Pneumococcal Surface Protein C Contributes to Sepsis Caused by *Streptococcus pneumoniae* in Mice

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The role of pneumococcal surface protein C (PspC; also called SpsA, CbpA, and Hic) in sepsis by *Streptococcus pneumoniae* was investigated in a murine infection model. The pspC gene was deleted in strains D39 (type 2) and A66 (type 3), and the mutants were tested by being injected intravenously into mice. The animals infected with the mutant strains showed a significant increase in survival, with the 50% lethal dose up to 250-fold higher than that for the wild type. Our findings indicate that PspC affords a decisive contribution to sepsis development.
from the wild type in growth and competence development (data not shown).

**Virulence of type 2 mutant.** Mouse-passaged pneumococci, prepared as previously described (17), were used for inocula. Before being infected, the mice were kept under an infrared lamp (200 W) for 2 to 3 min and then given an intravenous (i.v.) injection into the tail vein. Bacteria were delivered in a total volume of 200 μl. The animals were monitored for 10 days. Differences in survival were analyzed by the Mann-Whitney-Wilcoxon test, considering the time point when mice died; for statistical purposes, animals still alive after 10 days were assigned a time to death of 240 h. Groups of 7-week-old female outbred MF1 mice (n = 6 to 8) (Harlan Nossan) were inoculated with a range of bacterial inocula (from 10^3 to 10^8 CFU) of either FP58 (type 2) or its isogenic pspC mutant, FP30. Differences in survival were detected only at the lowest doses. At a dose of 10^4 CFU per animal, infection by wild-type pneumococci was lethal in 87.5% of mice, while none of those inoculated with the mutant died (P = 0.0016). The 50% lethal dose (LD_{50}) was 2 × 10^3 CFU for the wild type and 3.7 × 10^3 CFU for the mutant, indicating a 19-fold attenuation in virulence (Fig. 2A).

**Virulence of type 3 mutant.** Experimental sepsis was repeated with type 3 pneumococci by infecting both MF1 (outbred) and CBA/Jico (inbred) mice with HB565 (wild type) and FP20 (pspC mutant) (Fig. 2B and C). CBA/Jico mice were chosen because of their sensitivity to pneumococcal infection (8). MF1 mice infected with the mutant showed an increase in survival for inoculum doses ranging from 10^3 to 10^7 CFU. Differences in survival (by Mann-Whitney-Wilcoxon test) were significant at the dose of 10^6 CFU (P = 0.027) (Fig. 2B). Survival of CBA/Jico mice was also significantly different both at 10^5 (P = 0.0008) and 10^6 (P = 0.0019) CFU, as all mice infected with the mutant survived and all control mice died (Fig. 2C). In MF1 outbred mice, the LD_{50} was 10^5 CFU for the wild type and 2.5 × 10^7 CFU for the mutant, while for CBA/Jico inbred mice, the LD_{50} was 2 × 10^4 CFU for the wild type and 3.2 × 10^6 CFU for the mutant (Fig. 2B and C). Depending on the mouse strain, PspC-negative mutants of type 3 pneumococci showed a 160- to 250-fold reduction of virulence in the i.v. sepsis model.

Previous studies using sepsis infection models were not able to show a convincing virulence attenuation of pspC mutants. When tested by being injected intraperitoneally, pspC mutants were not significantly reduced in virulence (2, 19), and only minor differences in time to death were found with an i.v. sepsis model (1). The present data show that PspC affords a decisive contribution to sepsis development, with mutants showing an attenuation of virulence of up to 250-fold. Our data were obtained with two different pneumococcal serotypes and both inbred and outbred mice. In our opinion, the i.v. route of inoculation and the use of a wide range of infecting doses were indeed instrumental in showing the important role of PspC in pneumococcal sepsis.

While PspC is required for nasopharyngeal colonization and lung infection in the mouse model (1, 19, 21), here we show that it is also very important for sepsis. PspC binds fH of the complement system in different pneumococcal serotypes, and its fH binding efficacy was demonstrated to vary among different strains (7, 14). Pneumococci can escape complement attack and opsonophagocytosis by recruiting fH with PspC in vitro.

![Diagram](image)

**FIG. 1.** Representation of the genetic construct for pspC deletion. The construct is constituted of a gene conferring resistance to chloramphenicol (cat) under the control of the ami promoter (6), flanked by the regions upstream and downstream of pspC. Upon transformation, pspC flanking sequences allow integration of the amic/cat cassette and deletion of pspC. The upstream region (540 bp) is complementary to nt 6392 to 5843 on section 190 of the TIGR4 genome (GenBank accession no. AE007507). The region between nt 541 and 582 corresponds to the pneumococcal ami promoter (nt 175 to 216, GenBank accession no. X17337), whereas the sequence spanning nt 583 to 1390 derives from the *Staphylococcus aureus* pC194 plasmid containing the cat gene (nt 1103 to 1910, GenBank accession no. V01277). The downstream flanking segment (526 bp) is composed as follows: nt 1391 to 1721 correspond to nt 2268 to 2598 of the A66 pspC locus (GenBank accession no. AF252857), whereas nt 1722 to 1916 are complementary to nt 3541 to 3347 on section 190 of the TIGR4 genome.
FIG. 2. Experimental murine model of pneumococcal sepsis. (A) Twelve groups of MF1 outbred mice (n = 6 to 8) were injected i.v. with different inocula (10^3 to 10^8 CFU) of either the type 2 wild type (FP58, open triangles) or the pspC mutant (FP30, solid circles). (B) Eight groups of MF1 mice (n = 6) were inoculated i.v. with increasing bacterial doses (10^3 to 10^8 CFU) of the type 3 HB565 (wild type, open triangles) or pspC mutant FP20 (solid circles). (C) Twelve groups of CBA/Jc inbred mice (n = 6 to 8) were infected i.v. with bacterial inocula (10^3 to 10^8 CFU) of either HB565 (open triangles) or FP20 (solid circles). Survival was recorded for 10 days. The percentage of mice surviving versus the dose of bacteria is shown. The LD<sub>50</sub> are indicated by the dotted lines.

(15) Since the binding of <i>S. pneumoniae</i> to fH was shown to prevent complement-mediated phagocytosis, bacterial survival in the bloodstream should be compromised in PspC-deficient mutants. PspC-deficient mutants cannot bind fH (7, 14), and this inability is probably the key reason for the attenuation of virulence of these mutants in the sepsis model. This effect is more evident in type 3 than in type 2 <i>S. pneumoniae</i>, reflecting the higher binding affinity of fH observed for the type 3 strain.

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