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Cooperativity of streptococcal surface proteins in binding platelets and extracellular matrix

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Poster 34

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Abstract

Background The ability of the oral bacterium *Streptococcus gordonii* to bind platelets and extracellular matrix (ECM) contributes to its virulence in infective endocarditis. Surface protein PadA has recently been found to be crucial for platelet activation. The hypothesis is that PadA is dependent upon another surface protein (Hsa) for *S gordonii* to activate platelets and adhere to ECM. We aimed to determine the respective roles of Hsa and PadA in platelet adhesion, and ascertain PadA function in ECM binding.

Methods *S gordonii* DL1 Δ*padA* and Δ*padA*Δ*hsa* knockout mutants were generated by allelic replacement. Mutants were complemented using PadA or Hsa expression plasmids under the control of a nisin-inducible promoter. PadA expression by knock-out and knock-in strains was confirmed by western immunoblot of cell-wall protein extracts. Platelet adhesion to bacteria was measured under static conditions in a p-nitrophenol assay. Bacterial adhesion to ECM proteins was determined by crystal violet assay.

Findings Static platelet adhesion by *S gordonii* Δ*padA* mutant was reduced by 30% compared with wild-type. Δ*padA*Δ*hsa* was more than 80% reduced in binding platelets. Expression of *padA* in Δ*padA*Δ*hsa* failed to restore any platelet adhesion, whereas expression of *hsa* in Δ*padA*Δ*hsa* mutant restored binding to 70% of wild-type levels. The Δ*padA* mutant cells were reduced in binding cellular fibronectin by 25% and vitronectin by 60%. Deletion of *hsa* abrogated vitronectin binding. Complementation of Δ*padA*Δ*hsa* with either *hsa* or *padA* alone did not restore vitronectin binding.

Interpretation PadA requires the presence of Hsa to interact with platelets. PadA has a minor role in binding cellular fibronectin alongside other surface adhesins. In vitronectin binding, Hsa requires the presence of functional PadA for efficient binding. These results suggest that the *S gordonii* surface-anchored proteins Hsa and PadA work in concert to mediate processes relevant to host colonisation and pathogenesis.

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Contributors

HJ, AN, and SK conceptualised the study. HJ, AN, and SK devised methods. JH, JB, SK, and AN conducted investigations. JH drafted the abstract. HJ and AN reviewed and edited the abstract. HJ, AN, and SK supervised the study.

Declaration of interests

We declare no competing interests.