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# **Chapter 1**

## **General Introduction**

## **Introduction**

The tissues that surround and support the teeth are collectively called the periodontium (Socransky & Haffajee 1997). Their main functions are to support, protect, and provide nourishment to the teeth. The periodontium consists of cementum, alveolar bone, periodontal ligament, and gingiva. Microbial plaque accumulating in the gingival crevice region induces an inflammatory response. Gingivitis, the mildest form of periodontal inflammation, involves the marginal soft tissue structures surrounding the teeth, and is characterized by redness, swelling and bleeding of the gingiva. Gingivitis, one of the most common human diseases, affects 50-90% of adults worldwide and is readily reversible by simple, effective oral hygiene (Pihlstrom et al. 2005).

In certain susceptible individuals, gingivitis can extend deeper into the tissues, resulting in periodontitis, which involves the loss of supportive connective tissue and alveolar bone (Albandar & Rams 2002). The most common form of periodontitis, chronic periodontitis, has been reported to affect up to 30% of the adult population with approximately 7-13% of adults affected with severe disease (Nares 2003). Some loss of periodontal attachment and alveolar bone is to be expected in older persons, but age alone in a healthy adult does not lead to a critical loss of periodontal support (Burt 1994). Clinically, the disease is characterized by deepened periodontal pockets as a result of loss of connective tissue attachment and bone loss in conjunction with bleeding upon pocket probing due to the inflammation. With disease progression teeth become mobile and may show migration and in some cases excessive recession of the gums. If left untreated, teeth may eventually exfoliate. In humans, periodontitis is one of the most important causes of tooth loss in adult age (Akhter et al. 2008, Ong 1998). Treatment of periodontitis is generally good possible and should establish periodontal health, prevent recurrence of disease, and preserve the dentition in state of health, comfort, and function. This goal can be accomplished by various non-surgical and surgical therapies, depending on the specific treatment objective (Pihlstrom et al. 2005).

With a new awareness that not all individuals were equally susceptible to periodontal disease, scientists turned their attention to the “risk” for disease, i.e. the probability that an individual will develop periodontitis (Van der Velden et al. 2006, Williams 2008). In this respect, three types of variables are important (Beck 1994).

The first type are risk factors; i.e., characteristics that are thought to be aetiologic for the disease of interest and that have shown to increase one's odds for developing a disease, e.g. a specific bacterium. The second type of variable involves background characteristics that are not considered to be aetiologic, are immutable to change and are often referred to as a risk determinant (e.g. age, gender and race). The third type of variable are risk predictors. These are usually either biological markers that are indicative of disease or disease progression (e.g. cytokine production or gene polymorphism associated to periodontitis) and historical measures of the disease (e.g. past evidence of periodontal disease) (Beck 1994).

Periodontitis is a multifactorial infectious disease. There is no doubt that the interaction between host immune mechanisms and periodontal bacteria is fundamental in the different manifestations of chronic inflammatory periodontal disease (Gemmell & Seymour 1994b). The subgingival microflora in periodontitis can harbor hundreds of bacterial species, but only a small number is considered etiologically important and has been associated with progression of disease (Consensus report 1996). Due to the episodic nature of periodontal disease and our lack of sensitive diagnostic clinical tests that can detect disease activity, it is difficult to ascertain the causality of specific pathogens in periodontitis (Ezzo & Cutler 2003). However, three putative periodontal pathogens have been implicated as risk factor and risk predictors in periodontitis, i.e. *Aggregatibacter actinomycetemcomitans* (Aa) (Fine et al. 2007, Van der Velden et al. 2006) as a risk factor and *Porphyromonas gingivalis* (Pg) and *Tannerella forsythia* (Tf) as risk predictors (Ezzo & Cutler 2003, van Winkelhoff et al. 2002). Subgingival periodontal pathogens are essential for the initiation and progression of the disease, although it is the resulting host reaction that primarily mediates tissue damage in the susceptible host (Gaffen & Hajishengallis 2008). In some individuals, neutrophils and cell mediated immunity may limit the extent of attachment loss. However, in susceptible people as determined by genetic and lifestyle factors, the clearance of the periodontal pathogens by the host immune response seems to be unsatisfactory and therefore disease progression may occur (Gemmell & Seymour 2004).

