Vaccine xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Preparation and testing of a Vi conjugate vaccine using pneumococcal surface protein A (PspA) from *Streptococcus pneumoniae* as the carrier protein

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ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 1 August 2014
Accepted 15 August 2014
Available online xxx

Keywords:
Vaccine development
Pneumococcal surface protein A (PspA)
Vi polysaccharide
Conjugate vaccine
Typhoid fever vaccine

ABSTRACT

In the current study pneumococcal surface protein A (PspA) was conjugated to Vi capsular polysaccharide from *Salmonella* Typhi to make available a vaccine against typhoid fever that has the potential to also provide broad protection from *Streptococcus pneumoniae*. High yielding production processes were developed for the purification of PspAs from families 1 and 2. The purified PspAs were conjugated to Vi with high recovery of both Vi and PspA. The processes developed especially for PspA family 2 could readily be adapted for large scale production under cGMP conditions. Previously we have shown that conjugation of diphtheria toxoid (DT) to Vi polysaccharide improves the immune response to Vi but can also enhance the response to DT. In this study it was shown that conjugation of PspA to Vi enhanced the anti-PspA response and that PspA was a suitable carrier protein as demonstrated by the characteristics of a T-cell dependent response to the Vi. We propose that a bivalent vaccine consisting of PspA from families 1 and 2 bound to Vi polysaccharide would protect against typhoid fever and has the potential to also protect against pneumococcal disease and should be considered for use in developing countries.

1. Introduction

Typhoid fever continues to be a significant public health problem particularly in developing countries. To combat this problem a number of conjugate vaccines based on Vi capsular polysaccharide are being developed [1]. These conjugates mainly use diphtheria toxoid or the non toxic mutant diphtheria toxin (CRM_{197}) or tetanus toxoid as the carrier protein. Diphtheria and tetanus vaccination is covered by infant immunization programs. In the current study we investigated the possibility of using an alternative carrier protein to potentially provide additional protection against a significant public health problem namely pneumococcal disease.

Streptococcus pneumoniae infections remain a major source of morbidity and mortality and are responsible for approximately 11% of the worldwide deaths in children under 5 years of age [2]. Pneumococcal disease is a vaccine preventable illness and the current licensed vaccines include conjugate vaccines Prevenar 7 and 13 (Pfizer) [3] and Synflorix (GSK) and un-conjugated polysaccharide vaccines Pneumovax 23 (Merck) and Pneumo 23 (Sanofi). At least 95 serotypes of *S. pneumoniae* based on the capsular polysaccharide exist but licensed vaccines only target from 7 to 23 of these. Prevenar 7 was highly efficacious against invasive pneumococcal disease (IPD) caused by vaccine serotypes in children younger than 2 years of age [3]. A limited increase in IPD caused by non-vaccine serotypes (serotype replacement) has been observed [4]. The existing vaccines have made a major contribution to reducing the burden of disease caused by *S. pneumoniae* but given the current high cost of the conjugate vaccines, the possibility of serotype replacement and the limited serotype coverage offered by the current vaccines it is worth exploring other vaccine options.

Several pneumococcal protein antigens that have been shown to elicit protection in mice and are being considered as vaccine candidates include pneumolysin (Ply), pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC) and pneumococcal surface antigen A (PsaA) [5]. These proteins are virulence factors of *S. pneumoniae* [6].

Described here are methods for purification of PspA from families 1 and 2 and their subsequent conjugation to Vi polysaccharide.

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http://dx.doi.org/10.1016/j.vaccine.2014.08.041
0264-410X/

Immunogenicity testing of these conjugates confirmed our earlier observation [7] that binding of a carrier protein to Vi polysaccharide not only enhances the anti-Vi response but can also enhance the response to the carrier protein. Enhanced immunogenicity of proteins bound to high molecular weight polysaccharides was previously demonstrated with BSA when it was bound to dextran [8].

