Immunological aspects of the pathophysiology of periodontitis

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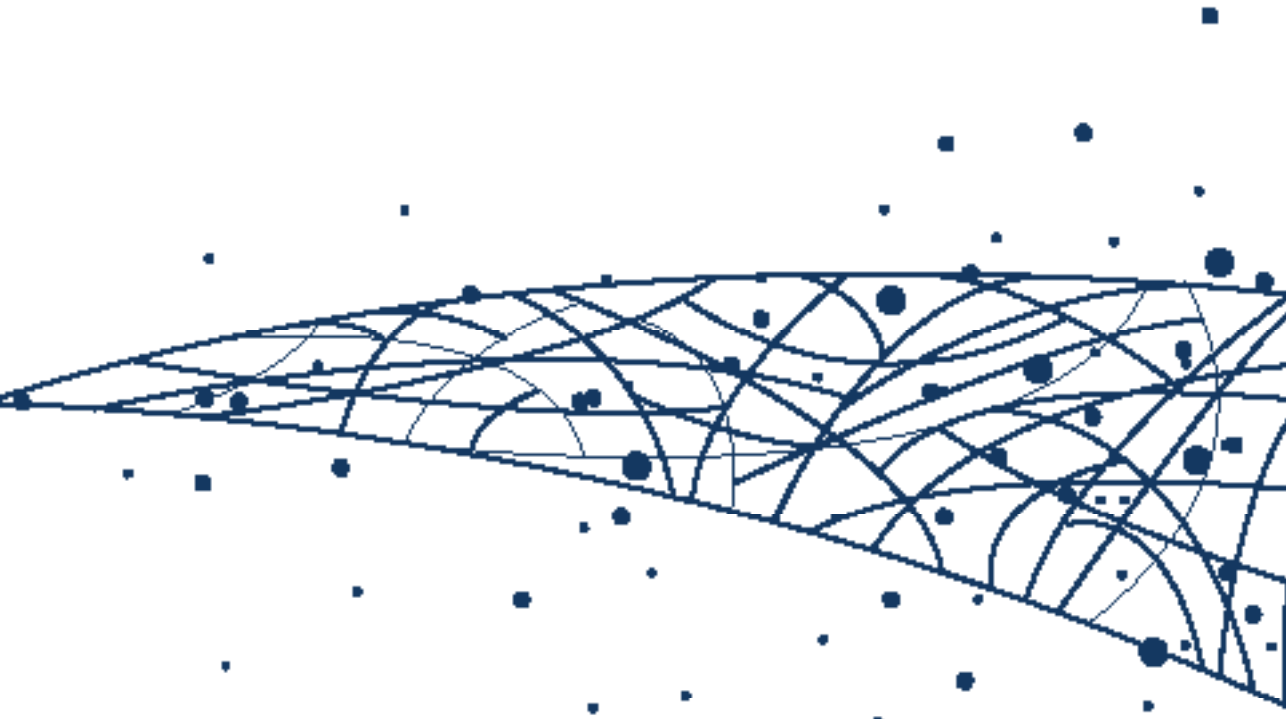
Summary



SUMMARY

Periodontitis, a complex chronic inflammatory disease of the tooth-supporting tissues, it is next to dental caries the second most common oral disease in humans. The disease is the result of complex interactions between the host response and the environment. The cause of periodontitis is still under investigation, however, it is suggested that an aberrant inflammatory response (inherited and acquired during life) exacerbates a shift in the periodontal symbiotic microbiome. This results in hyper- or hyporesponsiveness and/or lack of sufficient resolution of inflammation. Research into the key cellular players and immune mediators that stimulate alveolar bone resorption in periodontitis has been described in some detail. The immunological interactions leading to this aberrant inflammatory response and to alveolar bone resorption, are still not completely known. In this thesis, several immunological aspects of the pathophysiology of periodontitis are described.