Our understanding of the pathogenesis of periodontitis has continued to evolve as our understanding of the underlying mechanisms of the inflammatory immune response has become more sophisticated. Tissue injury mediated by inflammation is a consequence of the inability of the host to resolve the inflammation, not the initial inflammation itself. This is an important distinction, because inflammation is

necessary to protect the host from infection, but persistent inflammation can also cause disease and irreversible damage (Van Dyke & Serhan 2003). In this respect, the ultimate outcome of periodontal disease in adults depends on patient susceptibility which is modulated by the host immune response.

### **The host immune response in periodontitis**

In response to an inflammatory trigger, like periodontal pathogens, two distinct, yet intricately linked, immune responses occur: innate and adaptive. The innate immune response is the first line of defense against invading microorganisms. It acts through the recruitment of cells, activation of the complement system, identification and removal of foreign substances, and activation of the adaptive immune system. The main players in innate immunity are phagocytes such as neutrophils, macrophages, and dendritic cells. These cells can discriminate between pathogens and self by utilizing signals from the Toll-like receptors (TLRs). Toll-like receptors (TLR) are signal molecules essential for the cellular response to bacterial cell wall components (Folwaczny et al. 2004). In mammals there are at least 10 members of the TLR family that recognize specific components conserved among microorganisms. Stimulation of immune cells by the binding of various bacterial components (e.g. lipopolysaccharides, bacterial DNA) to TLRs causes an immediate defensive response (Akira 2003, Mahanonda & Pichyangkul 2007). Upon activation of TLRs, an intracellular signaling cascade is stimulated that leads to the activation of transcription factors and the production of various cytokines (Graves 2008). These chemical signals regulate the traffic of leukocytes and control the leukocyte response (Van Dyke & Serhan 2003). The production of appropriate cytokines in response to infection is necessary for the development of protective immunity (Seymour et al. 1996). 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 (Kinane & Lappin 2001). Essentially, two types of Th cells develop from the same CD4 + T cell precursor, termed Th1 and Th2 cells, which secrete different patterns of cytokine (Gemmell et al. 2007).

Cytokines are cell regulators that have a major influence on the production and activation of different effector cells. Monocytes and T cells are a major source, although cytokines are produced by a wide range of cells that play important roles in many physiological responses (Seymour & Gemmell 2001a). Cytokines produced by

monocytes and other antigen presenting cells (APC) drive polarization of non-committed T helper cells (Th) into either Th1 or Th2 (Kidd 2003). In particular, interleukin (IL)-12 produced by monocytes, macrophages, neutrophils and dendritic cells during innate immune responses, promotes naive T cells to differentiate into Th1, while the anti-inflammatory IL-10 cytokine favors Th2 differentiation (Gemmell & Seymour 2004). Th1 lymphocytes are characterized by the production of interferon (IFN)- $\gamma$  and IL-2, whereas Th2 cells produce mainly IL-4 and IL-13 (Mosmann & Coffman 1989, Seder 1994). In periodontitis it is generally accepted that the stable lesion is largely mediated by cells with a Th1 cytokine profile, while the progressive, unstable lesion involves Th2-like cells (Gemmell & Seymour 2004). It is clear that the immunoregulatory control of Th1/Th2 cytokine profiles is fundamental in determining the ultimate outcome of periodontitis. Advances in knowledge of the pathogenesis of periodontitis suggest that a group of disease modifiers, including smoking and genotype, contribute strongly to individual patient differences in the susceptibility to periodontitis (Kornman 2008). Indeed, many of the widespread systemic effects of smoking may provide mechanisms for the increased susceptibility to periodontitis and the poorer response to treatment.

### **Cigarette Smoking and periodontitis**

Smoking is recognized as an important risk predictor in periodontitis (Palmer et al. 2005). Various factors contribute to the deleterious periodontal effects of smoking, including alterations in both microbial and host response factors (Johnson & Hill 2004). Cigarette smoking affects the oral environment and ecology, the gingival tissues, the vasculature, the inflammatory response and the homeostasis and healing potential of periodontal connective tissues (Palmer et al. 2005). There are now a number of studies that suggest a trend for smokers to harbour more or greater numbers of potential periodontal pathogens than non-smokers without increasing the amount of plaque (Haffajee & Socransky 2001, Kamma et al. 1999, Zambon et al. 1996a). Gingival blood flow is suppressed by smoking (Morozumi et al. 2004). Indeed, Nair et al. (2003) have shown an increased gingival bleeding in subjects on a successful quit-smoking programme, suggesting certain recovery of the inflammatory response (Nair et al. 2003). Smoking may affect multiple functions of neutrophils and may shift the net balance of neutrophil activities into the more destructive direction (Palmer et al. 2005). It has also been shown that cigarette smoke results in reduced

