Inflammatory responses of gingival and periodontal ligament fibroblasts to Porphyromonas gingivalis

Porphyromonas gingivalis is an oral pathogen strongly associated with periodontitis, a chronic inflammatory disease of the tooth-supporting tissues. In periodontitis, a constant interaction between host cells and bacteria leads to tissue destruction. A major clinical symptom of periodontitis is degradation of the alveolar bone due to excessive resorptive activity by osteoclasts. Among host cells that are involved in the inflammatory response to bacteria in periodontitis are gingival and periodontal ligament fibroblasts. In this thesis we investigated the responses that viable P. gingivalis elicits in gingival and periodontal ligament fibroblasts from periodontitis patients and healthy controls, and the role that these responses may play in the pathogenesis of periodontitis.

We found that viable P. gingivalis induced the gene-expression of interleukins (IL)-1β, IL-6, IL-8, tumor necrosis factor-α (TNFα), monocyte chemotactic protein-1 (MCP-1), and RANTES (regulated upon activation, normal T-cell expressed and secreted) in both fibroblast subsets from periodontitis patients and from healthy donors. The polysaccharide capsule of P. gingivalis reduced these inflammatory responses in gingival fibroblasts, possibly by preventing its recognition by the host cell. The cytokine responses of both fibroblast subsets to P. gingivalis were quite similar in cells from periodontitis patients and healthy persons. Still, considerable heterogeneity in the scale of these cytokine responses existed, which was apparent not only between individuals, but also between gingival and periodontal ligament fibroblasts within a single individual. This heterogeneity possibly indicates a variable susceptibility towards P. gingivalis or periodontitis among different cell types, and among different individuals.

Interestingly, both fibroblast types from periodontitis patients appeared to be more prone to recognise and respond to P. gingivalis. Periodontal ligament fibroblasts from periodontitis patients had higher gene-expression levels of the receptors TLR1, TLR4, TLR7 and CD14. Furthermore, periodontal ligament fibroblasts from patients had a lower expression of NFκBIL-1, an inhibitor of activation of transcription factor NF-κB. Gingival fibroblasts from periodontitis patients had stronger responses to P. gingivalis with respect to the expression of the receptors TLR1, TLR2, and TLR7.

Periodontal ligament fibroblasts from persons that carried P. gingivalis in their subgingival plaque were more responsive to an in vitro P. gingivalis challenge, than fibroblasts from non-carriers. After a challenge with P. gingivalis, in periodontal ligament fibroblasts from carriers, gene-expression of the receptors TLR1 and TLR7, the transcription factor NF-κB1, the pro-inflammatory cytokine IL-1β, and the chemokines RANTES and MCP-1 were induced more strongly. A prior encounter with P. gingivalis may thus have rendered these periodontal ligament fibroblasts more responsive to a new challenge. Interestingly, this difference between carriers and non-carriers did not exist in gingival fibroblasts. Thus, gingival and periodontal ligament may differentially interact with and respond to P. gingivalis, in periodontitis and health.

Since the cytokines produced by gingival and periodontal ligament fibroblasts in response to P. gingivalis are known to play a role in osteoclast formation, we hypothesized that conditioned medium of gingival fibroblasts challenged with P. gingivalis influences the formation of osteoclasts in vitro. Also, P. gingivalis-produced factors may influence osteoclast formation directly. We found that both
conditioned medium from viable *P. gingivalis* alone, and conditioned medium from gingival fibroblasts challenged with viable *P. gingivalis*, stimulated the formation of osteoclasts in a similar way.

Conditioned medium from dead *P. gingivalis* alone, in contrast, had no stimulatory effect, whereas medium from gingival fibroblasts challenged with dead *P. gingivalis* did. This suggests that *P. gingivalis* can stimulate osteoclast formation in different ways; directly, via factors actively produced by viable *P. gingivalis*, and which we found to probably be LPS, and indirectly, by stimulating GF to secrete cytokines that induce osteoclast formation.

Surprisingly, the effect of conditioned medium on osteoclast formation depended on the culture conditions of the precursor cells, being the presence of RANKL and M-CSF. When RANKL and M-CSF were added, conditioned media inhibited osteoclast formation, in contrast to the stimulatory effect we had found without RANKL and M-CSF. The presence of bacterial components, pro-inflammatory cytokines, and RANKL and M-CSF during inflammation may thus all contribute to osteoclast formation in periodontitis.

In conclusion, the research presented in this thesis implies that gingival and periodontal ligament fibroblasts contribute significantly to the host response in *P. gingivalis*-induced periodontitis, through the production of pro-inflammatory cytokines and chemokines. The interactions between *P. gingivalis* and gingival or periodontal ligament fibroblasts lead to inflammatory responses that may play important roles not only in periodontitis-related osteoclast formation and bone destruction, but also in a hosts’ susceptibility towards periodontitis.