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Susceptibility to periodontitis. Studies with LPS-stimulated whole blood cell cultures

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CHAPTER 8

Summary

Chapter 1 introduces the general issue that has been addressed in the current thesis. Periodontal disease is a multifactorial infectious entity, affecting the tooth supporting tissues and eventually, when not treated, resulting in overall teeth loss. It is generally accepted that periodontitis results from the interaction of mainly Gram-negative bacteria, or their cell wall components like lipopolysaccharides (LPS), accumulating on the tooth surfaces and the host immune response. However, although so-called periodontal pathogens have frequently been associated with the disease, these pathogens are also detectable in subjects without destructive periodontal disease and have actually never been shown to cause periodontitis. Studying the natural history of periodontal disease in rural populations that never interfered with any dental care, only about 10% develops generalized severe post-adolescent periodontitis despite the abundant presence of periodontal pathogens in almost 100% of the population. Therefore, although bacteria are responsible for the induction of the inflammatory response in the periodontal tissues, they are insufficient to cause generalized severe periodontitis. In addition, it indicates that there exist differences in the susceptibility to periodontitis and that the host immune response is of fundamental importance. Moreover, different degrees of susceptibility implicate that differences in genetic backgrounds between susceptible and non-susceptible individuals might exist. Periodontal disease has indeed been aggregated in families and twin models have revealed that this aggregation is genetic and not environmental in nature.

Chapter 2 describes a study that investigated the host immune response in patients suffering from periodontitis in comparison to controls. The control subjects did not experience destructive periodontal disease as was analyzed on dental radiographs. In order to study the host immune response whole blood cell cultures (WBCC) were stimulated with LPS from *Escherichia coli* for 18 hours and the subsequent release of several pro- and anti-inflammatory mediators was measured. In addition, bacteriological samples were taken to further characterize both study populations, and the numbers of total and differential white blood cells were determined in venous blood samples. The data showed that patients as well as

controls were colonized by periodontal pathogens, although the number of patients displaying *Porphyromonas gingivalis* and *Bacteroides forsythus* in their subgingival plaque samples was higher. The number and percentages of peripheral white blood cells were not different between patients and controls. The release of prostaglandin (PG)E₂ was 2-fold higher in the patients' LPS-stimulated WBCC in comparison to the cultures of control subjects, whereas the release of interleukin (IL)-12p70 was 2-fold lower. In addition, the cultures from patients released also lower levels of IL-1 β and higher levels of IL-8 than the controls, while the concomitant release of tumor necrosis factor (TNF)- α , IL-6, IL-10 and IL-12p40 was not different. Since it is known from the literature that LPS-stimulated WBCC reflect specifically the behavior of the monocytes and that monocytes are the peripheral blood precursors of antigen-presenting cells (APCs) in the tissues, the following conclusions were drawn. Monocytes derived from periodontitis patients produce enhanced levels of PGE₂ and down-regulated levels of IL-12p70. Upon antigen exposure 2 different subsets of specific T helper (Th) cells develop, i.e. Th type 1 (Th1) and Th type 2 (Th2), and APCs are able to drive the polarization of Th cells into effector Th1 or Th2 cells by soluble molecules. In this respect, APC-derived IL-12p70 induces Th1 cells that promote cellular immunity, while APC-derived PGE₂ induces Th2 cells that promote humoral immunity. Therefore, it was postulated that APCs from periodontitis patients might have a bias in directing Th2 responses and thereby promoting the humoral immunity in periodontitis.

The suggested Th2-promoting phenotype of APCs from periodontitis patients implies either an intrinsic characteristic or a different priming of the monocytes, due to an altered tissue environment, i.e. the inflammatory periodontal lesion. In order to explore the intrinsic or acquired nature of this aberrant phenotype of the peripheral blood monocytes from periodontitis patients, the effect of periodontal therapy was studied in **chapter 3**. Therefore, untreated periodontitis patients underwent a specifically designed non-surgical periodontal therapy, including the systemic use of antibiotics for 7 days and 3 months rinsing with chlorhexidine twice daily. Clinical

