Human Vaccines

ISSN: 1554-8600 (Print) 1554-8619 (Online) Journal homepage: www.tandfonline.com/journals/khvi19

The clinical use of adjuvants in pneumococcal vaccination: Current status and future perspectives

Ole Søgaard

To cite this article: Ole Søgaard (2011) The clinical use of adjuvants in pneumococcal vaccination: Current status and future perspectives, Human Vaccines, 7:2, 276-280, DOI: 10.4161/hv.7.2.13919

To link to this article: https://doi.org/10.4161/hv.7.2.13919

Published online: 01 Feb 2011.

Submit your article to this journal

Article views: 1093

View related articles

Citing articles: 1 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=khvi20

Human Vaccines 7:2, 276-280; February 2011; © 2011 Landes Bioscience

The clinical use of adjuvants in pneumococcal vaccination

Current status and future perspectives

Ole Schmeltz Søgaard
Aarhus University Hospital-Skejby; Department of Infectious Diseases; Aarhus N, Denmark

©2011 Landes Bioscience. Do not distribute.

Pneumococcal infection disproportionally 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.

Key words: pneumococcal vaccines, vaccination, immunologic adjuvants, HIV, elderly, immunogenicity, *Streptococcus pneumoniae*

Submitted: 10/05/10

Accepted: 10/12/10

DOI: 10.4161/hv.7.2.13919

Correspondence to: Ole Schmeltz Søgaard; Email: olesoega@rm.dk

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,

Pfizer EX1035, Page 2 of 6

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 coworkers[23] 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 μg) 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 μg 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 μg 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

©2011 Landes Bioscience. Do not distribute.

Pfizer EX1035, Page 3 of 6

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	*Press release*[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 coworkers 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 (Prevnar) 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

©2011 Landes Bioscience. Do not distribute.

Pfizer EX1035, Page 4 of 6

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.

Financial Suppport

Grant support by Aarhus University, Denmark.

©2011 Landes Bioscience. Do not distribute.

References

1. Levine OS, O'Brien KL, Knoll M, Adegbola RA, Black S, Cherian T, et al. Pneumococcal vaccination in developing countries. Lancet 2006; 367:1880-2.
2. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet 2009; 374:893-902.
3. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1997; 46:1-24.
4. Obaro SK, Madhi SA. Bacterial pneumonia vaccines and childhood pneumonia: are we winning, refining or redefining? Lancet Infect Dis 2006; 6:150-61.
5. Lynch JP, III, Zhanel GG. *Streptococcus pneumoniae*: epidemiology, risk factors and strategies for prevention. Semin Respir Crit Care Med 2009; 30:189-209.
6. Rudan I, Campbell H. The deadly toll of *S* pneumoniae and *H. influenzae* type b. Lancet 2009; 374:854-6.
7. Pneumococcal conjugate vaccine for childhood immunization. WHO position paper. Releve epidemiologique hebdomadaire/Section d'hygiene du Secretariat de la Societe des Nations=Weekly epidemiological record/Health Section of the Secretariat of the League of Nations 2007; 82:93-104.
8. Trotter C, Scott P, Huss A, Egger M. Pneumococcal polysaccharide vaccine effectiveness: study quality must not be ignored. Lancet Infect Dis 2008; 8:664.
9. Jefferson T. Pneumococcal vaccines: confronting the confounders. Lancet 2009; 373:2008-9.
10. Simberkoff MS, El Sadr W, Schiffman G, Rahal JJ Jr. *Streptococcus pneumoniae* infections and bacteremia in patients with acquired immune deficiency syndrome, with report of a pneumococcal vaccine failure. Am Rev Respir Dis 1984; 130:1174-6.
11. Vila-Corcoles A. Advances in pneumococcal vaccines: what are the advantages for the elderly? Drugs Aging 2007; 24:791-800.

