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Review

Research progress of *Streptococcus pneumoniae* capsular polysaccharide-protein conjugate vaccines

Yayi Long[1], Yuanyuan Hou[2], Xue Feng[1], Dong Yang[2], Chao Ding[1] and Yuhong Zhen[1]*

[1]Pharmacy College, Dalian Medical University Dalian, China.
[2]Clinical Medicine of Seven-Year-Programme, Dalian Medical University, Dalian, China.

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***Streptococcus pneumoniae* is a major respiratory pathogen responsible for many diseases in young children and elderly. Because of the increasing antibiotic resistance of pneumococci recently, the need for development of an effective vaccine is more and more urgent. Since the 7-valent conjugate vaccine was licensed in the United States in 2000, its efficacy has been widely recognized. Effort on researching polysaccharide-protein conjugate vaccine never stopped. This article reviewed the conjugate vaccines in view of the carrier proteins.**

Key words: Conjugate vaccine, *Streptococcus pneumoniae*, capsular polysaccharide, carrier protein.

INTRODUCTION

Stretococcus pneumoniae (the pneumococcus) is a kind of gram-positive diplococci and is the major cause of many invasive disease, such as acute otitis media (AOM), pneumonia, bacteremia (blood stream infections), meningitis, sinusitis and conjunctivitis (Williams et al., 2008; Beery and Paton, 2000; Hausdorff et al., 2005). Pneumococcal disease causes a large number of deaths every year. According to the estimate of World Health Organization (WHO) that 1.6 million die every year from the invasive disease, 0.7-1 million of which are children aged < 5 years (Bravo, 2009). Because the use of antibiotics is more and more extensive, there is more bacterium strains resistance to multiple antibiotics. Even with reasonable antibiotic therapy, pneumococcal infections still cause high morbidity and mortality. To date, vaccine is still an effective method to prevent pneumococcal invasive disease (Beery and Paton, 2000; Michon et al., 1998).

According to the chemical compositions of polysaccharide capsules, there are 91 pneumococcal serotypes that have been identified and the 91 serotypes are grouped in 46 serogroups based on immunological similarities (Hausdorff et al., 2005). Polysaccharide capsule of *S. pneumoniae* is the major and important virulence factor and still considered as the target of all pneumococcal vaccines up to now. But the pneumococcal polysaccharide capsule has serotype specificity, which determines the virulence, invasiveness and the ability to obtain drug resistance (Ho and Lin, 2005).

Several decades, the vaccines that contained polysaccharide capsule antigen have contributed to reduce pneumococcal invasive disease. Polysaccharide-based vaccine provides wide serotype coverage at low cost but is useless in young children. Since more efforts were put on pneumococcal conjugate vaccines, this review will focus on the current status of pneumococcal polysaccharide-protein conjugate vaccines (Reinert et al., 2010).

PNEUMOCOCCAL VACCINE AVAILABLE UNDER LICENCE

There are currently two types of pneumococcal vaccines available under license. One is 23-valent pneumococcal polysaccharide vaccine and the other one is 7-valent

*Corresponding author. E-mail: zhenyuhong@gmail.com. Tel: +86-411-86110414. Fax: +86-411-86110414.

pneumococcal conjugate vaccine.

Twenty-three-valent pneumococcal polysaccharide vaccines (PPV23)

In 1983, PPV23 was recommended for adults and children selected aged ≥2 years (Reinert et al., 2010). PPV23 was made of 23 serotypes capsular polysaccharide including 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 20, 22F, 23F and 33F. These serotypes covered about 90% of invasive pneumococcal infection (Ho and Lin, 2005). It is reported that PPV23 is effective in elder children and adults. However, the vaccine is useless in children aged ≤2 years as it cannot elicit adequate antibody responses to most of the capsular polysaccharides (Briles et al., 1998). Polysaccharide capsule is T-cell-independent antigen and poorly immunogenic in children under 2 years of age and there is no convincing evidence that children under 2 years old can respond well to these types of antigens. And some of these serotypes cause most case of disease namely 6A, 14, 19F and 23F do not induce a good immune response to polysaccharide vaccine until a child is 5 years old (Williams et al., 2008). PPV23 also did not reduce nasopharyngeal colonization of *S. pneumoniae* among children because of replacement of non-vaccine serotypes carriages. The epidemiological importance remains unclear. At the same time, one dose of this vaccine decrease the incidence of AOM and the efficacy persists for only 6 months and revaccination gives no further benefit.

The limitations of PPV23 can be induced into two points. These include the facts that PPV23 covers only 23 of the 91 known serotypes and polysaccharide capsule are T-cell-independent antigens. So protein-polysaccharide capsule vaccines are currently undergoing clinical trials.

