Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Production and efficacy of a low-cost recombinant pneumococcal protein polysaccharide conjugate vaccine

Jenny A. Herbert [a,1,2], Emily J. Kay [b,1], Sian E. Faustini [c,d], Alex Richter [c,d,e], Sherif Abouelhadid [b], Jon Cuccui [b], Brendan Wren [b], Timothy J. Mitchell [a,*]

[a] Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, England, UK
[b] Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London, UK
[c] Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, England, UK
[d] Department of Immunology, Queen Elizabeth Hospital, Birmingham, UK
[e] University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

ARTICLE INFO

Article history:
Received 30 January 2018
Received in revised form 1 May 2018
Accepted 5 May 2018
Available online xxxx

Keywords:
Streptococcus pneumoniae
Conjugate vaccine
Protein glycan coupling technology
Glycoengineering
Pneumonia

ABSTRACT

Streptococcus pneumoniae is the leading cause of bacterial pneumonia. Although this is a vaccine preventable disease, *S. pneumoniae* still causes over 1 million deaths per year, mainly in children under the age of five. The biggest disease burden is in the developing world, which is mainly due to unavailability of vaccines due to their high costs. Protein polysaccharide conjugate vaccines are given routinely in the developed world to children to induce a protective antibody response against *S. pneumoniae*. One of these vaccines is Prevnar13, which targets 13 of the 95 known capsular types. Current vaccine production requires growth of large amounts of the 13 serotypes, and isolation of the capsular polysaccharide that is then chemically coupled to a protein, such as the diphtheria toxoid CRM_{197}, in a multistep expensive procedure. In this study, we design, purify and produce novel recombinant pneumococcal protein polysaccharide conjugate vaccines in *Escherichia coli*, which act as mini factories for the low-cost production of conjugate vaccines. Recombinant vaccine efficacy was tested in a murine model of pneumococcal pneumonia; ability to protect against invasive disease was compared to that of Prevnar13. This study provides the first proof of principle that protein polysaccharide conjugate vaccines produced in *E. coli* can be used to prevent pneumococcal infection. Vaccines produced in this manner may provide a low-cost alternative to the current vaccine production methodology.

1. Introduction

Streptococcus pneumoniae (the pneumococcus) is the leading cause of bacterial pneumonia. The highest disease burden is observed in the developing world due to limited vaccine availability [1]. In the developed world, since the introduction of pneumococcal conjugate vaccines, *S. pneumoniae* disease burden in children has drastically reduced (PCV7/10/13) [2,3]. Prevnar7 was the first pneumococcal conjugate vaccine produced and was introduced in the UK in 2006. Post introduction, invasive pneumococcal disease caused by vaccine serotypes dropped by 41% [2]. After introduction of Prevnar13 (PCV13) in 2010 invasive disease caused by the additional 6 serotypes dropped by 75% [4].

PCV13 is a component of the childhood vaccine schedule in the UK and is given to all children in a three-dose schedule at 2, 4 and 12 months of age. This vaccine targets the capsular polysaccharide surrounding the bacteria. PCV13 protects against the 13 (out of 95) pneumococcal serotypes found to be most prevalent in disease [5]. Polysaccharide alone is not immunogenic in children under 2 years of age, and does not produce a lasting immune response [6]. Conjugate vaccines work by coupling the polysaccharide component to a protein carrier [7], resulting in a protective T-cell dependent memory response [8]. This technology was first used for the production of a *Haemophilus influenzae* type B vaccine [9], followed by conjugate vaccines to prevent *Neisseria meningitidis* serogroup C [10], and subsequently pneumococcal infection [11]. Pneumococcal conjugate vaccines are the world's best-selling vaccines, and in 2014 PCV13 sales produced revenues of £2.9 billion [12].

Although these glycoconjugate vaccines are very effective, there are some limitations to their use. Serotype distribution of disease causing isolates varies geographically [13]. The PCV7 vaccine

* Corresponding author.
E-mail address: t.j.mitchell@bham.ac.uk (T.J. Mitchell).
[1] These authors contributed equally to this work.
[2] Current address: UCL Great Ormond Street Institute of Child Health, London, UK.

https://doi.org/10.1016/j.vaccine.2018.05.036
0264-410X/

serotypes are more prevalent in the western world, therefore this vaccine did not provide adequate protection against serotypes prevalent in developing countries. However, the introduction of an additional 6 serotypes in PCV13 includes the main disease-causing serotypes in the developing world [5,13]. Serotype replacement remains a problem, and introduction of vaccines has resulted in increased incidence of disease from non-vaccine serotypes [2,14,15]. The most recent emerging serotypes (22F and 33F) are included in a new 15-valent vaccine preparation [16]. Finally, the high cost of conjugate vaccines means they are often not available to the poorest regions, which have the greatest disease burden.

The complex nature of the production process of the conjugate vaccine is one contributing factor to the high cost. Using standard methods, growth of large quantities of pathogenic pneumococci is required for isolation of the polysaccharide. Post purification the polysaccharide must then be chemically coupled to the carrier protein, in the case of PCV13 a diphtheria toxoid (CRM_{197}). This process is time consuming, requires several rounds of purification to remove toxic chemicals and by products, and can often result in batch to batch variation [17].

