TITLE: CppA is involved in of *Streptococcus sanguinis* evasion to complement immunity

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CppA is a putative C3 degrading proteinase found in the genome of *Streptococcus sanguinis*, a commensal species of the oral cavity further associated with cardiovascular opportunistic infections. The aim of this study was to investigate the role of CppA in *S. sanguinis* susceptibility to complement-mediated immunity. An isogenic *cppA* mutant (SKcppA) was obtained in strain SK36 by double cross-over recombination with a null allele. A complemented strain (SKcppA+) was also generated by transforming SKcppA with shutte plasmid containing a copy of *cppA*. C3b deposition and opsonophagocytosis by PMN were compared between SKcppA and parent (SK36) or complemented mutant (SKcppA+). Briefly, strains were incubated with 20% human serum (30 min; 37°C) or PBS, and surface C3b probed with FITC-conjugated anti-human C3b antibody (1:300, 40 min). Mean fluorescent intensity (MFI) of surface bound C3b was determined by flow cytometry. FITC-labeled strains were also used to assess opsonophagocytosis of PMN isolated from human blood. Briefly FITC-labeled strains were treated with 20% human serum or PBS and exposed to PMN (MOI 200:1 at 37ºC, 5 min.), and the numbers of PMN with intracellular bacteria determined by flow cytometry. The mean levels of C3b deposition were 38% higher in SKcppA (mean MFI: 874 $\pm$ 138) compared to SK36 (mean MFI: 539 $\pm$ 176) (Kruskal-Wallis, $p<0.05$). The percentages of PMN with intracellular was significantly higher in SKcppA (44.5% $\pm$ 5.56) than in SK36 (25.15% ± 3.14) ($p <0.05$) when strains were treated with serum. No significant difference in the frequencies of phagocytoses was observed between strains treated with PBS. Additionaly, the complemented mutant (SKcppA+) showed phenotypes of C3b deposition and opsonophagocytosis similar to those of SK36 ($p>0.05$). These results show that *cppA* is required for *S. sanguinis* evasion to C3b deposition and serum-mediated phagocytosis by human PMNs, implying important roles of *cppA* in *S. sanguinis* systemic virulence.

KEYWORDS: *Streptococcus sanguinis*, C3b deposition, complement system, CppA, neutrophils.
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