Structural and functional characterization of streptococcal SpnA-host interactions

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Every major health organization has identified infectious diseases and the concomitant increased resistance to antibiotics as serious global threats. We address these problems by studying the *Streptococcus pyogenes* bacterium; an important pathogen, ranking globally among the top ten causes of mortality from infectious diseases with an estimated >150 000 annual deaths. During infection, *S. pyogenes* encodes for a variety of virulence factors; many of these virulence factors have not been thoroughly studied. One of these virulence factors is the *S. pyogenes* nuclease A, SpnA, originally described as a cell-wall associated DNAse degrading host neutrophil extracellular traps (NETs) via its C-terminal endo/exonuclease domain.

We study the structure and function of SpnA by combining quantitative and structural proteomics mass spectrometry with integrative structural biology methods. Our results combining AlphaFold modeling, single-particle cryoEM and hydrogen-deuterium exchange mass spectrometry demonstrate that SpnA harbors disordered regions in its N-terminus. We have demonstrated by combining affinity-purification and bacterial surface adsorption mass spectrometry, that in human blood plasma SpnA binds the complement system membrane attack complex (MAC). By combining affinity-purification with crosslinking mass spectrometry we have further been able to determine that SpnA specifically targets the MAC assembly intermediate C5b-C7. We hypothesize that SpnA acts like another streptococcal virulence factor SIC, streptococcal inhibitor of complement. Which targets the C5b-C7 complex preventing its insertion into the streptococcal membranes, hence preventing bacterial lysis. Our results indicate that in addition to degrading host DNA in NETs, SpnA mediates other central functions in host immune evasion in streptococcal pathogenesis.