Seven-valent pneumococcal conjugate vaccine (PCV7)

To overcome the shortage of PPV23, scientists conjugate the polysaccharide capsule to protein carriers and reverse the T-cell-independent antigens to T-cell-dependent antigens. In this way, the conjugate vaccine can promote B-cell proliferation, affinity maturation and immunological memory in early life. But serotypes coverage will be more limited (Berry and Paton, 2000; Ho and Lin, 2005). In 2000, American Wyeth developed PCV7, in which pneumococcal polysaccharide is linked to a carrier protein CRM197 (a nontoxic mutant of diphtheria toxoid). The PCV7 vaccine was designed to cover seven serotype 4, 6B, 9V, 14, 18C, 19F and 23F. These serotypes account for about 80% of invasive infections and 65% AOM in children under 6 years in the United States (William et al., 2008). The licensed PCV7 was recommended for routine use in infancy in the United States by the Advisory Committee on Immunization Practices. It recommended that all children aged ≥23 months should be vaccinate with PCV7 (Ho and Lin, 2005). To the developing countries where they needed most, PCV7 is too expensive to limit their development (Ogunniyi et al., 2000).

PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE VACCINES UNDER TRIAL

Although serotype coverage is limited, pneumococcal conjugate vaccines are still safe and well-tolerated formulations to prevent pneumococcal infections. Currently, the carrier proteins used for conjugating to polysaccharide can be divided into two categories: exogenous carrier protein and self carrier protein.

Extrinsic carrier protein-polysaccharide capsular conjugate vaccines

As early as 1929, Avery and co-workers demonstrated that immunogenicity of polysaccharide increases when capsular polysaccharide was coupled covalently to proteins. This comprises linkage of capsular polysaccharides to a protein carrier either by covalent binding or reactive group (Bogaert et al., 2004). In the case of *Haemophilus influenzae* type b (Hib) vaccine, polysaccharide have been coupled to large immunogenic and good immunocopetent carriers such as tetanus toxoid (TT), diphtheria toxoid (DT), outer membrane protein complex (OMPC) from meningococcus group B. These proteins are proven in most studies that they have been employed for human vaccination for many years and they did not have untoward side effects (Peeters et al., 1991). In addition, TT and DT mixture is also a potential carrier protein.

Tetanus toxoid-*Streptococcus pneumoniae* capsular polysaccharides conjugate vaccines

TT is produced by tetanus clostridia via detoxification by formaldehyde and it is the most widely used exogenous carrier protein. TT is a commonly used carrier protein in conjugate vaccines undergoing clinical trials. Polysaccharide capsular-TT conjugate vaccine can induce protective level of antibody and reduce the incidence of pneumococcal infection in both infants and adults (Kroll-Amir. et al., 2003). A study was performed to compare antibody levels and opsonic activities in sera of infants and adults who injected with pneumococcal polysaccharide type 6B (Pn6B) conjugated to tetanus toxoid. In adult, level of IgM, IgG, IgA, IgG1 and IgG2

were all increased. For infants, the level of IgG1 anti-Pn6B reached adult level after primary injection, the level of total IgG and IgM anti-Pn6B were similar to adults after booster injection. IgG1 became the dominant infant anti-Pn6B isotype and its level was higher than vaccinated adults after booster injections. The result indicates that Pn6B-TT conjugate vaccine has protective potential for young children (Vidarsson et al., 1998). In animal experiments, the immune response of pneumococcal polysaccharide type 9V (Pn9V)-TT conjugate vaccine was quantified, mouse anti-Pn9V IgG and IgM reference standards were established. Immunized young mice aged 2 weeks produced IgM antibody in response to Pn9V alone or Pn9V-TT conjugate vaccine. However, only the Pn9V-TT conjugate generated an IgG antibody response and an anamnestic effect (Lu et al., 1994). Above all, TT-polysaccharide conjugate vaccines are efficacious.

But carrier-induced epitope suppression was described for synthetic peptides coupled to TT in human. This phenomenon also occurred upon polysaccharide-protein conjugate vaccines (Peeters et al., 1991). Preimmunization with low dose of TT can enhance the antibody response to TT-polysaccharide conjugate vaccine. However, multivalent conjugates may cause a carrier overload when some people might be immunized with tetanus vaccine before. In order to solve this problem, tetanus toxoid and diphtheria toxoid were both used as carriers in an 11-valent conjugate vaccine which contains 1, 3, 5 and 7F serotypes on the basis of PVC7. And this can reduce the epitope suppression caused by different vaccines with the same epitope protein (Puumalainen et al., 2002). In some countries, this 11-valent conjugate vaccine showed good effects and covered a wider range of serotypes in adults and infants (Ugpo et al., 2009; Capeding et al., 2003; Puumalainen et al., 2003; Dagan et al., 2004).

