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CHAPTER 1

Susceptibility to Destructive Periodontal Disease

General Introduction

Prevalence to periodontitis

Periodontal disease is an infection that has a number of properties in common with infections in other parts of the body, but has unique features resulting from the passage of the tooth through the soft tissue integument into the oral cavity (1). Periodontitis is a chronic inflammatory and destructive disease of the teeth-supporting tissues, i.e. connective tissue from the periodontal ligament and alveolar bone. This inflammatory condition will, if left untreated, eventually lead to loosened teeth and subsequent exfoliation. Clinically, the disease is characterized by deep probing pocket depths as a result of connective tissue attachment loss and bleeding upon probing due to the inflammation. Periodontitis results from the interaction of mainly Gram-negative bacteria, or their cell wall components like lipopolysaccharides (LPS), accumulating on the solid, non-shedding tooth surfaces and the host immune response (2, 3). Major periodontal pathogens, like *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Bacteroides forsythus*, have frequently been associated with periodontitis (4-8), but it has never been shown that they actually cause the disease. Above that, periodontal pathogens are also detectable in subjects without periodontitis (9-12).

Longitudinal human studies on the natural history of periodontal disease in rural populations, refraining from any dental care, showed that despite the high prevalence of (major) periodontal pathogens, only 8% of the population developed a generalized severe form of periodontitis leading to an overall tooth loss before the age of 45 years (6, 13, 14). More than 80% of the population developed a moderate type of periodontitis that gradually deteriorated during life. In addition, also studies in industrialized countries revealed that a small portion of the population is on a high risk for developing severe periodontitis, resulting in an overall tooth loss before the age of 45 years, and therefore is not a consequence of aging (15-17). Moreover, evaluating the long-term effect of periodontal therapy in patients receiving regular periodontal care, again a small subpopulation of the treated patients exhibited progressive disease leading to almost overall tooth loss, irrespective of the type of therapy applied (18, 19).

Since periodontal pathogens are believed to play an important role in the pathogenesis of destructive periodontal disease, the prevalence of periodontal pathogens in families of severe periodontitis patients was studied (20-23). Despite the fact that major periodontal pathogens, such as *A. actinomycetemcomitans* and *P. gingivalis*, were sometimes transmitted within families, the presence of these bacteria did not correlate with the degree of periodontal inflammation and breakdown in the infected family members. In addition, a study carried out in a rural population, not receiving regular dental care and therefore developing natural periodontitis, showed that periodontal pathogens aggregated in families (24). However, despite this aggregation in families, the microbiological parameters did not correlate with the amount of periodontal breakdown in these families. Whereas, the periodontal breakdown itself did aggregate in the families. Reexamination of the same families after a period of 7 years, demonstrated that cohabitation has no effect on the periodontal condition of the spouses (25). Therefore, the fact that infected probands would put their family members on a higher risk for developing destructive periodontal disease by the transmission of pathogens is far too simplistic. As stated already a decade ago, patient susceptibility is of outmost importance to the outcome of periodontitis, and the host immune response to the colonized bacteria is of fundamental importance (26).

Therefore, although bacteria are essential in the initiation of the inflammatory reaction, they are insufficient to cause generalized severe periodontitis. It is well accepted nowadays that not everyone is equally susceptible to the disease (2).

Genetics in periodontitis

Different degrees of susceptibility to periodontitis implicates that differences in genetic backgrounds between susceptible and non-susceptible individuals might exist. Currently, it is believed that individual differences in the host immune response are also genetically controlled (27). In this respect, probably the most powerful method to study genetic aspects of periodontal disease are twin models; especially,

when monozygous twins, who are separated at birth and reunited in adulthood, are studied. In this way the effect of shared genes can be examined without the confounding effects of a common environment. Such studies have indicated that between 38 and 82% of the population variance for periodontitis parameters may be attributed to genetic factors, and these percentages are unaltered when adjustments for environmental/behavioral factors are made (28, 29). Moreover, it was also shown that the prevalence of periodontal pathogens is not different between heterozygous as well as homozygous twins, indicating that the familial aggregation of periodontal diseases appears to be genetic and not environmental in nature (30).