The oral polymorphonuclear leukocytes (oPMN) are the most abundant innate immune responders present in the oral cavity. Circulatory PMNs (obtained through venipuncture) have been widely studied using various functional assays. In the first experimental chapter of this thesis (**Chapter 2**), the potential of oral polymorphonuclear leukocytes (oPMNs) in maintaining oral health was compared to circulatory PMNs (cPMNs) with respect to their chemotactic, phagocytic, and NET formation properties. In contrast to cPMNs, oPMNs showed exhausted capacity for efficient directional movement which was explained by the significantly lower response to and membrane receptor expression of N-formylmethionyl-leucyl-phenylalanine (fMLP), which is a chemotactic factor for PMNs. This may be the result of their migration through the oral tissues into the oral cavity, being a highly hostile' ecosystem. Furthermore, increased adhesion and internalization of various micro-organisms by oPMNs was observed. oPMNs formed 13 times more NETs than stimulated cPMNs and 32 times more than unstimulated cPMNs. Thus, oPMNs most likely contribute to maintaining a balanced oral ecosystem, as their ability to internalize microbes in conjunction with their abundant NET production remains after entering the oral cavity.

The inflammatory immune response in periodontitis involves the activation of PMNs that can entrap and eliminate pathogens by releasing neutrophil extracellular traps (NETs). Adequate NET clearance is a prerequisite for periodontal healing and abnormal NET degradation may negatively affect periodontal inflammation and its systemic co-morbidities such as atherosclerotic cardiovascular disease (ACVD). NETs play an important protective role by preventing bacterial dissemination into the vasculature and possibly lymphatics. On the other hand, NETs have been found to play a causative role in the formation of atherosclerotic plaques and venous thrombi Interestingly, previous research has shown beneficial effects of non-surgical periodontal therapy on several clinical and biochemical parameters of ACVD, including flow-mediated dilatation, intima media thickness, systolic blood pressure, and a decrease in activated platelets.

In **Chapter 3**, we aimed to investigate the ex vivo NET degradation capacity of plasma from 38 periodontitis patients compared to 38 controls (part 1). The NET degradation levels did not differ significantly between periodontitis patients and controls. In contrast to other studies, we did not match patients with control subjects. Therefore, differences in socio-demographic characteristics, smoking habits, and BMI may have influenced the comparison of NET degradation in the first part of our study and may have been why no significant difference was found between two groups. Despite this, analyses of covariance resulted in non-significant differences, suggesting that this was not a result of a multifactorial cause-effect relationship. The second objective of this study (part 2) was to investigate the effect of non-surgical periodontal therapy on the NET degradation levels in 91 patients over time. In this part, we investigated whether periodontal treatment would benefit NET degradation and thus could be a possible reason for the improved ACVD profiles of these patients. NET degradation levels significantly increased by 10% after non-surgical periodontal therapy and this increased capacity was maintained at 6 and 12 months, irrespective of systemic usage of antibiotics. We conclude that non-surgical periodontal therapy is beneficial for NET degradation capacity and could be an explanation for the improved ACVD profiles of these patients.

The chronic inflammatory cell infiltration of the periodontal soft tissues is accompanied by osteoclast-induced alveolar bone resorption, the hallmark of periodontitis progression. The ligand of the receptor activator of NF-kB (RANKL) is a key molecule in the formation of the bone resorbing cells: osteoclasts. PMNs are one of the most prominent cells in periodontitis lesions where they are often activated or in a hyperactive state. Therefore, we hypothesized that PMNs could conceivably play an important role in providing signals to trigger osteoclastogenesis activating pathological bone resorption in periodontitis. In **Chapter 4**, we attempted to validate the aforementioned hypothesis in two ways. In part A, we investigated whether oPMNs, as a model representing the activated PMNs from periodontitis lesions, express RANKL and whether they can be primed and activated in response to the continuous presence of extracellular stimulants (saliva, oral bacteria, shed epithelial cells, and cell debris) that are present in the gingival sulcus and oral cavity. Thus, RANKL expression was investigated on cPMNs and oPMNs taken from both healthy controls and periodontitis patients. In both cPMNs and oPMNs, the RANKL expression was minimal and did not differ significantly between periodontitis patients and controls. *In vivo*, a phenotypic transition must take place from cPMNs that come from a relatively sterile environment and that are triggered and phenotypically altered by the various inflammatory challenges after egressing into the periodontal tissues. In part B of this study, we, therefore, investigated whether cPMNs, after activation by the (bacterial or immunological) modulators lipopolysaccharides, interleukin (IL)-6, or tumor necrosis factor (TNF)-α, have the capacity to contribute to osteoclast formation. A caveat in this kind of studies, is the long duration of osteoclastogenesis assays, whereas PMNs are shortlived. To overcome this discrepancy, we fixed PMNs, ensuring enduring surface expression of RANKL, as used before in the context of