concentration of immunoglobulin G (IgG) antibodies (Graswinckel et al. 2004). Increased numbers of T-cells and elevated T-cell responsiveness in patients who smoke may be one of several explanations why smoking increases the risk for periodontitis (Loos et al. 2004). The majority of clinical trials show significantly greater reductions in probing depths and bleeding on probing, and significantly greater gain of clinical attachment following non-surgical and surgical treatments in non-smokers compared with smokers (Heasman et al. 2006). Furthermore, after periodontal therapy, smoker patients remain culture positive for periodontal pathogens, which may contribute to the often observed unfavourable treatment results in smoker periodontitis patients (Van der Velden et al. 2003). However, the mechanism of how the different effects of smoking contributes to increase the severity of periodontal breakdown in smoker periodontitis patients needs to be further investigated.

### **Genetics in Periodontitis**

Genetic variance, environmental exposures and life style factors are the key determinants to phenotypic differences between individuals. Most diseases have a genetic component in their etiology. However, the extent of the genetic contribution to disease can and does vary greatly. Life style factors may diversely affect the phenotypic expression of the genotype of different individuals (Hodge & Michalowicz 2001). Therefore, differences in disease susceptibility may have, besides a genetic and environmental component, a life style constituent. In addition, according to the model proposed by Kinane and Hart (2003), the interaction between genetic, environmental and life style factors is as important as these factors alone, and may be pivotal to determine periodontitis (Kinane & Hart 2003).

Evidence for a genetic predisposition to periodontitis comes from three areas of research: (1) population studies, (2) family studies, and (3) twin studies. In this respect, probably the most powerful method to study genetic aspects of periodontal disease is the twin model. Studying phenotypic characteristics of twins is a method of differentiating variations due to environmental and genetic factors. Despite the twin model being a powerful method of providing evidence of a genetic predisposition to periodontitis, very few twin studies of chronic adult periodontitis have been conducted, possibly, due to the enormous difficulty in gathering a homogeneous twin population representative for the disease. Existent twin studies in periodontitis have

suggested a substantial role of genetic factors in the etiology (Corey et al. 1993, Michalowicz et al. 1991, Michalowicz et al. 2000, Mucci et al. 2005). Nevertheless the main limitation of these studies is the fact that subjects were selected based on their twinship rather than their periodontal condition, resulting in populations with mild periodontal breakdown.

Genetic polymorphisms in a candidate gene approach have been explored as risk predictors for periodontitis. There is limited evidence that some polymorphisms in the genes encoding interleukin (IL)-1, Fc gamma receptors, IL-10 and the vitamin D receptor, may be associated with periodontitis in certain ethnic groups. However relatively large variations in carriage rates of the Rare (R)-alleles among studies on any polymorphism were observed (Loos et al. 2005). To date, genetic studies in relation to periodontitis have revealed only one major disease gene, a functional polymorphism for the cathepsin C gene, displaying decreased cathepsin C activity and responsible for the occurrence of pre-pubertal periodontitis (Hart et al. 2000). However till today there is no strong evidence for target genes and gene polymorphisms that play a key role in the susceptibility to and severity of periodontitis.

### **Aim and scope of this thesis**

Periodontitis results from the inflammatory response to periodontopathic bacteria. However the susceptibility of the patient determines the ultimate outcome of the disease progress (Gemmell et al. 2002). Lifestyle factors, such as smoking, not only modify the host response, but they also may diversely affect the phenotypic expression of the genotype of different individuals, thereby being major determinants of the enormous variation in susceptibility (Hodge & Michalowicz 2001). In the genetically susceptible host, inappropriate immunological mechanisms can be triggered by environmental factors. In the present thesis, we attempted to get more insight in the susceptibility to periodontitis.

