Extracellular polysaccharide influences on the susceptibility of *Streptococcus mutans* to opsonization by the complement system

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Resumo:

Streptococcus mutans (SM) is a major pathogen of dental caries and is commonly involved in infective endocarditis. Caries virulence of SM is dependent of expression of genes (*gtfB, gtfC, gbpB, ftf*) for the production of and interaction with extracellular polysaccharides (EPS), major components of the extracellular matrix of cariogenic biofilms produced from sucrose. The aim of this study was to investigate the role of EPS in SM susceptibility to deposition of C3b of the complement system, major opsonins of blood and host tissues. To this end, we compared intensities of C3b/iC3b deposition between the SM wild type strain UA159 and UAcov, an isogenic mutant of the transcriptional repressor of *gtfB/C, gbpB* and *ftf* (CovR), which overproduces surface-associated EPS from sucrose (UAcov). Strains were grown from fresh cultures in BHI or Chemically Defined Medium (CDM) added or not of 0.01 or 0.1% sucrose. Then, after incubation with 20% serum (30 min; 37°C) or PBS, surface C3b was probed with FITC-conjugated anti-human C3 (1:300), detected by flow cytometry and expressed as measures of geometric means of fluorescent intensity (MFI). In BHI, amounts of surface C3b in UAcov (mean MFI: 187.54 ± 95.35) were 4.1-fold lower than in UA159 (mean MFI: 771.14 ± 91.23) (Kruskal-Wallis, *post hoc* Tukey: $p<0.05$). Grow in 0.1% sucrose BHI impaired C3b deposition in UAcov (36.22 ± 3.21) and UA159 (307.76 ± 23.48), compared to strains grown in BHI ($p< 0.05$). Growth in CDM (w/o trace amounts of sucrose) promoted 2.7-fold higher levels of C3b in UAcov (507.4 ± 201.7) and 1.2-fold higher in UA159 (991.5 ± 202.53), when compared the same strains grown in BHI ($p<0.05$). Deposition of C3b decreased with addition of sucrose to CDM in a dose dependent manner. In UA159, mean MFIs in 0.01 and 0.1% sucrose were respectively 674.66 (± 56.83) and 326.4 (± 74.3). In UAcov, these values were respectively 181.40 (± 94.61) and 82.31 (± 59.80). However, even in CDM w/o, UAcov showed a 2-fold lower level of C3b than UA159 ($p <0.05$), suggesting that other surface components than EPS regulated by CovR influences on SM susceptibility to C3b deposition. The data show that inactivation of CovR reduces the deposition of complement C3b and this results, in part, from the overproduction of EPS from sucrose.

Palavras-chaves: *Streptococcus mutans*, complement system, infective endocarditis, virulence, extracellular polysaccharide.

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