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The clinical use of adjuvants in pneumococcal vaccination

Current status and future perspectives

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Pneumococcal infection disproportionately affects infants, elderly and persons with chronic diseases such as HIV and cancer. Healthy infants normally mount a protective antibody response following pneumococcal conjugate vaccination, but persons in other risk groups are often hypo-responsive to immunization with the licensed pneumococcal polysaccharide and pneumococcal conjugate vaccines. The addition of vaccine adjuvants can potentially overcome hypo-responsiveness in these individuals by augmenting the innate and adaptive immune responses. Vaccine adjuvants may also enhance the immunogenicity of highly purified or recombinant antigens, lead to antigen sparring and reduce the number of immunizations required to achieve protective immunity. Numerous adjuvants have been tested in conjunction with pneumococcal polysaccharides and protein-conjugated polysaccharides in animal immunization studies. However, only a small portion of these adjuvants has progressed to clinical trials. This commentary looks at the clinical experiences with the use of adjuvants in pneumococcal immunization and discusses future perspectives for the development of adjuvanted pneumococcal vaccines.

Introduction

Streptococcus pneumoniae is one most prevalent bacterial infectious organism with a major effect on public health in both industrialized and developing countries.^{1,2} *Streptococcus pneumoniae* mainly causes disease at the extremities of ages, in the very young (<2 yrs) and in the elderly

(>65 yrs).²⁻⁴ Ninety-one different capsular serotypes can cause pneumococcal disease such as pneumonia, sepsis or meningitis and pneumococcal infections kill more people than all other vaccine-preventable diseases combined.^{3,5,6} In developing countries, one out of six childhood deaths is attributed to pneumococcal infections.⁷ Currently licensed vaccines are based on capsular polysaccharides that induce immune protection primarily via opsonophagocytic antibodies.

Vaccine Hypo-Responsiveness in High-Risk Groups

More than six decades after the first publication of pneumococcal vaccination, the effectiveness of pneumococcal polysaccharide vaccines (PPV) in adults at increased risk of pneumococcal infection (e.g. splenectomized, cancer, and HIV patients) remains controversial.^{8,9} Vaccine failures—acquiring infection with vaccine-serotypes following PPV immunization—are relatively common in the literature.¹⁰⁻¹³ Furthermore, several randomized trials have questioned the efficacy of PPV and systematic reviews and meta-analyses do not demonstrate a consistent protective effect from the vaccine.^{14,15} The lack of PPV efficacy has prompted research into other vaccine formulations for high-risk populations, such as pneumococcal conjugate vaccines (PCV). Plain (unconjugated) polysaccharide vaccines are not efficacious in children less than two years old.⁷ To recruit CD4⁺ T cells for antibody responses against capsular polysaccharides, exogenous CD4⁺ T cell epitopes must be provided,

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usually in the form of a carrier protein. Pneumococcal conjugate vaccines (PCV) were developed for use in infants. In PCV, modified or unmodified immunogenic bacterial proteins are used as carriers coupled to capsular polysaccharides. PCVs are highly effective in reducing pneumococcal disease in children.¹⁶ Further, in a recent trial, 7-valent PCV (Prevenar®, Pfizer) was protective against vaccine serotype-specific invasive pneumococcal disease in a cohort consisting primarily of HIV-infected Malawian adolescents and adults who had recovered from documented invasive pneumococcal disease.¹⁷ While this was the first trial showing clinical efficacy of PCV among adults, concerns about partial coverage, duration of immunity and serotype replacement,¹⁸ as well as the cost and complexity of manufacturing multivalent pneumococcal conjugate vaccines, persist. This conflict highlights the critical need for alternative approaches to pneumococcal vaccination. Many of these alternative techniques include the use of vaccine adjuvants.

Adjuvants

Adjuvants augment the innate immune responses to vaccine antigens; they have been used in immunization since the 1930s. Adjuvants are used for several purposes including immunogenicity enhancement of highly purified or recombinant antigen, antigen sparring, reduction of the number of immunizations required for protective immunity and improvement of vaccine efficacy in immunocompromised persons.¹⁹ Alum salts, primarily aluminum phosphate or hydroxide, are the most widely used adjuvants in human vaccines. Alum salts cause antigen depot formation at the injection site, resulting in the slow release of antigen from local tissues.¹⁹ This in turn increases the duration of antigen interaction with the immune system and boosts complement, eosinophil and macrophage activation, as well as increasing antigen uptake by antigen presenting cells.²⁰ In the widely used 7/13-valent PCV (Prevenar), protein-conjugates are absorbed on aluminum phosphate. Unfortunately, alum salts are relatively weak adjuvants and have little or no adjuvant effect when co-administered

with type II T cell-independent antigens such as pneumococcal polysaccharides.²¹ As a consequence, numerous other adjuvants have been tested in conjunction with pneumococcal polysaccharides and protein-conjugated polysaccharides in animal immunization studies. However, only a small portion of these adjuvants has progressed to clinical trials.

