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Neutrophil Serine Proteases in Health and Disease

Akademisk avhandling

som för avläggande av odontologie doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, den 7 Juni 2022, klockan 09:00

av Felix P Sanchez Klose

Fakultetsopponent:
Per-Arne Oldenborg, Professor
Umeå Universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. Björnsdottir H, Dahlstrand Rudin A, Klose FP, Elmwall J, Welin A, Stylianou M, Christenson K, Urban CF, Forsman H, Dahlgren C, Karlsson A, Bylund J. **Phenol-Soluble Modulin α Peptide Toxins from Aggressive *Staphylococcus aureus* Induce Rapid Formation of Neutrophil Extracellular Traps through a Reactive Oxygen Species-Independent Pathway.** *Frontiers in Immunology 2017;8:257.*

II. Sanchez Klose FP, Björnsdottir H, Dahlstrand Rudin A, Persson T, Khamzeh A, Sundqvist M, Thorbert-Mros S, Dieckmann R, Christenson K, Bylund J. **A rare *CTSC* mutation in Papillon-Lefèvre Syndrome results in abolished serine protease activity and reduced NET formation but otherwise normal neutrophil function.** *PLoS One 2021;16:e0261724.*

III. Sanchez Klose FP, Björnsdottir H, Dahlstrand Rudin A, Kårelius A, Persson T, Khamzeh A, Thorbert-Mros S, Ransjö M, Christenson K, Bylund J. **Regulation of Bone Resorption and Inflammation by Neutrophil Serine Proteases.** *In manuscript.*

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR ODONTOLOGI

GÖTEBORGS UNIVERSITET

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Neutrophil Serine Proteases in Health and Disease

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Abstract

Neutrophils are filled with many antimicrobial agents, including the neutrophil serine proteases (NSPs); a group of proteases including Elastase, Proteinase 3, and Cathepsin G. Synthesized as inactive proforms, the NSPs are activated through proteolytic processing by Cathepsin C (CTSC). The NSPs have been demonstrated to degrade microbes *in vitro*. Therefore, NSPs have been described as crucial for microbial killing and are also believed to be critical for NETosis, a neutrophil-specific type of cell death capable of ensnaring extracellular microbes.

Periodontitis is a destructive inflammation of the tooth supporting tissues initiated by colonizing bacteria. Neutrophils are abundantly present in the gingival crevice and more are recruited during periodontitis. Certain defects in neutrophil functions, like the Papillon-Lefèvre Syndrome (PLS), are associated with severe forms of periodontitis. PLS is a rare autosomal recessive loss-of-function mutation in the *CTSC* gene. The subsequent absence of CTSC activity in PLS neutrophils, results in absence of NSP activity. Interestingly, patients with PLS do not typically display increased susceptibility to opportunistic infections. The cardinal symptom of PLS is instead a rapidly progressing periodontitis with prepubertal onset.

By studying PLS neutrophils, this thesis shows that NSP activity is indeed important for certain but not all types of NETosis (**paper I**). The work also demonstrates that PLS neutrophils from two families with distinct *CTSC* mutations, are indeed devoid of CTSC as well as NSP activities (**paper II**). Despite this, PLS neutrophils appeared to function normally with the exception of NETosis. To explain how lack of NSP activity results in periodontitis, it was hypothesised that NSPs may regulate inflammation by the cleavage of cytokines. *In vitro*, it was shown that whereas normal neutrophils were capable of degrading certain cytokines, PLS neutrophils were unable to do so (**paper III**). Most notably, IL-17A, IL-23, CCL20, and RANKL, all of potential importance for driving periodontal pathology, were quite susceptible to NSP-mediated cleavage. Other cytokines, e.g., IL-17F and CCL2, were resistant to NSP mediated cleavage indicating that degradation was not indiscriminate.

In conclusion, this thesis shows that NSP activity is important for certain types of NET formation, but otherwise dispensable for basic neutrophil function. It also demonstrates that NSP activity may be able to regulate inflammatory processes and could help to explain the aggressive periodontal pathology seen in patients with PLS.

Keywords: Neutrophil, Periodontitis, PLS, CTSC, NSP, Elastase, Proteinase 3, Cathepsin G, NETosis.

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