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Effect of genetic variability on the inflammatory response to periodontal infection

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Abstract

Aim: To review the association between genetic variability and the inflammatory response induced by periodontal infection.

Material and Methods: A search of MEDLINE-PubMed was performed from January 2000 up to and including March 2005. The search included all types of publications, published in English without other limitations. The following search terms were used: ''cytokine polymorphism'', ''gene polymorphism'', ''periodontitis'', ''gingivitis'', ''inflammation'' and ''host-response''. The papers resulting from the above search were used as an additional source for relevant articles.

Results: Genetic variability was examined for the correlation to clinical indicators of inflammation such as bleeding on probing (BOP), gingival inflammation, cytokine in gingival crevicular fluid (GCF) and cytokine production by inflammatory cells. According to the current literature, most of the studies found no association between genetic variability and BOP, gingival inflammation or cytokine concentrations in the GCF. These studies were hampered by inappropriate study designs and the use of inflammatory parameters as secondary rather than primary outcome variables. The data suggest that the production of inflammatory mediators by inflammatory cells may be affected by different genetic traits but further studies are needed in order to establish this association.

Conclusions: To date, there is no clear correlation between any of the gene polymorphisms and clinical indicators of inflammation. The powering of studies to reveal associations between single or multiple nucleotide polymorphisms and inflammatory parameters will need to involve a much larger number of subjects than were used in the past. The available data (including the interleukin-1 composite genotype) do not currently support the utility of such tests in the diagnosis and prognostic assessments of periodontal diseases.

Key words: cytokine polymorphism; gene polymorphism; genetic variability; inflammation

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Role of Inflammation in the Periodontal Tissues

The primary aetiologic agent in the initiation of the periodontal diseases is the accumulation of plaque bacteria in the gingival crevice. Irritation of the gingival tissues by these bacteria induces an ''inflammatory response''. Inflammation comprises a group of physiologic responses that are the first organized reaction to any injurious challenge to the body, including bacterial infection. The rapid, generalized inflammatory processes that occur in response to challenges constitute an early step in the initiation of the immune response and are part of the *innate immune* system, so called to reflect that they are part of the inherent inborn biological responses that require no prior learning or experience. Inflammation is a well-coordinated process that involves increased vascular permeability followed by migration of polymorphonuclear (PMN) leucocytes, monocytes and lymphocytes from the blood stream into the irritated or infected tissues and the activation of these cells to secrete inflammatory mediators that guide an amplifying cascade of biochemical and cellular events (Larsen & Henson 1983, Sharma & Mohsin 1990). Although inflammation was once considered a non-specific arm of the immune response, current knowledge leads to the conclusion that the inflammatory response is a specific event, and a wideranging repertoire of receptors and corresponding ligands is involved. The specific nature of inflammation

allows rapid identification and a better tailored response to infection (Janeway & Medzhitov 2002) or to other threatening external stimuli (Matzinger 2002). For example, bacterial lipopolysaccharide (LPS), a common antigen of Gram-negative bacteria, is specifically recognized by host receptors such as soluble LPS-binding protein (LBP), membrane-associated CD14 and Toll-like receptors (TLR). The interactions between LPS and these host proteins activate an intracellular cascade of events that leads to secretion of specific inflammatory mediators. These specific interactions may explain why the inflammatory response to Gram-positive bacteria is less pronounced than the inflammatory response to Gram-negative bacteria during in vitro and in vivo inflammatory assays (Dixon et al. 2004). In addition, the discovery that there is a group of TLRs that can recognize a wide range but limited set of pathogen-associated molecular structures may explain how different bacteria may induce different inflammatory responses. For example, LPS extracted from different Gram-negative bacteria was found to activate different TLRs and induce a different response (Bainbridge & Darveau 2001, Martin et al. 2001). These interactions enable the host to sample and sort their current environmental condition, to discriminate between pathogenic bacterial challenges and to mount a selective appropriate response (Dixon et al. 2004). Recent data have indicated that TLRs may respond not only to bacterial but also to non-bacterial challenges, such as oxidized low-density lipoprotein cholesterol (Binder et al. 2002, 2003). Thus, the host may respond through inflammation to a wide variety of challenges, ranging from a Gram-negative bacterial infection to excess cholesterol (Dixon et al. 2004). However, the nature of the response differs and its character will depend on specific receptors and signal transduction pathways.