Clinical Experience with Adjuvanted Pneumococcal Vaccination

PPV-14 and indomethacin. Indomethacin is a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis.^{22,23} Animal studies demonstrate prostaglandin-induced inhibition of both cellular and humoral immune responses, and a reduction in prostaglandin's immune modulatory effect with indomethacin treatment. In 1981, Lafferty and coworkers²³ investigated whether indomethacin administration would augment the antibody response to pneumococcal vaccination in a group of healthy elderly individuals (Table 1). However, the authors found no significant difference in mean increases in antibody levels to 12 pneumococcal polysaccharide types between the 20 subjects who received indomethacin 25 mg q.i.d. for five days after immunization versus controls.

PPV-23 and recombinant human interleukin-12 (rhIL-12). Interleukin (IL)-12 is a potent inducer of cytokine production, in particular T cell secretion of interferon- γ . IL-12 also enhances the production of certain antibody isotypes in response to exogenous antigens.^{24,25} In a study by Hedlund and colleagues,²⁶ co-administration of rhIL-12 and PPV-23 was tested in healthy immunocompetent volunteers. Subjects were randomized to PPV-23 and concurrently treated subcutaneously with either rhIL-12 (1 or 4 μ g) or placebo. Serum anti-pneumococcal antibody titers were measured prior to vaccination and at weeks 4 and 8 after vaccination. Co-administration of rhIL-12 did not produce a significant general upregulation of antibody production, a shift in the isotype profile, or an increase in antibody avidity. Further, the observed

frequency and severity of study treatment-related complications in relation to rhIL-12 were unacceptable according to the authors.

PPV-23 and QS-21. QS-21 is a saponin-based adjuvant isolated from the bark of *Quillaja saponaria* Molina. QS-21 stimulates both Th1 immune responses and the proliferation of cytotoxic T-lymphocytes (CTLs) against exogenous antigens.^{27,28} Two unpublished trials compared vaccine-specific antibody responses to QS-21 adjuvanted PPV-23 (Quilimmune-P, Antigenic) and PPV-23 immunization alone.^{29,30} More than 400 elderly adults were enrolled. The investigators found no consistent, statistically significant differences between Quilimmune-P formulations and the control vaccine in the induction of two-fold or greater increase in antibody titer following a single dose of vaccine. Following these negative results further development of Quilimmune-P was stopped.

PCV-9 and monophosphoryl lipid A (MPL). MPL® (Corixa) adjuvant is a chemically modified derivative of lipopolysaccharide (LPS). MPL has less toxicity than LPS while maintaining its immuno-stimulatory potential primarily through Toll-like receptor 4 (TLR4) activation.³¹ Vernacchio and colleagues immunized healthy toddlers with two doses of nine-valent pneumococcal-CRM197 protein conjugate vaccine (PCV9) combined with 10, 25 or 50 μ g of MPL with or without alum. Thirty days after the second dose of study vaccine there were no differences in vaccine-specific IgG titers between the MPL and non-MPL groups. However, the investigators did find enhanced CRM197-specific T cell proliferation and interferon- γ production in the 10 μ g MPL group compared with controls who received PCV9 with alum. The study thus confirmed that MPL stimulates TH1-responses to the carrier protein, but the significance of this finding remains unknown and further development of MPL-adjuvanted PCV has not been pursued.

PPV-23 and granulocyte-macrophage-colony-stimulating factor (GM-CSF). GM-CSF is a powerful chemoattractant for neutrophils and enhances the microbicidal activity, oxidative metabolism and

Table 1. Overview of human trials which have investigated the effectiveness of various adjuvants in pneumococcal vaccination

Vaccine	Adjuvant	Action	Administration (vaccine/adjuvant)	Trial population	n	End of trial	Effect on IgG response	Reference
PPV-14	Indomethacin	Non-selective COX inhibitor	IM/oral (concurrent)	Elderly	20	1981*	None	Lafferty ²³
PPV-23	RhIL-12	IL-12 agonist	IM (concurrent)	Adults aged 55–64	34	1998	None	Hedlund ²⁶
PPV-23	QS-21	Lytic/DC maturation	IM (mixed)	Adults aged +65	400+	2002	None	<i>Press release</i> ^{29,30}
PCV-9	MPL	TLR2/4 agonist	IM (mixed)	Toddlers	129	2002*	None	Vernacchio ³²
PPV-23	GM-CSF	Granulocyte-macrophage-colony-stimulating factor	IM/SC (concurrent)	Adults with CLL	32	2006	None	Safdar ³³
PPV-23	Abatacept	Selective T cell co-stimulatory modulator	IM/IV (concurrent)	Adults aged 18–65	80	2007*	None	Tay ³⁴
PPV-23	CPG 7909	TLR9 agonist	IM (mixed)	Adults with HIV	96	2009	None	Søgaard ³⁶
PCV-7	CPG 7909	TLR9 agonist	IM (mixed)	Adults with HIV	96	2009	Positive effect	Søgaard ³⁶