Diphteria toxoid -*Streptococcus pneumoniae* capsular polysaccharides conjugate vaccines

CRM197 is a genetically detoxified form of diphtheria toxin that is a nontoxic, immunologically cross-reacting mutant protein of diphtheria toxin contains 39 lysine residues with a free α amino terminus for attachment of multiple oligosaccharides chains (Usonis et al., 2008; Mawas et al., 2002). The most widely used 7-valent conjugate vaccine (PCV7) uses it as carrier protein. Post-PCV7 introductions, the proportion of invasive infections caused by six other *S. pneumoniae* serotypes (1, 3, 5, 6A, 7F and 19A) has increased (Reinert et al., 2010). Because the process of producing conjugate vaccine is very complex, this vaccine is likely to be expensive. It covers only seven serotypes, rather than 23. There are another two types of conjugate vaccines currently undergoing clinical trials. The 9-valent formulation is 7-valent plus serotype 1 and 5. The 11-valent formulation is 9-valent plus serotype 3 and 7F (Ogunniyi et al., 2000). To some extent, this solved the problem of serotype coverage in different regions. Pneumococcal nasopharyngeal colonization is important for transmission of the organisms. But these conjugate vaccines are not efficacious in prevention of colonization (Crane et al., 1997; Ron et al., 1997). Therefore, new conjugate needed to be developed.

Outer membrane protein complex-*Streptococcus pneumoniae* capsular polysaccharides conjugate vaccines

H. influenzae type b (Hib) conjugate vaccines have dramatically reduced Hib disease when they were first introduced for routine use in infants nearly two decades ago. On the basis of the success of this conjugate vaccine, pneumococcal conjugate vaccines were developed subsequently. Because Hib conjugate vaccines use outer membrane protein complex (OMPC) as carrier protein. This carrier protein is from *Neisseria meningitidis* serotype B and is an effective carrier which has been shown to potentiate antibody levels to Hib capsular polysaccharide in animal and humans (Vella et al., 1992; Usonis et al., 2008). Vella et al. linked polysaccharide of types 6B, 14, 19F and 23F to OMPC covalently and examined their immunogenicity in mice and infant monkeys. The immunogenicity data in infant monkeys and mice both suggest T-cell-dependent properties (Vella et al., 1992). In addition, OMPC showed another role in a current research. The Hib conjugate vaccine elicits protective levels of anti-capsular polysaccharide antibody after a single dose, but other conjugate vaccines require multiple doses. In previous research, it has been shown that OMPC robustly engages Toll-like receptor 2 (TLR2) and enhanced the early anti-Hib capsular polysaccharide antibody titer associated with an increasing in TLR2-mediated induction of cytokines. Lai et al. added OMPC to the PCV7 during immunization and found it can increase significantly the anti-capsular polysaccharide IgG and IgM response to most serotypes of pneumococcal contained in the vaccine. At the same time, addition of OMPC also increased the likelihood of anti-capsular polysaccharide IgG3 production against serotypes 4, 6B, 9V, 18C, 19F and 23F (Lai and Schreiber, 2011).

Self carrier protein-polysaccharide capsular conjugate vaccines

Despite existing conjugate vaccines achieved success on a certain degree, the complexity of producing polysaccharide-protein conjugate vaccines makes them expensive. It is almost impossible to use it in poverty countries where most of the annual 3-5 million fatal

respiratory infections occur in young children (Briles et al., 2003a). The reasonable coverage of vaccines requires 7 to 13 serotypes. Moreover, because the conjugate vaccines are composed of a limited number of serotypes, it can result in serotype replacement due to the decrease in the prevalence of vaccinal serotypes. This is aggravated in a pneumococcal vaccine where at least 90 different serotypes have been identified in humans (Csordas et al., 2008). To obtain increased coverage with fewer components, some pneumococcal proteins were used as carrier proteins. These proteins are not serotype-specific; they have potential to provide broader immunity. Alternatively, it can increase immunity to polysaccharide and provide an additional protective antigen for broad protection against pneumococcal infection (Lee et al., 2001; Csordas et al., 2008). Up to date, three proteins including pneumolysin (Ply), pneumococcal surface protein A (PspA) and pneumococcal surface adhesion A (PsaA) have shown the greatest promise as carrier proteins. They are virulence factors common to all serotypes and can be used in pneumococcal vaccines either alone or in conjugation to capsular polysaccharide (Godfroid et al., 2011; Ogunniyi et al., 2000).