An increase in the understanding of bacterial protein glycosylation has led to development of novel ways to couple protein and polysaccharide (reviewed in [18]). The approach, often referred to as protein glycan coupling technology (PGCT) allows production of protein polysaccharide conjugate vaccines in *Escherichia coli* [19]. This technology utilises an oligosaccharyltransferase enzyme, PglB, from the general protein glycosylation locus (Pgl) of *Campylobacter jejuni* [19]. This locus encodes the genes required for the production of a *C. jejuni* heptasaccharide along with PglB, which is required for coupling of the heptasaccharide to a carrier protein. PglB couples over sixty *C. jejuni* proteins to this heptasaccharide [20]. These proteins contain an amino acid acceptor sequence, which is recognised by PglB [21]. The consensus, or glycotag, sequence can be engineered into any protein carrier, allowing recognition by PglB [21]. The glycan specificity of *C. jejuni* PglB has been well characterised, using this knowledge researchers have been able to couple a number of different polysaccharides to chosen carrier proteins using PglB [21–23]. In simple terms, PGCT can be divided into three procedures. In the first stage, genes encoding the target glycan are faithfully cloned and expressed in *E. coli* on a suitable plasmid. In the second stage, the target carrier protein containing the appropriate consensus sequon and purification tag are cloned into a suitable plasmid, and targeted to the periplasm. Finally, the coupling enzyme, CjPglB, recognises the initial sugar on the glycan and transfers it to the carrier protein. The plasmids are introduced into an appropriate *E. coli* host strain to produce an inexhaustible supply of recombinant glycoprotein that can be readily purified [19].

There are a number of vaccines that have been produced using this technology that show excellent promise in both animal models and in clinical trials [24–27]. Vaccines produced using PGCT will reduce vaccine costs, speed up the production process, and negate the need for growth of large volumes of pathogenic bacteria. Other benefits of using this technology include the ability to readily change the carrier protein, and to add further compatible polysaccharide types. Further, this technology could allow rapid addition of polysaccharides to vaccine preparations to protect against emerging serotypes. Vaccines produced in this manner could also be tailored to specific geographical regions, by protecting against the most prevalent serotypes. To date pneumococcal polysaccharides of type 4, 5, 8 and 12F have been expressed in *E. coli* [28], the first stage of PGCT.

In the current study, we provide the first evidence that recombinant protein polysaccharide conjugate vaccines can be produced in *E. coli* and protect against pneumococcal invasive disease in a murine infection model.

2. Materials and methods

2.1. Bacterial strains and plasmids

Escherichia coli strains were grown in modified super optimal broth, SSOB (Tryptone 2%, Yeast extract 0.5%, NaCl 10 mM, KCl 2.5 mM, $MgCl_2$ 10 mM, $MgSO_4$ 10 mM) at 28 °C, with shaking. Antibiotics were added as necessary for plasmid maintenance: tetracycline 20 μg ml^{-1}; ampicillin 100 μg ml^{-1}; chloramphenicol 30 μg ml^{-1}. A table of strains and plasmids used in this study can be found in the supplementary information (Table S1).

Streptococcus pneumoniae strain (TIGR4) was cultured on BHI agar with 5% horse blood, or statically in BHI broth, in an atmosphere containing 5% CO_2.

2.2. Vaccine production

Recombinant serotype 4 polysaccharide was produced in *E. coli*, as previously described [28]. Conjugation to AcrA was carried out using protein glycan coupling technology [25]. *E. coli* cultures were grown for 16 h. These starter cultures were used to inoculate 2 L of SSOB to an OD600 of 0.03 and incubated with shaking at 28 °C. Once OD600 had reached 0.4–0.6, expression of PglB was induced with the addition of 1 mM IPTG. $MnCl_2$ was also added to a final concentration of 4 mM. After 20 h growth at 28 °C cells were pelleted by centrifugation at 5400*g* for 30 min at 4 °C. Pellets were resuspended in lysis buffer (50 mM NaH_2PO_4, 0.3 M NaCl and 10 mM imidazole, pH 7.5) with 1 mg/ml lysozyme, and lysed using a FastPrep instrument (MP Biomedicals) with lysing matrix B. Supernatant was treated with 250 units benzonase for 10 min. Insoluble debris was removed by centrifugation at 7800*g* for 60 min at 4 °C and the supernatant passed through a 0.2 μm filter. The protein/polysaccharide conjugate labeled with a polyhistidine affinity tag was purified using HisTrap columns (GE Healthcare) using an imidazole gradient of 20–300 mM on an AKTA protein purification system (GE Healthcare).

2.3. SDS-PAGE and immuno blot analysis

To verify glycoconjugate production and to select AKTA fractions for pooling, samples were subject to SDS-PAGE followed by coomassie staining or immunoblot. Rabbit anti-serotype 4 capsule antibody from the Statens Serum Institut, (SSI) Denmark was used at a dilution of 1:1000 and mouse anti-His monoclonal antibody (Abcam, UK) was used at a dilution of 1:10,000 to detect recombinant serotype 4 capsule and His-tagged AcrA respectively. HR6 antiserum was used to detect the *Campylobacter* heptasaccharide (S. Amber and M. Aebi, unpublished data). Secondary goat anti-rabbit IgG IRDye 800 and goat anti-mouse IgG IRDye 680 conjugates were used at a dilution of 1: 10 000. Fluorescent signal was detected using an Odyssey LI-COR detection system (LI-COR Biosciences UK Ltd.).

2.4. Protein and polysaccharide (PS) quantification in vaccine preparations

Selected AKTA fractions were concentrated using Vivaspin protein concentrator spin columns with 10 KDa MWCO (GE Healthcare) and protein was quantified using a Qubit protein assay (Thermo Fisher Scientific). Levels of Type 4 polysaccharide in vaccine preparations was quantified by ELISA using type 4 antiserum and a standard curve generated using purified type 4 polysaccharide (SSI, Denmark).

Please cite this article in press as: Herbert JA et al. Production and efficacy of a low-cost recombinant pneumococcal protein polysaccharide conjugate vaccine. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.05.036