2. Materials and methods

2.1. PspA antigens

Recombinant truncated PspA molecules were expressed as cytoplasmic proteins in *E.coli* as described previously [9]. Recombinant plasmids were transformed into *E. coli* strain RosettaBlue(DE3)pLysS which contains a chromosomal copy of the T7 promoter under the control of the inducible UV5 promoter. Upon induction with isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma) the following truncated recombinant PspA molecules were produced: PspA/Rx1which contains amino acids 1–302 of PspA protein from strain Rx1 plus a 6x His tag on the C-terminus [10] and PspA/V24 which contains amino acids 1–411 of PspA protein from strain V24 (without His-tag). The PspA from Rx1 belongs to family 1, clade 2, the PspA from V24 belongs to family 2, clade 3.

2.2. Cultivation in a bioreactor

The growth of the recombinant *E. coli* was performed in a Biostat B-DCU bioreactor (Sartorius-Stedim) using fed batch conditions with a starting volume of 2.75 L. Base media contained 10 g/L yeast extract (Oxoid), 10 g/L glucose (Sigma), 1.5 g/L $(NH_4)_2HPO_4$ (UBS), 11.0 g/L KH_2PO_4 (UBS), 3.5 g/L $MgSO_4{\cdot}7H_2O$ (Junsei Chemical) plus 5 mL/L of trace elements (consisting of 10 g/L $FeSO_4{\cdot}7H_2O$ (Junsei Chemical), 2 g/L $CaCl_2{\cdot}2H_2O$ (Sigma), 2.2 g/L $ZnSO_4{\cdot}7H_2O$ (Sigma), 0.5 g/L $MnSO_4{\cdot}5H_2O$ (Junsei Chemical), 1.0 g/L $CuSO_4{\cdot}5H_2O$ (Sigma), 0.4 g/L $Na_2MoO_4{\cdot}2H_2O$ (Sigma), 0.1 g/L H_3BO_3 (Sigma) in 5 M HCl (Junsei Chemical) and 100 mg/L kanamycin sulphate (Sigma) adjusted to pH 7.1 before addition to the bioreactor. Feeding (100 g/L glucose, 100 g/L yeast extract, 3.5 g/L $MgSO_4{\cdot}7H_2O$, 100 mg/L kanamycin sulphate and 5 mL/L trace elements) was initiated when the glucose concentration dropped to 1 g/L. Prior to induction, the culture broth pH was controlled at 7.1, dissolved oxygen at 35% and the temperature at 37 °C. At 9 h culture time when the OD_{600} had reached 25 (mid log phase) the temperature was reduced to 25 °C. IPTG was then added to a final concentration of 1.5 mM to induce production of PspA. Fermentation was continued for another 3 h. At the end of fermentation the culture was harvested (3.4 L and 3.8 L for families 1 and 2, respectively) and the bacterial cells were pelleted by centrifugation at 7000 rpm for 30 min.

2.3. Lysis of bacterial cells

Lysis buffer was added to the pellets (340 g and 377 g for families 1 and 2, respectively) at a rate of 5 mL per g of pellet. Lysis buffer contained 0.52 g/L NaH_2PO_4 H_2O (USB) 4.32 g/L $Na_2HPO_4{\cdot}7H_2O$ (USB), 29.22 g/L NaCl (USB), 5 mL/L triton X100 (Merck), 0.154 g/L dithiothreitol (Biorad), 100 mL/L glycerol (Merck), 0.4 mL/L ≥ 250 units/μL benzonase nuclease (Sigma) and 4 mL/L protease inhibitor cocktail (Calbiochem) pH adjusted to 7.5. The mixture was stirred for 18 h at 4 °C, sonicated (Labsonic, B. Braun Biotech) on ice for 20 (30 s) cycles at an amplitude of 100. Supernatant was collected after centrifugation at 10,000 rpm (7000 × *g*) for 45 min (Beckman coulter Avanti J-20 XP) and sterilized by filtration through a 0.2 μm (300 cm^2) Sartopore 2 300 (Sartorius-Stedim). Volumes obtained were 1.7 L and 1.9 L for families 1 and 2, respectively.