The main purpose of this thesis was to determine the contribution of the host genetic make up in chronic adult periodontitis by means of the classical twin model. Since smoking could be a confounding factor in the analysis of the twin population, it was decided to explore the influence of smoking on the immune response in periodontitis, in a non-twin population, previous to the start of the twin study. Stable periodontal lesions are regarded to be predominantly Th1, whereas the active lesions

express a Th2 profile. In addition, the Th1/Th2 balance in periodontitis has been investigated and currently periodontitis is considered as a Th2-type disease. We hypothesized that the Th2 pattern in periodontitis may be accentuated by smoking, accelerating disease progression and relapse in treated periodontitis patients. Since monocytes are considered as orchestrating cells in the innate and adaptive immunity, we firstly studied the monocytic cytokines secreted after *ex vivo* stimulation. We hypothesized that in treated periodontitis patients the monocytic cytokines representative for a shift towards Th2 immune response was increased in smokers compared to non-smokers (*Chapter 2*).

The monocytic cytokine production gives only an indication of the ensuing adaptive immune response. However, to investigate the actual Th1/Th2 balance, the measurement of the lymphocytic cytokine production was needed. It was supposed that the cytokine pattern to be found in the lymphocytic cytokine production should be in line with the findings from monocytes. In this respect, if indeed smoking potentiates the monocytic Th2 immune response in the studied periodontitis population (i.e. lower IL-12 p40/IL-10 ratio in smokers) consistently, the lymphocyte cytokine production should reflect and confirm this finding (i.e. higher IL-13 cytokine production in smokers) (*Chapter 3*).

Previous twin studies on periodontal disease have suggested that in the population approximately half of the variance in the clinical parameters of periodontitis is attributed to genetic variance (Michalowicz et al. 1991, Michalowicz et al. 2000). Contrary, the presence of periodontal pathogens has been shown not to be under the genetic control (Michalowicz et al. 1999). In their twin study on the presence of periodontal bacteria, Michalowicz et al. (1999) concluded that any effect of the genetic make up on the presence of the periodontal microorganisms is not apparent in adulthood. However, since their conclusions are founded on the clinical and microbiological results of twins mildly affected by periodontal breakdown, they warned that their results may not necessarily be extrapolated to more advanced disease states. Thus, the question whether these findings would be also applicable for moderate to severe periodontitis was an important one. Therefore we studied in monozygotic (MZ) and dizygotic (DZ) twin pairs, selected on the basis of one sib of a twin pair having moderate to severe chronic periodontitis, the contribution of genetics, life style factors and periodontal pathogens to the clinical phenotype of the disease (*Chapter 4*). In addition, we investigated the extent of concordance in number of

white blood cells and monocytic and lymphocytic cytokine secretion after *ex vivo* stimulation among the previously studied twin population selected on the basis of one sib of a twin pair suffering from moderate to severe chronic periodontitis (*Chapter 5*). The main findings and conclusions of these studies are summarized in *Chapter 6* and future lines of investigations are suggested.

## References

- Consensus report (1996). Periodontal diseases: pathogenesis and microbial factors. *Annals of Periodontology* **1**, 926-932.
- Akhter, R., Hassan, N. M., Aida, J., Zaman, K. U. & Morita, M. (2008) Risk indicators for tooth loss due to caries and periodontal disease in recipients of free dental treatment in an adult population in Bangladesh. *Oral Health & Preventive Dentistry* **6**, 199-207.
- Akira, S. (2003) Toll-like receptor signaling. *Journal of Biological Chemistry* **278**, 38105-38108.
- Albandar, J. M. & Rams, T. E. (2002) Global epidemiology of periodontal diseases: an overview. *Periodontology 2000* **29**, 7-10.
- Beck, J. D. (1994) Methods of assessing risk for periodontitis and developing multifactorial models. *Journal of Periodontology* **65**, 468-478.
- Burt, B. A. (1994) Periodontitis and aging: reviewing recent evidence. *Journal of the American Dental Association* **125**, 273-279.
- Corey, L. A., Nance, W. E., Hofstede, P. & Schenkein, H. A. (1993) Self-reported periodontal disease in a Virginia twin population. *Journal of Periodontology* **64**, 1205-1208.
- Ezzo, P. J. & Cutler, C. W. (2003) Microorganisms as risk indicators for periodontal disease. *Periodontology 2000* **32**, 24-35.
- Fine, D. H., Markowitz, K., Furgang, D., Fairlie, K., Ferrandiz, J., Nasri, C., McKiernan, M. & Gunsolley, J. (2007) *Aggregatibacter actinomycetemcomitans* and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. *Journal of Clinical Microbiology* **45**, 3859-3869.