*Year of publication—no published trial dates. PPV-14/23, 14- or 23-valent pneumococcal polysaccharide vaccine; PCV-7/9, 7- or 9-valent pneumococcal conjugate vaccine; IM, intra-muscular; IV, intravenous; SC, subcutaneous; CLL, chronic lymphatic leukemia.

phagocytotic activity of both neutrophils and macrophages.²⁴ In a clinical study, multiple-doses of GM-CSF were administered in an attempt to improve responses to PPV-23 in patients with chronic lymphocytic leukemia.³³ Patients received PPV-23 either alone or with three doses of GM-CSF (250 µg) given either before or after vaccination. Serum antibodies were measured prior to vaccination and at four weeks and 12 weeks after vaccination. GM-CSF was well tolerated, but there were no differences in geometric mean IgG levels between any of the three groups at four weeks or 12 weeks after vaccination. Thus, at least in this patient population the effect of GM-CSF as adjuvant to PPV-23 was negligible.

PPV-23 and abatacept. Tay and co-workers tested the effect of a single infusion of abatacept (Orencia®, Bristol-Myers Squibb), a selective T-cell co-stimulation modulator, on the antibody response to PPV-23 and tetanus toxoid vaccine among healthy volunteers.³⁴ Subjects were randomized to receive one of four treatments: (1) vaccines on day 1 only (control group); (2) vaccines 2 weeks before abatacept; (3) vaccines 2 weeks after abatacept; and (4) vaccines 8 weeks after abatacept. Anti-pneumococcal IgG titers were measured 14 and 28 days after vaccination. Approximately 70% of abatacept-treated subjects responded to at least three pneumococcal serotypes compared to 100% of

control subjects. Approximately 25–30% of abatacept-treated subjects versus 45% of control subjects responded to at least six serotypes. The lowest titers were found among subjects who were vaccinated two weeks after receiving abatacept. Hence, abatacept blunts the immune response to PPV-23.

PCV-7, PPV-23 and CPG 7909. CPG 7909 is a synthetic oligodeoxynucleotide (ODN) containing CpG motifs that activate human B cells and plasmacytoid dendritic cells via toll-like receptor 9 (TLR9), which promotes antigen-specific antibody secretion.³⁵ In a clinical trial, HIV-positive patients were randomized to receive double doses of PCV-7 (Pneumovax) at 0 and 3 months and 1 dose of PPV-23 at 9 months, with experimental patients receiving 1 mg of CPG 7909 added to each of their three vaccine doses. Immunogenicity and safety were evaluated for up to 10 months.³⁶ The addition of CPG 7909 to PCV-7 doubled the proportion of subjects achieving a high vaccine-specific IgG antibody response at 9 months (pre-PPV-23 immunization). The TLR9 agonist-adjuvant also induced a more durable antibody response of higher magnitude than administration of PCV-7 alone. Subsequent boosting with PPV-23 resulted in increased production of antibodies with higher opsonophagocytic activity in the experimental group compared with the control group. However, mild systemic and injection-site

reactions to PCV-7 were more common in the experimental group. The addition of CPG 7909 to PPV-23 did not enhance the antibody response to non-PCV-7 serotypes but increased the incidence of moderate to severe influenza-like reactions. Thus, this study was the first human trial to document a significant positive effect on antibody production from an adjuvanted pneumococcal conjugate vaccine.