2.4. Capture of PspA family 1

PspA family 1 supernatant was concentrated 3 fold to 0.55 L then diafiltered against 5 volume changes (2.8 L) of binding buffer using a 10 kDa Hydrosart 0.1 m^2 cassette (Sartorius-Stedim) installed in a Sartocon Slice 200 holding device (Sartorius-Stedim). The binding buffer contained 0.253 g/L $NaH_2PO_4.H_2O$ (USB), 4.86 g/L $Na_2HPO_4.7H_2O$ (USB), 29.22 g/L NaCl (USB) and 0.34 g/L imidazole (Sigma) pH adjusted to 7.9.

A Nickel Sepharose 6 fast flow column (HisPrepFF16/10—GE healthcare) connected to an AKTA explorer (Amersham Biosciences) was equilibrated by passage of 200 mL of binding buffer at a rate of 2 mL/min. A 20 mL sample of PspA family 1 (in binding buffer) was pumped onto the column at a rate of 0.5 mL/min. The column was then washed with 100 mL of binding buffer, then 40 mL of wash buffer (same as binding buffer except 2.04 g/L imidazole). Elution was performed by pumping 100 mL of elution buffer (same as binding buffer except 34.04 g/L imidazole) through the column. Peak fractions were pooled and stored at 4 °C.

2.5. Capture of PspA family 2

The PspA family 2 supernatant was concentrated 9.5 fold (to 0.2 L) then diafiltered against 10 volume changes (2 L) of 10 mM phosphate buffered saline (PBS) pH 5.8 using a 30 kDa Hydrosart 0.1 m^2 cassette (Sartorius-Stedim) installed in a Sartocon Slice 200 holding device (Sartorius-Stedim). After concentration retentate was very viscous so was diluted to a final volume of 0.8 L.

A Sartobind Q150 (Sartorius-Stedim) membrane absorber (quaternary ammonium functional groups bound to cellulose membrane) was equilibrated by passage of 1.5 L of 10 mM PBS pH 5.8 at a flow rate of 200 mL/min. A 390 mL sample of PspA family 2 (in PBS) was pumped through the Q150 at a rate of 50 mL/min. The Q150 was then washed with 1.5 L of PBS at a flow rate of 100 mL/min. The Q150 was then washed with 1.5 L of PBS containing 0.2 M NaCl. Elution was performed by pumping 1.0 L of PBS containing 0.4 M NaCl through the Q150. Peak fractions were pooled and stored at 4 °C.

2.6. Vi polysaccharide

Vi purified using the method previously described [11] was concentrated to 5.0 mg/mL then diafiltered against 80 mM MES buffer using a 100 kDa Hydrosart membrane (Sartorius-Stedim) then sterile filtered (0.2 μm Sartopore 2 300).

2.7. Preparation of conjugates

Conjugates were prepared using a two step procedure as described previously [1] with the following changes.

2.7.1. Derivatization of PspA

The PspAs were concentrated to about 5 mg/mL then diafiltered against 10 volume changes of 80 mM MES buffer (2-(*N*-morpholino)ethanesulfonic acid) (Sigma) pH 5.6 using a 30 kDa Hydrosart (0.1 m^2) membrane. Derivatization reactions were performed by first adding adipic acid dihydrazide (ADH) followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Sigma) to the PspA preparations so that the final concentration of PspA, ADH and EDC were 4.5, 9.0 and 0.3 mg/mL in 80 mM MES. The final volume of PspA families 1 and 2 derivatization reactions was 50 mL. The reaction time was 60 min and the pH was maintained at 5.6 throughout the reaction. Unbound ADH and EDC were removed by

Please cite this article in press as: Kothari N, et al. Preparation and testing of a Vi conjugate vaccine using pneumococcal surface protein A (PspA) from *Streptococcus pneumoniae* as the carrier protein. Vaccine (2014), http://dx.doi.org/10.1016/j.vaccine.2014.08.041