- Folwaczny, M., Glas, J., Torok, H. P., Limbersky, O. & Folwaczny, C. (2004) Toll-like receptor (TLR) 2 and 4 mutations in periodontal disease. *Clinical & Experimental Immunology* **135**, 330-335.
- Gaffen, S. L. & Hajishengallis, G. (2008) A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *Journal of Dental Research* **87**, 817-828.
- Gemmell, E. & Seymour, G. J. (1994) Modulation of immune responses to periodontal bacteria. *Current Opinion in Periodontology*, 2nd edition, eds. Williams, R., Yukna, R. & Newman, M. Current Science. 28-38.
- Gemmell, E. & Seymour, G. J. (2004) Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontology 2000* **35**, 21-41.
- Gemmell, E., Yamazaki, K. & Seymour, G. J. (2002) Destructive periodontitis lesions are determined by the nature of the lymphocytic response. *Critical Reviews in Oral Biology & Medicine* **13**, 17-34.
- Gemmell, E., Yamazaki, K. & Seymour, G. J. (2007) The role of T cells in periodontal disease: homeostasis and autoimmunity. *Periodontology 2000* **43**, 14-40.
- Graswinckel, J. E., van der Velden, U., van Winkelhoff, A. J., Hoek, F. J. & Loos, B. G. (2004) Plasma antibody levels in periodontitis patients and controls. *Journal of Clinical Periodontology* **31**, 562-568.
- Graves, D. (2008) Cytokines that promote periodontal tissue destruction. *Journal of Periodontology* **79**, 1585-1591.
- Haffajee, A. D. & Socransky, S. S. (2001) Relationship of cigarette smoking to attachment level profiles. *Journal of Clinical Periodontology* **28**, 283-295.
- Hart, T. C., Hart, P. S., Michalec, M. D., Zhang, Y., Marazita, M. L., Cooper, M., Yassin, O. M., Nusier, M. & Walker, S. (2000) Localisation of a gene for prepubertal periodontitis to chromosome 11q14 and identification of a cathepsin C gene mutation. *Journal of Medical Genetics* **37**, 95-101.
- Heasman, L., Stacey, F., Preshaw, P. M., McCracken, G. I., Hepburn, S. & Heasman, P. A. (2006) The effect of smoking on periodontal treatment response: a review of clinical evidence. *Journal of Clinical Periodontology* **33**, 241-253.
- Hodge, P. & Michalowicz, B. (2001) Genetic predisposition to periodontitis in children and young adults. *Periodontology 2000* **26**, 113-134.

- Johnson, G. K. & Hill, M. (2004) Cigarette smoking and the periodontal patient. *Journal of Periodontology* **75**, 196-209.
- Kamma, J. J., Nakou, M. & Baehni, P. C. (1999) Clinical and microbiological characteristics of smokers with early onset periodontitis. *Journal of Periodontal Research* **34**, 25-33.
- Kidd, P. (2003) Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Alternative Medicine Reviews* **8**, 223-246.
- Kinane, D. F. & Hart, T. C. (2003) Genes and gene polymorphisms associated with periodontal disease. *Critical Reviews in Oral Biology & Medicine* **14**, 430-449.
- Kinane, D. F. & Lappin, D. F. (2001) Clinical, pathological and immunological aspects of periodontal disease. *Acta Odontologica Scandinavica* **59**, 154-160.
- Kornman, K. S. (2008) Mapping the pathogenesis of periodontitis: a new look. *Journal of Periodontology* **79**, 1560-1568.
- Loos, B. G., John, R. P. & Laine, M. L. (2005) Identification of genetic risk factors for periodontitis and possible mechanisms of action. *Journal of Clinical Periodontology* **32 Suppl 6**, 159-179.
- Loos, B. G., Roos, M. T., Schellekens, P. T., van der Velden, U. & Miedema, F. (2004) Lymphocyte numbers and function in relation to periodontitis and smoking. *Journal of Periodontology* **75**, 557-564.
- Mahanonda, R. & Pichyangkul, S. (2007) Toll-like receptors and their role in periodontal health and disease. *Periodontology 2000* **43**, 41-55.
- Michalowicz, B. S., Aepli, D., Virag, J. G., Klump, D. G., Hinrichs, J. E., Segal, N. L., Bouchard, T. J., Jr. & Pihlstrom, B. L. (1991) Periodontal findings in adult twins. *Journal of Periodontology* **62**, 293-299.
- Michalowicz, B. S., Diehl, S. R., Gunsolley, J. C., Sparks, B. S., Brooks, C. N., Koertge, T. E., Califano, J. V., Burmeister, J. A. & Schenkein, H. A. (2000) Evidence of a substantial genetic basis for risk of adult periodontitis. *Journal of Periodontology* **71**, 1699-1707.
- Michalowicz, B. S., Wolff, L. F., Klump, D., Hinrichs, J. E., Aepli, D. M., Bouchard, T. J., Jr. & Pihlstrom, B. L. (1999) Periodontal bacteria in adult twins. *Journal of Periodontology* **70**, 263-273.