Pfizer EX1035, Page 5 of 6

12. Ortqvist A, Henckaerts I, Hedlund J, Poolman J. Non-response to specific serotypes likely cause for failure to 23-valent pneumococcal polysaccharide vaccine in the elderly. Vaccine 2007; 25:2445-50.
13. French N, Nakiyingi J, Carpenter LM, Lugada E, Watera C, Moi K, et al. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. Lancet 2000; 355:2106-11.
14. Huss A, Scott P, Stuck AE, Trotter C, Egger M. Efficacy of pneumococcal vaccination in adults: a meta-analysis. CMAJ 2009; 180:48-58.
15. Pedersen R, Lohse N, Ostergaard L, Søgaard O. The effectiveness of pneumococcal polysaccharide vaccination among HIV-infected adults: a systematic review. HIV Med 2010; In Press.
16. Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. northern California Kaiser Permanente Vaccine Study Center Group. Pediatr Infect Dis J 2000; 19:187-95.
17. French N, Gordon SB, Mwalukomo T, White SA, Mwafulirwa G, Longwe H, et al. A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. N Engl J Med 2010; 362:812-22.
18. Singleton RJ, Hennessy TW, Bulkow LR, Hammitt LL, Zulz T, Hurlburt DA, et al. Invasive pneumococcal disease caused by nonvaccine serotypes among alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. JAMA 2007; 297:1784-92.
19. Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. Immunol Cell Biol 2004; 82:488-96.
20. Schmidt CS, Morrow WJ, Sheikh NA. Smart adjuvants. Expert Rev Vaccines 2007; 6:391-400.
21. Flebbe LM, Braley-Mullen H. Immunopotentiating effects of the adjuvants SGP and Quil A. I. Antibody responses to T-dependent and T-independent antigens. Cell Immunol 1986; 99:119-27.
22. Graber CD, Goust JM, Glassman AD, Kendall R, Loadholt CB. Immunomodulating properties of dimethylglycine in humans. J Infect Dis 1981; 143:101-5.
23. Lafferty WP, Selinger DS, Schiffman G, Goodwin JS. Response to pneumococcal vaccine in the elderly: no enhancement by indomethacin. J Immunopharmacol 1981; 3:241-50.
24. Tovey MG, Lallemand C. Adjuvant activity of cytokines. Methods Mol Biol 2010; 626:287-309.
25. Buchanan RM, Briles DE, Arulanandam BP, Westerink MA, Raeder RH, Metzger DW. IL-12-mediated increases in protection elicited by pneumococcal and meningococcal conjugate vaccines. Vaccine 2001; 19:2020-8.
26. Hedlund J, Langer B, Konradsen HB, Ortqvist A. Negligible adjuvant effect for antibody responses and frequent adverse events associated with IL-12 treatment in humans vaccinated with pneumococcal polysaccharide. Vaccine 2001; 20:164-9.
27. DeVelasco EA, Dekker HA, Antal P, Jalink KP, van Strijp JA, Verheul AF, et al. Adjuvant Quil A improves protection in mice and enhances opsonic capacity of antisera induced by pneumococcal polysaccharide conjugate vaccines. Vaccine 1994; 12:1419-22.
28. Garg M, Subbarao B. Immune responses of systemic and mucosal lymphoid organs to Pnu-Imune vaccine as a function of age and the efficacy of monophosphoryl lipid A as an adjuvant. Infect Immun 1992; 60:2329-36.
29. CareCureCommunity. Antigenics' Quilimmune-P Pneumococcal Vaccine Induces Potent Immune Response in the Elderly. 2001. http://sci.rutgers.edu/forum/showthread.php?t=4298. Accessed 5.10.2010.
30. The Free Library. Aquila Announces Initial Results Of Phase II Clinical Trial Of Quilimmune-P(TM) To Prevent Pneumococcal Infections 1998. http://www.thefreelibrary.com/_/print/PrintArticle.aspx?id=53080854. Accessed 5.10.2010.
31. Evans JT, Cluff CW, Johnson DA, Lacy MJ, Persing DH, Baldridge JR. Enhancement of antigen-specific immunity via the TLR4 ligands MPL adjuvant and Ribi.529. Expert Rev Vaccines 2003; 2:219-29.
32. Vernacchio L, Bernstein H, Pelton S, Allen C, MacDonald K, Dunn J, et al. Effect of monophosphoryl lipid A (MPL) on T-helper cells when administered as an adjuvant with pneumococcal-CRM197 conjugate vaccine in healthy toddlers. Vaccine 2002; 20:3658-67.
33. Safdar A, Rodriguez GH, Rueda AM, Wierda WG, Ferrajoli A, Musher DM, et al. Multiple-dose granulocyte-macrophage-colony-stimulating factor plus 23-valent polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia: a prospective, randomized trial of safety and immunogenicity. Cancer 2008; 113:383-7.
34. Tay L, Leon F, Vratsanos G, Raymond R, Corbo M. Vaccination response to tetanus toxoid and 23-valent pneumococcal vaccines following administration of a single dose of abatacept: a randomized, open-label, parallel group study in healthy subjects. Arthritis Res Ther 2007; 9:38.
35. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. Nature Rev Drug Discov 2006; 5:471-84.
36. Sogaard OS, Lohse N, Harboe ZB, Offersen R, Bukh AR, Davis HL, et al. Improving the immunogenicity of pneumococcal conjugate vaccine in HIV-infected adults with a toll-like receptor 9 agonist adjuvant: a randomized, controlled trial. Clin Infect Dis 2010; 51:42-50.
37. Yin JZ, Bell MK, Thorbecke GJ. Effect of various adjuvants on the antibody response of mice to pneumococcal polysaccharides. J Biol Response Mod 1989; 8:190-205.
38. Astronomo RD, Burton DR. Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat Rev Drug Discov 2010; 9:308-24.
39. Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. Annu Rev Immunol 1995; 13:655-92.
40. Daubenberger CA. TLR9 agonists as adjuvants for prophylactic and therapeutic vaccines. Curr Opin Mol Ther 2007; 9:45-52.
41. Taillardet M, Haffar G, Mondiere P, Asensio MJ, Gheit H, Burdin N, et al. The thymus-independent immunity conferred by a pneumococcal polysaccharide is mediated by long-lived plasma cells. Blood 2009; 114:4432-40.
42. Taillardet M, Haffar G, Mondiere P, Asensio MJ, Pleau-Pison T, Burdin N, et al. Toll-like receptor agonists allow generation of long-lasting antipneumococcal humoral immunity in response to a plain polysaccharidic vaccine. J Infect Dis 2010; 202:470-9.
43. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. Science 2002; 298:2199-202.
44. Li WM, Bally MB, Schutze-Redelmeier MP. Enhanced immune response to T-independent antigen by using CpG oligodeoxynucleotides encapsulated in liposomes. Vaccine 2001; 20:148-57.
45. Malley R. Antibody and cell-mediated immunity to *Streptococcus pneumoniae*: implications for vaccine development. J Mol Med 2010; 88:135-42.
46. Basset A, Thompson CM, Hollingshead SK, Briles DE, Ades EW, Lipsitch M, et al. Antibody-independent, $CD4^+$ T-cell-dependent protection against pneumococcal colonization elicited by intranasal immunization with purified pneumococcal proteins. Infect Immun 2007; 75:5460-4.

©2011 Landes Bioscience. Do not distribute.

Pfizer EX1035, Page 6 of 6