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Human antibody response towards the pneumococcal surface proteins PspA and PspC during invasive pneumococcal infection☆

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Abstract

IgG antibodies against pneumococcal surface protein A, family 1 (PspA1) and family 2 (PspA2), protein C (PspC), and protein Hic were investigated in 41 patients with invasive pneumococcal disease. Pre-existing antibody levels against the four pneumococcal proteins were not significantly different from those found in 40 patients with non-pneumococcal bacteremia or 80 healthy controls. However, during convalescense a strong immune response developed especially against PspA, and there was a high degree of cross-reactivity between PspA- and PspC-antibodies. Our findings on immunogenicity and cross-reactivity suggest that in a future pneumococcal protein based vaccine, only a limited number of proteins could be sufficient.

Keywords: *Streptococcus pneumoniae*; PspA; PspC; Antibody response

1. Introduction

Streptococcus pneumoniae, a ubiquitous human pathogen, is a major cause of invasive infections such as community-acquired pneumonia and meningitis, and is a common cause of otitis media, sinusitis and bronchitis. Risk groups are primarily young children, the elderly, and patients with immunodeficiencies. Invasive disease still carries significant mortality, and together with the emergence of pneumococcal strains with multiple antimicrobial resistances the need for preventive strategies against this common pathogen has been emphasized. The potential advantage of pneumococcal protein based vaccines compared to polysaccharide vaccines would be protection against multiple pneumococcal serotypes and induction of memory responses with a longer duration and immunogenicity in all ages. In animal models, several pneumococcal proteins have been shown to elicit a protective immune response [1].

Pneumococcal surface protein A (PspA) is a surface protein expressed in all strains of pneumococci. The molecule has an N-terminal α-helical domain exposed on the bacterial surface and a C-terminal choline-binding repeat region responsible for the attachment to the cell wall [2,3]. PspA sequences are variable, especially in the N-terminal domain, and have been classified into three families based on sequence homologies. Ninety-five percent of pneumococcal strains carry PspA of family 1 (PspA1) or family 2 (PspA2) [4], and there is a significant serological cross-reactivity between the different families. PspA interferes with complement function and has been shown to function as a virulence factor for pneumococcal infection [5].

Pneumococcal surface protein C (PspC) is strucurally related to PspA but has a distinct α-helical N-terminal region

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[6]. The protein is present in approximately 75% of all strains of *S. pneumoniae* and interferes with the complement system by the binding of factor H. Hic, the factor H-binding inhibitor of complement, is a distinct variant of PspC, which is found mainly in serotype 3 strains of pneumococci. Sequence homology to PspC proteins of other strains is most dominant in the N-terminal region [7,8].

In the present study, we investigated the immune response towards PspA1, PspA2, PspC, and Hic in patients with pneumonia and pneumococcal bacteremia, and correlated the levels of specific IgG antibodies to the presence of the respective surface proteins in strains isolated from the patients.

2. Materials and methods

2.1. Patients and sera

Serum samples were obtained from 41 patients with invasive infection with pneumococcal bacteremia (median age 51 years, age range 30–83 years, male/female ratio 22/19) and 40 patients with non-pneumococcal bacteremia (median age 63 years, age range 21–92 years, male/female ratio 13/27) that were hospitalized at the Clinic of Infectious Diseases, Lund University Hospital, Sweden, between 1992 and 2003. No patients with bronchial asthma, chronic obstructive pulmonary disease, previous pneumonia, malignancy or immunosuppressant therapy were included in the study. In the pneumococcal bacteremia group all patients had characteristic pneumonial infiltrates on chest X-rays together with positive blood cultures. The patients in the non-pneumococcal bacteremia group had growth in blood cultures of *Escherichia coli* ($n=28$), *Salmonella species* ($n=3$), *Staphylococcus aureus* ($n=3$), *Neisseria meningitidis* ($n=2$), *Campylobacter jejuni*, *Capnocytophaga canimorsus*, *Klebsiella pneumoniae* or *Streptococcus bovis* ($n=1$). Acute sera were obtained within 1–4 days after onset of infection (mean/median: 2.6/3 days) from the pneumococcal bacteremia group and after 1–5 days (mean/median: 2.3/3 days) from the non-pneumococcal bacteremia group. Convalescent sera were obtained from 22 patients with pneumococcal bacteremia >7 days after onset of infection. Serum samples from 80 healthy Swedish blood donors (median age 50 years, male/female ratio 40/40) were also collected.

2.2. Bacterial isolates

Serotyping of the pneumococcal strains was performed at the Department of Clinical Microbiology, Lund University Hospital, using the quellung reaction [9] with antisera from Statens Seruminstitut, Copenhagen, Denmark.

2.3. PCR analysis

To analyze the presence of *psp* genes in different pneumococcal isolates, PCR template DNA was prepared by boiling bacteria for 5 min in sterile water. Cell debris was removed by centrifugation and 1 μl of the boiled lysate was used for PCR amplifications. The primer pspA-F (5′-CCGGATCCAGCGTCGCTATCTTAGGGGCTGGTT-3′) is located in the highly conserved 5′-end of the *pspA* gene, while the primers pspA-R1 (5′-TTTCTGGCTCAT(CT)AACTGCTTTC-3′) and pspA-R2 (5′-TGGGGGTGGAGTTTCTTTCTTCTTCATCT-3′) correspond to diverging regions and distinguish between *pspA* families 1 and 2, respectively. Similarly, the primer pspC-F (5′-CGACGAATAGCTGAAGAGG-3′) is located in a conserved region upstream of the *pspC* locus, while the primers pspC-R (5′-CCACATACCGTTTTCTTGTTTCCAGCC-3′) and hic-R (5′-TAGATCC(AT)GT(AT)GATGGCAA-3′) correspond to regions that discriminate between *pspC* and the less common *hic*-like alleles. Each isolate was analysed with four primer pair combinations, thus determining the type of gene found in the *pspA* and *pspC* loci. PCR products were analyzed by agarose (0.8%) gel electrophoresis.

2.4. Proteins

PspA family 1 (aa 1–303), PspA family 2 (aa 1–411) and PspC (aa 1–445) proteins were from recombinant cloned fragments in pET20b (Novagen Inc.) as previously described [5,6]. The three recombinant proteins were purified by nickel affinity chromatography according to the Novagen system. A fragment of Hic, covering aa 1–223 of the mature Hic protein, was expressed as a GST fusion protein as previously described [7].

2.5. ELISA methods

ELISA experiments were performed as previously described [10]. After coating microtiter plates (Maxisorb, NUNC) with different pneumococcal antigens (1 μg/ml), a fixed dilution (1/400) of serum samples from patients and from healthy individuals were added to the wells. Bound antibodies were detected by a horseradish peroxide-conjugated antibody against human IgG diluted 1/3000 (Bio-Rad). After the substrate reaction the OD at 415 nm was determined. A blank without serum was included in quadruplicate on each plate and these OD values were subtracted from the values obtained with the serum samples. An ELISA index was calculated by dividing the mean OD value for each serum sample by the mean OD value for a standard positive serum sample run in quadruplicate on each plate.

2.6. Statistical evaluation

The two-sample z-test, assuming unequal variances, was used for comparing antibody levels in different groups. p values <0.05 were regarded as statistically significant.