- Morozumi, T., Kubota, T., Sato, T., Okuda, K. & Yoshie, H. (2004) Smoking cessation increases gingival blood flow and gingival crevicular fluid. *Journal of Clinical Periodontology* **31**, 267-272.
- Mosmann, T. R. & Coffman, R. L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* **7**, 145-173.
- Mucci, L. A., Bjorkman, L., Douglass, C. W. & Pedersen, N. L. (2005) Environmental and heritable factors in the etiology of oral diseases--a population-based study of Swedish twins. *Journal of Dental Research* **84**, 800-805.
- Nair, P., Sutherland, G., Palmer, R. M., Wilson, R. F. & Scott, D. A. (2003) Gingival bleeding on probing increases after quitting smoking. *Journal of Clinical Periodontology* **30**, 435-437.
- Nares, S. (2003) The genetic relationship to periodontal disease. *Periodontology 2000* **32**, 36-49.
- Ong, G. (1998) Periodontal disease and tooth loss. *Int Dent J* **48**, 233-238.
- Palmer, R. M., Wilson, R. F., Hasan, A. S. & Scott, D. A. (2005) Mechanisms of action of environmental factors--tobacco smoking. *Journal of Clinical Periodontology* **32 Suppl 6**, 180-195.
- Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. (2005) Periodontal diseases. *Lancet* **366**, 1809-1820.
- Seder, R. A. (1994) Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Journal of Allergy and Clinical Immunology* **94**, 1195-1202.
- Seymour, G. J. & Gemmell, E. (2001) Cytokines in periodontal disease: where to from here? *Acta Odontologica Scandinavica* **59**, 167-173.
- Seymour, G. J., Gemmell, E., Kjeldsen, M., Yamazaki, K., Nakajima, T. & Hara, K. (1996) Cellular immunity and hypersensitivity as components of periodontal destruction. *Oral Diseases* **2**, 96-101.
- Socransky, S. S. & Haffajee, A. D. (1997) The nature of periodontal diseases. *Annals of Periodontology* **2**, 3-10.
- van der Velden, U., Abbas, F., Armand, S., Loos, B. G., Timmerman, M. F., Van der Weijden, G. A., Van Winkelhoff, A. J. & Winkel, E. G. (2006) Java project on periodontal diseases. The natural development of periodontitis: risk factors,

- risk predictors and risk determinants. *Journal of Clinical Periodontology* **33**, 540-548.
- van der Velden, U., Varoufaki, A., Hutter, J. W., Xu, L., Timmerman, M. F., Van Winkelhoff, A. J. & Loos, B. G. (2003) Effect of smoking and periodontal treatment on the subgingival microflora. *Journal of Clinical Periodontology* **30**, 603-610.
- van Dyke, T. E. & Serhan, C. N. (2003) Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *Journal of Dental Research* **82**, 82-90.
- van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. A. & van der Velden, U. (2002) Porphyromonas gingivalis, Bacteroides forsythus and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* **29**, 1023-1028.
- Williams, R. C. (2008) Understanding and managing periodontal diseases: a notable past, a promising future. *Journal of Periodontology* **79**, 1552-1559.
- Zambon, J. J., Grossi, S. G., Machtei, E. E., Ho, A. W., Dunford, R. & Genco, R. J. (1996) Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. *Journal of Periodontology* **67**, 1050-1054.