rheumatoid arthritis. Again, limited RANKL expression was detected on (stimulated) cPMNs. Furthermore, these limited levels of expression did not induce osteoclastogenesis when cocultured with pre-osteoclasts for 10 days. We report that, under the aforementioned experimental conditions, neither oPMNs nor naive or inflammatory triggered cPMNs induced osteoclastogenesis.

Gingival fibroblasts (GFs) present in the gingiva have the capacity to induce osteoclastogenesis and are thought to play a role in the recruitment of immune cells toward the inflamed periodontium. In **Chapter 5**, we aimed to study the cellular interactions of GFs with immune cells, including the contribution of GFs to osteoclast formation and their possible role in the proliferation of these immune cells. After 21 days, comparable numbers of osteoclasts were observed in GF cocultures with monocytes and cultures with PBMCs, indicating that the combination of T, B, and NK cells did not contribute extra. In GF-PBMC and GF-PBL cocultures, persisting mononuclear cells were interacting with osteoclasts. Significantly more T, B, and NK cells were identified in both GF-PBMC and GF-PBL cocultures compared to monocultures without GFs at all time points. This observed lymphocyte retention correlated with the expression of lymphocyte-function-associated antigen-1 (LFA-1) expression, which was significantly higher in GF-PBL cultures compared to GF-monocyte cultures. Furthermore, we investigated the expression of inflammatory cytokines that are evoked by this interaction. High tumor necrosis factor alpha (TNF-α) expression was only observed in the GF-PBMC cultures, indicating that this tripartite presence of GFs, monocytes, and lymphocytes was required for such an induction. Finally, we demonstrated that only the T cells proliferated in the presence of GFs. With this study, we concluded that GFs mediate the retention, survival, and selective proliferation of T lymphocytes.

During inflammation of the gums, GFs interact with heterogeneous cell populations of the innate and adaptive immune system that play a crucial role in protecting the host from pathogenic infectious agents. In the last experimental chapter of this thesis (**Chapter 6**), the effect of chronic inflammation, by exposing PBMCs or PBLs monocultures, and GF-PBMC cocultures to toll-like receptor (TLR)2 and TLR4 activators was investigated. Here, we assessed whether this influenced the leukocyte retention, survival, and proliferation. Chronic stimulation of PBMC-GF cocultures with TLR2 and TLR4 agonists induced a reduction of NK, T, and B cells, whereas the number of TLR-expressing monocytes were unaffected. TLR2 agonists doubled the T cell proliferation in GF-PBMC cocultures and PBMC monocultures, but given the net loss of T cells, a selection of T cells must be prone to divide upon TLR stimulation. Furthermore, chronic TLR-2 activation of PBMCs without GFs induced a pro-inflammatory cytokine production of TNF-α and IL-1β up to 21 days, while this was not detected in PBL monocultures, nor in TLR4 activated cells. We conclude that TLR2 activation-induced T cell proliferation and pro-inflammatory cytokine production only when monocytes were present, suggesting that TLR2 activation represents a bridge between innate and adaptive immunity.

Collectively, investigating cellular players and immune mediators that stimulate alveolar bone resorption will help to unravel the pathogenesis of periodontitis. This thesis contributes to the more specified understanding of PMNs in maintaining oral health and the role of GFs in the retention and survival of lymphocytes and by specifically activating T cell proliferation. A better understanding of osteoimmunological processes in which tooth-associated fibroblasts interact with immune cells has led to further insight into the pathogenesis of periodontitis and can be used to achieve the desired homeostasis for periodontal health.