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Human and murine PMN migration is reduced by SpyCEP, a streptococcal protease responsible for IL-8 cleavage

N. Chiappini*, A.K. Seubert, M. Gallotta, V. Nardi Dei, C. Faralla, D. Serruto et al

Novartis V&D Siena, Italy

Streptococcus pyogenes is a frequent cause of infection, ranging from common and mostly innocuous conditions such as bacterial pharyngitis and erysipelas, to invasive and serious diseases. A recently identified virulence factor is SpyCEP, a subtilisin-like protease that cleaves and inactivates human IL-8, thereby impeding recruitment of neutrophils. SpyCEP has been also characterized as vaccine antigen against infection mediated by *S. pyogenes*. In this work we attempt to further understand the activity of SpyCEP applying biochemical, genetic and cellular approaches. Analyzing protein extracts from the bacterial surface and spent medium we show that SpyCEP is progressively released throughout growth, with very little SpyCEP remaining surface-associated in stationary phase. In exponential phase bacteria, surface-associated SpyCEP hydrolyzes IL-8, while two mutant strains deficient in SpyCEP show no such effect. Recombinant SpyCEP was purified, and the activity versus IL-8 was comparable to native SpyCEP in bacterial extracts. To better understand SpyCEP substrate recognition, the IL-8 structure was analyzed for potential amino acid residues involved in recognition of the cleavage site. W.t. IL-8 and a mutant IL-8(R60E) was cloned, expressed and purified. A time-course cleavage assay showed that processing of IL-8(R60E) by SpyCEP is less efficient compared to w.t. IL-8. Using a murine and human *in vitro* model of PMN recruitment, we show that preincubation of the chemoattractants IL-8 and KC with SpyCEP inhibited transmigration of PMN. On the contrary, preincubation of SpyCEP with rabbit serum raised against SpyCEP partially restores IL-8/KC dependent PMN migration suggesting that an efficient immune response against this vaccine antigen may interfere with its enzymatic activity.

Keywords: GAS, SpyCEP, IL-8, PMN