Pneumolysin- polysaccharide capsular conjugate vaccines

Ply is a multifunctional protein with both cytotoxic and complements activation properties. It is coated in cytoplasm but is released when pneumococci undergo autolysis (Berry et al., 2000). A genetically detoxified pneumolysin, pneumolysoid (PLD) was investigated as a carrier protein for pneumococcal capsular polysaccharide (CPS). Such a CPS-PLD conjugate might provide additional protection against pneumococcal infections and result in tissue damage (Michon et al., 1998) Ply is a potent hemolytic cytotoxin, which can be produced by all essential strains of *S. pneumoniae* isolated clinically. Its status as an important virulence protein has been confirmed by previous demonstration that genetically defined Ply-negative mutant pneumococcus strains have significantly reduced virulence (Paton et al., 1991). PLD-polysaccharide conjugate vaccines were prepared from CPS serotypes 6B, 14, 19F and 23F by reductive amination and TT-polysaccharide conjugate vaccines were similarly prepared and used for comparison. The PLD conjugates were generally equivalent to or better than the TT conjugates in CPS-specific responses (Michon et al., 1998). The pneumococcal toxin pneumolysin is an important pneumococcal virulence factor, which appears to satisfy the necessary criteria for use in vaccines. All clinical isolates of *S. pneumoniae* produce pneumolysin and there appears to be very little variation in its primary structure. Immunization with pneumolysin protects mice from subsequent challenge with virulent pneumococci. Native pneumolysin is unsuitable for inclusion in a vaccine for humans because of its toxicity. Lee et al. conjugated type 19F polysaccharide capsular to PLD and immunized infant mice with this conjugate vaccine. The bacteria were cleared more effectively from the blood of immunized mice than from control mice when the infant mice were challenge with type 19F pneumococci (Lee et al., 1994).

Pneumococcal surface protein A- polysaccharide capsular conjugate vaccines

PspA is a member of a family of structurally and related to choline-binding surface proteins, its precise function is uncertain, although it has recently been shown to be capable of binding human lactoferrin (Berry et al., 2000). PspA is able to bind apolactoferrin and thereby reduce the ability of apolactoferrin to kill pneumococci. PspA also inhibits C3 activation through a yet unknown mechanism and can even reduce the amount of C3 deposited by antibody to capsule (Briles et al., 2003b). Like other two pneumococcal proteins, PspA is also potential to offer a broad range of protection as a candidate carrier protein (Heeg et al., 2007). Huang et al. prepared conjugate vaccine consisting of serotype 5 polysaccharide capsular. The result showed this conjugate vaccine can enhance the level of both IgG and IgG2a antibody titer and have some cross-protection function against serotypes 1, 5, 6B, 19F and 23F. Passive and active protection experiments against intraperitoneal challenge with *S. pneumoniae* were carried out to validate the protection of the conjugate vaccine (Huang et al., 2008).

Pneumocaccal adhesion protein A- polysaccharide capsular conjugate vaccines

As a common pneumococcal protein, PsaA has been shown to be immunogenic and reduce nasopharyngeal colonization in a mouse model (Whaley et al., 2010). It has been identified as a cell-membrane-associated lipoprotein expressed by pneumococcal strains. PsaA has been advanced as a suitable protein target for pneumococcal vaccine development and has the potential to provide broader immunity. Comparing with the traditional carrier proteins, PsaA could be used as protein carrier in a conjugate vaccine to increase immunity to polysaccharide capsular and to provide an additional protection. Huang et al. prepared PsaA-polysaccharide conjugate vaccine and detected its immunity. The experimental data showed that this conjugate vaccine induced a T-cell-dependent immune response, which led to an increase of polysaccharide-specific antibody titer and development of immunological memory and maturation of the immune response. Besides these functions, this conjugate vaccine can induce high titer antibodies in serum. This antibody is valuable for

additional protection against pneumococcal infection. So it is successful that PsaA was used as the protein carrier replacing other proteins from foreign source (Lin et al., 2010). On the other hand, PsaA can be used as an auxiliary agent. By co-administering PCV7 and PsaA, there is a reduction of pneumococcal colonization for serotypes 4, 14 and 19A in mice (Whaley et al., 2010).

CONCLUSION

S. pneumoniae is part of the normal resident oropharyngeal flora and also an opportunistic pathogen. The effective way of treatment and prevention on this infection is vaccine (Huo et al., 2004). Protection by the PPV23 is not effective for subjects <2 years of age, particularly infants, in whom pneumococcal disease is most prevalent. The PCV7 has had a dramatic effect on the epidemiology of pneumococcal infections (Lu et al., 2009). To overcome these shortcomings the vaccine based on protein, particularly common surface proteins of pneumococcal are conserved (Lin et al., 2010).

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