measurements and bacteriological samples were performed at baseline and 3 months after therapy to evaluate the effectiveness of the therapy. Furthermore, the release of inflammatory mediators was measured in WBCC stimulated with *E. coli*-derived LPS and the total and differential numbers of peripheral white blood cells were determined before and after therapy. Patients were successfully treated, since the number of deep probing depths and the bleeding on probing scores were strongly reduced. In addition, the major periodontal pathogens, *Actinobacillus actinomycetemcomitans*, *P. gingivalis* and *B. forsythus*, were no longer detectable, whereas the prevalence of the other cultured bacteria decreased. The total and differential numbers of peripheral white blood cells, such as the neutrophils, were either significantly decreased or showed a trend towards reduction after therapy. In contrast, the number of peripheral blood monocytes was unchanged after periodontal therapy. In addition, monocytes in the LPS-stimulated WBCC derived from the treated patients produced 2-fold higher levels of IL-12p70 compared to baseline, whereas the levels of PGE₂ showed a trend towards reduction. However, the ratio of the PGE₂ and IL-12p70 concentrations decreased on average 2 times after the periodontal therapy. The levels of TNF- α , IL-1 β , IL-6, IL-8, IL-10, IL-12p40 did not change after therapy. Since the net-IFN- γ production of Th cells is largely determined by the ratio of APC-derived PGE₂ and IL-12p70 at the time of T cell activation, the decreased PGE₂ to IL-12p70 ratio indicated that APCs from periodontitis patients would favor the promotion of Th1 cell responses instead of Th2 responses after periodontal therapy. It was concluded that the Th2-promoting phenotype of APCs from untreated periodontitis patients reverses after therapy and is therefore not an intrinsic characteristic. Moreover, after periodontal therapy the functional phenotype of peripheral blood monocytes from patients resembles that of control subjects without destructive disease.

The cytokine IL-12p70 is a crucial and dominant factor in the development of Th1 cell responses and in the maintenance of such cellular immune responses. A growing body of evidence indicates that a subgroup of chemokines, i.e. CC chemokines

structurally lacking an amino acid separating the first two cysteine residues, have effect on the differentiation of Th cells either directly or through their effects on APC cytokine secretion. In this respect, it has been shown that a selective set of CC chemokines, i.e. macrophage chemoattractant proteins (MCP), is able to suppress the production of IL-12p70 by human monocytes, and in that way participate in the development of T cell-mediated immune responses. Since the IL-12p70 levels were strongly enhanced after periodontal therapy, the production of monocyte-derived CC chemokines was measured in the same untreated and treated periodontitis patients and matched control subjects (**chapter 4**). The studied CC chemokines were MCP-1, RANTES (regulated on activation normal T cell expressed and secreted), monocyte derived chemokine (MDC), and macrophage inflammatory protein (MIP)-1 α and MIP-1 β . In addition, this study investigated whether the release of MCP-1 in *E. coli* derived LPS-stimulated WBCC correlated with the levels of IL-12p70. Results showed that the untreated periodontitis patients released 2-fold higher levels of RANTES and a trend towards higher levels of MCP-1 in comparison to controls, while the IL-12p70 levels were 2-fold lower. Concomitant release of MDC, MIP-1 α and MIP-1 β were not different between patients and controls. After periodontal therapy no changes were seen with regard to the levels of MDC, MIP-1 α , MIP-1 β and RANTES, whereas the MCP-1 levels decreased and reciprocally the IL-12p70 levels strongly increased. Moreover, the levels of MCP-1 and IL-12p70 consistently correlated in an inversed way, suggesting a role for MCP-1 in the regulation of IL-12p70 production by peripheral blood monocytes from periodontitis patients. Furthermore, the therapy-induced changes in the MCP-1 and IL-12p70 release corresponded with the clinical improvements, and the expression of both molecules reflects therefore the periodontal inflammation. The persistent augmented levels of RANTES after periodontal therapy suggest a possible role for this chemokine in the susceptibility to periodontal disease.