The Nature of Pneumococcal Polysaccharides May Explain the Failure of Adjuvants in Combination with PPV-23

As highlighted above, human trials of pneumococcal polysaccharide vaccines combined with adjuvants have produced disappointing results. It seems unlikely that any of these strategies in their present form will be brought forward in more advanced clinical trials. The reason for the apparent failure of potent adjuvants to improve PPV immunogenicity may be related to the nature of carbohydrate antigens.^{37,38} Pneumococcal polysaccharides are type II T cell-independent (TI-II) antigens characterized by large molecular weight, repeating antigenic epitopes, ability to activate the complement cascade, poor in vivo degradability and inability to stimulate MHC class II-dependent T cells.^{38,39} B-cell receptor crosslinking through binding of repetitive

motifs activates antigen-specific B cells independent of CD4⁺ helper T cells. Such T cell-independent responses are less robust, short-lived and primarily consist of immunoglobulin M (IgM) antibodies. The adjuvants mentioned above do not activate naïve B cells directly; rather they target T cells, memory B cells and antigen presenting cells. For instance, CPG 7909 activates plasmacytoid dendritic cells and mature B cells via the intracellular pattern recognition receptor TLR9.³⁵ TLR9 agonists are very potent TH1 adjuvants whose excellent adjuvant potential in combination with other vaccines such as the hepatitis B vaccine has been demonstrated.⁴⁰ Interestingly, CPG 7909 adjuvanted PPV-23 significantly enhanced IgG and OPA titers for PCV-7 serotypes (for which trial participants had been primed), but the combination failed to boost antibodies directed towards non-PCV-7 serotypes compared with PPV-23 immunization alone. Thus, a molecule showing great adjuvant potential combination with protein-antigens such as hepatitis B surface antigen (HBsAg) or CRM197 may not necessarily be as efficacious when combined with other vaccine-antigens like the TI-II-dependent pneumococcal polysaccharides.

A question of timing and formulation?

In the two human trials evaluating the use of differential timing of vaccine and adjuvant (abatacept and GM-CSF), there was no enhancement of antibody responses. Despite this, differential timing may have an effect for other and more potent adjuvants. Two animal studies^{41,42} suggest that TLR agonists (TLR9 agonists in particular) can boost the primary antibody response to plain pneumococcal polysaccharide (serotype 3) in mice, provided that they are administered twodays after vaccination. In these experiments, the humoral immunity induced by a single immunization with the plain TLR9-adjuvanted polysaccharide vaccine was comparable, in terms of efficiency and longevity, to that conferred by a prime/boost immunization with the pneumococcal conjugate vaccine.⁴² The authors suggested that the effect of delayed TLR9 agonist administration was due to increased survival rates of antigen-stimulated B cells and a greater stimulation of plasmablasts. This

hypothesis is in line with the perception that, in B cells, TLR9 receptors are only expressed on antigen-exposed B cells,⁴³ and that the delay allows B cells time to become susceptible to TLR9 stimulation. Altered formulations of adjuvants (i.e., encapsulated in liposomes) may also impact the interaction with polysaccharides and the immune system,⁴⁴ but the importance of timing and formulation of adjuvants must still be confirmed in humans.

What is the Future for Adjuvanted Pneumococcal Vaccines?

The overwhelming success in developed countries of pneumococcal conjugate vaccines with extensive serotype coverage provided by new generation conjugate vaccines led to hopes that pneumococcal disease could be globally controlled. However, serotype replacement by pathogenic strains like serotype 19A,¹⁸ a lack of superiority compared to PPV-23 in high-risk adults, the requirement of multiple injections, as well as concerns about the cost and complexity of manufacturing multivalent pneumococcal conjugate vaccines have underscored the need for alternative vaccine formulations. We demonstrated proof-of-concept that TLR9 agonists can be effective adjuvants in pneumococcal conjugate vaccines in immunocompromised adults. The immunogenicity and toxicity of this combination may be further improved by modulating the co-formulation of PCV and TLR9 agonist. Alternatively, following PPV-23 immunization the administration of adjuvants such as TLR9 agonists could be delayed to improve antigen-specific B cell survival, but the feasibility, tolerability and efficacy of this strategy remains to be demonstrated in humans.

Another approach is protein-based pneumococcal vaccines that, in rodents, provide protection against both nasopharyngeal colonization and invasive disease by most or all pneumococcal serotypes.⁴⁵ Protein-based pneumococcal vaccines can potentially provide broad protection against almost all *Streptococcus pneumoniae* strains, but the modest immunogenicity of pneumococcal proteins may necessitate the use of adjuvants.⁴⁵ In

fact, preparations of protein-based pneumococcal vaccines in combination with adjuvants such as cholera toxin and TLR4 agonists have yielded promising results in pneumococcal pharyngeal colonization and challenge mice models.⁴⁶

Despite intense activity in the field of adjuvant research and development, there remain a relatively small number of adjuvants used clinically. The main challenge for new formulations to enter clinical practice is that the safety requirements for prophylactic vaccines are extraordinarily high, in particular for vaccines aimed for use in healthy infants. However, as more and safer adjuvants are being development and used in commercial vaccines, the general acceptance of vaccine adjuvants appears to be increasing. Thus, despite past failures, future pneumococcal vaccines may very well be formulated with potent but safe adjuvants.

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