were noted in NALT and cervical lymph nodes of aged mice (61). In addition, increased numbers of mature-type CD8 or CD11b-expressing DCs were detected in mucosal tissues of aged mice (61). Importantly, aged mice given PspA plus a combination of pFL and CpG ODN showed protective immunity against nasal *S. pneumoniae* colonization (61). In contrast, both aged and young adult mice given nasal PspA alone fail to provide sufficient protection after nasal challenge. Thus, high numbers of *S. pneumoniae* CFUs were seen the NWs and NPs of both groups of mice. Further, aged mice given PspA plus pFL or CpG ODN revealed high numbers of bacterial CFUs in both NWs and NPs. The numbers of *S. pneumoniae* CFUs were essentially the same as those mice given PspA alone. (61). These results demonstrate that nasal delivery of a combined DNA adjuvant offers an attractive possibility for protection against *S. pneumoniae* in the elderly.

CLOSING REMARKS

Our focus has targeted the NALT for development of nasal vaccines containing a novel adjuvant combination which stimulates mucosal DCs in order to elicit pathogen-specific SIgA Ab responses in the elderly. Our studies have shown that vaccine/adjuvant-triggered NALT DCs from aged mice possess normal APC functions and thus fully support Ag-specific $CD4^+$ T cell responses. In particular, nasal immunization with PspA given with pFL and CpG ODN elicited increased levels of Ag-specific SIgA Abs for subsequent protection from *S. pneumonmiae* infection in aged mice. The elderly have already experienced numerous UR infections and have received prior vaccines. Thus, although they possess specific Abs, their titers are both too low and too clonally restricted to effectively combat reinfection. In the future, it would be of great benefit to the aged population if one could use pFL and CpG ODN alone, in order to immediately enhance pre-existing mucosal immunity which had developed in response to past respiratory infections and/or deliberate (annual) vaccination. Thus, subsequent nasal vaccine boosting could then more effectively expand pathogen-specific memory T and B cell responses to provide prolonged protection.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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