Although the periodontal condition improved substantially after the non-surgical periodontal therapy, no complete resolution of the disease process was possible. Still

about 10% of the sites displayed deepened probing depths and 20 to nearly 30% of the sites showed bleeding on probing. This residual periodontal infection could still have impact on the responsiveness of peripheral blood monocytes. In order to exclude any influence of the periodontal infection on the responsiveness of the peripheral blood monocytes, the inflamed periodontium was completely eliminated in a case. **Chapter 5** describes this case report studying the responsiveness of peripheral blood monocytes before and during a period of 3 years after full mouth tooth extraction therapy in a patient with generalized terminal adult periodontitis. Before and 3, 9, 20 and 32 months after therapy venous blood was collected. Total and differential white blood cell counts were determined and WBCC were incubated with LPS from *E. coli* to stimulate the production of inflammatory mediators in monocytes. After full mouth tooth extraction, the numbers of total peripheral white blood cells and neutrophils decreased over time. The release of the chemokines IL-8 and macrophage MCP-1 decreased 2-fold over time, whereas no changes were found for the other studied cytokines, CC chemokines and PGE₂. It was concluded that the higher expression of IL-8 and MCP-1 in the peripheral blood monocytes from periodontitis patients is most likely acquired, as their levels decreased over time when the periodontal infection was controlled. Since both chemokines are regarded as important factors related to atherosclerosis, the possible connection between periodontitis, IL-8, MCP-1 and atherosclerosis were discussed.

It is believed that the periodontal infection contributes to the development and progression of atherosclerosis by eliciting systemic responses. Upon infection an acute-phase response is elicited locally but also systemically, such as the induction of plasma protein synthesis in the liver and activation of the complement system. The production of these acute-phase proteins is regulated by the early-response cytokines IL-1 β , TNF- α , IL-6 and IL-8, which are released by alarmed monocytes or tissue macrophages. **Chapter 6** describes a study that investigated whether periodontal disease results in systemically elevated levels of the early response cytokines and the chemokines MCP-1 and C5a, and what the effect of therapy on

these levels is. Peripheral blood samples were taken from the same untreated and treated periodontitis patients and the control subjects without periodontal breakdown. The plasma IL-8 levels were not different between untreated patients and controls, however the levels decreased after therapy. In contrast, the plasma MCP-1 levels were higher in patients than controls, but did not change after therapy. Furthermore, more patients than controls (8 versus 1) displayed elevated plasma levels of the complement product C5a, although no differences existed between the average levels of the patients and control subjects. It was concluded that either reduction or elimination of the periodontal infection results in systemically decreased IL-8 levels, most likely since circulating monocytes are no longer activated. The plasma MCP-1 levels do not change after therapy and continued to be higher in periodontitis patients, suggesting that periodontitis may have contributed to an (inflammatory) vascular response that is not resolved by periodontal therapy.

Chapter 7 discusses the central function of peripheral blood monocytes in the systemic immune responses induced by periodontitis. Being, highly responsive to danger molecules that are released in the blood stream by alarmed tissue or immune cells from the periodontally infected lesion. In addition, being instructive to leukocytes in the peripheral blood and in the peripheral tissues, and in that way orchestrating the modulation of the immune response. Finally, being flexible to changes in the local environment and as a result of that being capable to adapt their functional profile. In case of periodontitis, the chronic infection induces activation of peripheral blood monocytes, i.e. enhanced production of the major chemokines IL-8 and MCP-1, and an up-regulation of the PGE₂ production. Subsequently, MCP-1 regulatory suppresses the production of IL-12p70 by monocytes, in order to prevent collateral damage when arriving in the periodontal lesion. The enhanced production of PGE₂ contributes to the down-regulation of the IL-12p70 production. These periodontitis-induced regulatory processes by peripheral blood monocytes are compensatory, since after therapy the production of IL-12p70 is strongly enhanced, while the production of MCP-1, PGE₂ and of IL-8 is down-regulated. In contrast, the high production of RANTES by the monocytes both before and after therapy, suggests

that it could play an important role in the susceptibility to the disease. RANTES could ameliorate the periodontal destruction through antigen-independent stimulation of Th cells. Finally, the activated peripheral blood monocytes in periodontitis patients also result in elevated circulating IL-8 and MCP-1 levels, potentially contributing to atherosclerosis and increasing the risk for cardiovascular diseases.