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Enhanced protective immunity against pneumococcal infection with PspA DNA and protein

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Abstract

The effect of priming and boosting with *pspA*/EF5668 and purified recombinant PspA/EF5668 was examined. With this strategy CBA/N mice were protected against fatal challenge with *Streptococcus pneumoniae* EF5668. Anti-PspA antibody titers were elevated, and Western analysis with the immune serum demonstrated cross-reactivity with PspA from several different pneumococcal isolates, representing different PspA clades. Immune serum localized cross-reactive epitopes to the alpha-helical domain of PspA/Rx1 and PspA/EF5668. We demonstrated that DNA/protein prime-boost immunizations can enhance protective immunity against pneumococcal challenge.

Keywords: PspA; Prime-boost; DNA vaccine

1. Introduction

Streptococcus pneumoniae (pneumococcus) is an important human pathogen associated with significant morbidity and mortality [1]. Pneumococci colonize the upper respiratory tract and are a major cause of bacterial pneumonia, meningitis, bacteremia, and otitis media [2]. Pneumococcal infections are prevalent in children under the age of 5 and the elderly (>65 years of age). Prevention of these diseases is of great medical importance. Current pneumococcal vaccines provide limited coverage against more than 90 different capsular serotypes. Another shortcoming of these vaccines is cost, which limits usage in developing countries. Recently, much attention has been focused on the potential role of pneumococcal proteins as vaccine candidates [1]. One of the best characterized candidates is pneumococcal surface protein A (PspA) [3].

PspA is an important virulence factor found on the surface of all pneumococci [4]. PspA, pneumococcal surface protein C (PspC) [5,6], and pneumolysin [7], are important in colonization of the host and contribute to the ability of the pneumococcus to cause invasive disease. PspA plays a role in slowing the clearance of pneumococci from the blood of infected mice [4]. PspA has also been identified as a specific receptor for human lactoferrin [8], and hence may protect the pneumococcus from the bactericidal effects of apolactoferrin [9].

PspA is serologically variable [4,10] and is composed of four distinct domains [11–13]. The N-terminal half of the protein is the α-helical domain which contains protection-eliciting epitopes based on immunization studies with the full length and truncated PspA fragments [14–16]. The clade-defining region (CDR) located at the C-terminal end of the α-helical domain has been used to classify PspA into six clades based on their relatedness at the amino acid sequence level. This classification was further grouped into three families [17]. Based on the restricted cross-reactivity between families, it is proposed that PspA from family 1 and 2 should be incorporated in a vaccine formulation [18].

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Previous immunization studies using PspA from a single pneumococcus have demonstrated cross-protection against pneumococcal strains of different capsular serotypes [12,19]. PspA has been investigated for use in human vaccines because of its ability to elicit protective immunity, which prevents carriage, pneumonia, and bacteremia in mice. Recombinant PspA (rPspA) has been found to be safe and highly immunogenic in humans during Phase I clinical trials [20]. Genetic immunization, however, is less costly, lending itself to use in a worldwide vaccine effort. It is an effective means of eliciting both antibody and cell-mediated immune responses [21].

Genetic immunization with *pspA* was previously shown to elicit protection against pneumococcal infection in a mouse model [19,22]. Previous cross-protection studies have shown that PspA molecules from clades 1–4 can elicit protective antibody responses to pneumococci that differ from the immunogen by more than 50% within the α-helical portion of the molecule at the amino acid level [3,12,23].

In this study, we evaluated the potential of a fragment encoding the α-helical domain of *pspA*/EF5668 to elicit protection following genetic immunization. This PspA variant represents a PspA clade distinct from that of PspA/Rx1 [12]. EF5668 represents PspA from clade 4, family 2, which is a difficult pneumococcal strain to protect against. Our results demonstrate that genetic immunization with *pspA*/EF5668 can elicit protection that is mediated by specific antibody responses. Furthermore, we have demonstrated that the incorporation of DNA and protein in a prime-boost strategy leads to an enhanced response to PspA and protects mice from a lethal challenge.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The *S. pneumoniae* strains used in this study are described in Table 1. Pneumococci were grown to mid log phase in Todd–Hewitt broth supplemented with 0.5% yeast extract at 37 °C and harvested by centrifugation. The frozen stocks were maintained in 10% glycerol at −80 °C. Serial dilutions were plated on blood agar and incubated at 37 °C in 5% CO_2 to enumerate bacteria colony forming units (CFU/ml). *Escherichia coli* DH5α (Invitrogen, Carlsbad, CA, USA) with plasmids was grown in Luria–Bertani medium supplemented with kanamycin (50 μg/ml) at 37 °C with shaking.

Table 1
Pneumococcal strains used in the study

Strain	Capsule serotype	PspA clade	Reference
D39	2	2	[32]
A66	3	1	[32]
WU2	3	2	[4]
EF3296	4	3	[33]
EF5668	4	4	[12]
EF6796	6A	2	[15]
DBL6A	6A	1	[34]
Rx1	Nonencapsulated	2	[35]

2.2. Cloning of α-helical domain of PspA/EF5668 in eukaryotic expression vector

Construction and preparation of plasmids for genetic immunization were carried out as previously described [19]. Briefly, the 1 kb DNA fragment that encodes the α-helical domain of PspA/EF5668 was amplified by PCR using the primers described below and cloned into the pNGVL3 expression vector containing a cytomegalovirus (CMV) immediate-early enhancer and promoter upstream of a multiple cloning site [19]. The 5′ primer LSM302 (5′GGGGTACCCCATTATGGCTCCTGTAGCTAACCAG3′) was designed with a *KpnI* site at the 5′ end and a translational start site within the sequence. The 3′ primer LSM301 (5′GCTCTAGAGCTCATTCATCTCCATCAGGGCC3′) contained a *XbaI* site and a stop codon. After amplification, the product was subsequently cloned into the TA-TOPO vector (Invitrogen, Carlsbad, CA, USA) which was transformed into *E. coli* TOP-10 competent cells and then screened by PCR. Positive products were confirmed by restriction digestion analysis with *EcoRI* to ensure presence of the DNA insert. The DNA insert was subcloned into the pNGVL3 vector. The resulting plasmid was designated pJB100EF. After confirmation of the PspA/EF5668 coding sequence, plasmid DNA was purified using a Qiagen Endofree Plasmid Giga kit (Qiagen, Santa Clarita, CA, USA).

2.3. In vitro expression of PspA

Expression of PspA from pJB100EF was analyzed in transiently transfected COS-7 (Green monkey fibroblasts) cells by using GenePorter™ 2 Transfection Reagent (Gene Therapy Systems, San Diego, CA, USA) according to the manufacturer's instructions. Cells were then suspended in sodium dodecyl sulfate loading buffer with 100 μg/ml of phenylmethylsulfonyl fluoride (PMSF) and boiled for 5 min. Western analysis was performed using the anti-PspA monoclonal antibody, XiR278 [24]. A histidine-tagged recombinant EF5668/PspA purified from *E. coli* was used as a positive control.

2.4. Mouse immunizations and challenge

Six to eight-week-old CBA/N (CBA/CAHN-BTK XID/J) mice (Jackson laboratory, Bar Harbor, ME, USA) received intramuscular (i.m.) injections of plasmid DNA on days 0 and 14. The plasmid was diluted to a concentration of 20 μg in 50 μl of Lactated Ringer's (LR) and given lingually. Mice were challenged on day 28 by intravenous (i.v.) injection of the designated pneumococcal strain diluted in 200 μl of LR.