To date, genetic studies in relation to periodontitis have revealed only one major disease gene, a functional polymorphism for the cathepsin C gene, localized on chromosome 11, displaying decreased cathepsin C activity and responsible for the occurrence of pre-pubertal periodontitis (31). In addition, a genetic polymorphism for the cytokine interleukin (IL)-1 β has been associated with the severity of periodontitis in non-smoking Caucasian patients (32). However, this IL-1 polymorphism has no functional consequences, since gene-positive and gene-negative periodontitis patients do not differ in the production of the cytokine by stimulated monocytes (33). In conclusion, periodontitis is a multifactorial infectious disease in which host factors, but also environmental factors may play an important role (2).

Host immune responses and the role of antigen-presenting cells

In response to an antigen the host reacts with a non-specific innate immune response and a subsequent specific adaptive immune response. During the early events in an immune response, inflammatory mediators are produced that subsequently modulate the development of the adaptive immunity (34). It has been shown that these released mediators direct the subsequent development of differential specific immune responses by eliciting subsets of T helper (Th) cells (34, 35). Essentially, two types of Th cells develop from the same T cell precursor: Th

type 1 (Th1) and Th type 2 (Th2) cells that direct cellular and humoral immune responses, respectively (36-38). These Th subsets are characterized by their different profiles of regulatory cytokines (35, 39). However, the development of such specific Th subsets is dependent upon the presentation of antigens by the antigen-presenting cells (APCs). The APCs seem to play a crucial role in the control of the immunity by orchestrating the modulation of the immune response, and therefore the APCs interface the innate and adaptive immunity (40-42).

The cells belonging to this group of APCs are monocytes, macrophages and dendritic cells. The monocytes are in fact the blood derived precursor cells that originate from the bone marrow and develop into macrophages or either dendritic cells after traversing the endothelium (43). Within the peripheral tissues the macrophages are important scavenger cells, while the dendritic cells (DCs) are the highest specialized APCs that mature upon activation by pathogens or their products, or by tissue-derived factors (44, 45). As a matter of fact, APCs transfer information from an infected environment to the T cells and select thereby the most effective immune response to the pathogenic antigens associated with that microenvironment (41, 46). APCs present pathogen-derived peptides to specific T cell receptors in the context of class II major histocompatibility complex, determining the specificity of the response, and provide T cells with co-stimulation through CD28, determining whether or not an immune response will occur and how strong this response will be (47, 48). In addition to that, APCs adapt the type of immune response to the nature of the invading pathogen by providing T cells with factors, that drive the polarization of Th cells into effector Th1 or Th2 cells (49, 45). In this respect, bioactive IL-12p70 is an important and crucial factor, directing the development of Th1 cells and maintaining such responses (35, 50). Alternatively, Th2 polarization may be induced by prostaglandin (PG)E₂ either directly (51, 52), or indirectly through the inhibition of IL-12P70 production by APCs (53, 54). Both IL-12p70 and PGE₂ are normally produced by APCs in response to infection by bacteria or viruses, or following exposure to their compounds, i.e. LPS or DNA (53, 55, 56).

Scope of this thesis

Peripheral blood monocytes are the precursor cells for APCs in the peripheral tissues, e.g. the periodontal lesion. In addition, the functional role of the APCs is based on *in vitro* experiments with these blood precursors, and monocyte-derived APCs seem to resemble the *in vivo* types. Since the APCs play an important orchestrating role in the host immune response by the production of inflammatory mediators, the present thesis focused on the responsiveness of peripheral blood monocytes in relation to the susceptibility to periodontitis. To study the monocyte response in periodontitis patients compared to controls without destructive periodontal disease, LPS-stimulated whole blood cell cultures were used and inflammatory mediators assessed (**chapters 2 and 4**). To study the intrinsic or acquired nature of the functional phenotype of monocytes, patients were subsequently treated (**chapters 3 and 5**). In addition to the monocyte response, the levels of inflammatory mediators in the circulation of untreated patients, controls and treated patients were assessed (**chapter 6**). Finally, the general discussion (**chapter 7**) addressed a central role for peripheral blood monocytes in the systemic immune responses induced by periodontitis.

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