The primary role of the inflammatory cascade is to protect the host against bacterial invasion. The initial recruitment of leucocytes to the infected site is directed by chemoattractants or the release of chemical mediators from damaged cells. They migrate into the infected site, creating an ''inflammatory infiltrate'' within the periodontal tissues, close to the colonized tooth root surfaces (Kornman et al. 1997b). The adhesion molecules E-selectin and intercellular adhesion molecule 1 (ICAM-1) are involved in the extravasation process of neutrophils out of the blood vessels and through the gingival tissues (Page & Schroeder 1976, Moughal et al. 1992). Bacterial products and inflammatory mediators induce the expression of adhesion molecules on endothelial cells. These receptors enhance the interaction between leucocytes and the endothelial-lined blood vessel wall, allowing the neutrophils to roll on the endothelium, the first stage in extravasation (Springer 1994). In the tissue, the neutrophil follows the gradient of chemokines such as interleukin (IL)-8 and crawl up the chemotactic gradient using the ICAM receptor. Humoral factors, specific antibodies and complement, assist the neutrophils in their protective response, particularly in opsonization and phagocytosis of bacteria. It remains controversial whether or not antibody is protective in periodontal disease, but an effective antibody response is one of the main mechanisms by which the body generally responds to bacterial infection and invasion. During the chronic phase of the disease, the antibody response has been suggested to be generally protective, facilitating bacterial clearance and arresting disease progression (Gemmell & Seymour 2004). Antibodies are produced and secreted from cells of the B-lymphocyte lineage, but the B-cell response requires T-cell help. T cells stimulate B cells for expansion and for differentiation using cell-to-cell contact and the secretion of cytokines, resulting in the development of plasma cells and in antibody-class switching (O'Garra 1989). T cells and their products are necessary for both specific antibody production and the non-specific polyclonal B-cell activation (Rosenkoetter et al. 1984), and thus they play a fundamental role in the protection against bacteria-induced periodontal disease. If the result of B-cell differentiation into antibody-producing plasma cells is antibodies that can induce protection against specific bacteria, elimination of the causative organism from the tissues should prevent further destruction of the periodontal apparatus. As these responses are regulated by immunoregulatory genes, it may be that antibody responses are protective in one individual but not in another (Gemmell & Seymour 2004). The inability of specific antibodies to eliminate the periodontal pathogens could be because of the production of antibodies with poor anti-bacterial properties, or poor recognition of the antibodies by inflammatory cells.

Neutrophils utilize Fc receptors that bind immunoglobulins (Ig) and constitutes a crucial step in opsonization and phagocytosis of bacteria. Any structural changes because of genetic variability in these Fc receptors may alter the ability of neutrophils to respond to immune complexes and to eliminate the infection. The orchestration of neutrophil migration, antibody production and complement activation by regulatory cells (T cells and macrophages) and their cytokines enable the host to eliminate bacterial invasion and to reduce the risk of disease development.

However, inflammation in periodontitis can be a double-edged sword. On the one hand, the inflammatory response is protective and aims to eliminate bacterial invasion into the tissues. Once the insult is eliminated, inflammation is resolved, and the subsequent immune reactions diminish. On the other hand, in more chronic forms of inflammation, the persistence of excessive inflammatory mediators may lead to destruction of the tooth-supporting tissues and results in irreversible pathological changes such as the clinical condition of periodontitis. Thus, periodontitis can be considered one of the chronic inflammatory disorders that includes inflammatory arthritis, inflammatory bowl disease, inflammatory skin diseases and other conditions. There are common pathological features to most chronic inflammatory diseases: these include inflammatory cell infiltration and the loss of tissue architecture in the affected organ. In periodontal disease, the affected organ includes the dental attachment apparatus, epithelial attachment, connective tissue attachment, periodontal ligament and the alveolar bone.

Progression of periodontal disease is because of a combination of environment, host derived and genetic factors, including the presence of pathogenic bacteria, high tissue levels of inflammatory cytokines, tissue-destructive enzymes (including matrix metalloproteinases) and prostaglandins (PGs) and low levels of anti-inflammatory cytokines (Page et al. 1997). High levels of inflammatory cytokines such as IL-1β and tumour necrosis factor (TNF)-α have been described in diseased periodontal tissues (Stashenko et al. 1991b) and are bioactive (Kinane et al. 1991). These and other cytokines stimulate the

production of many mediators that accelerate the inflammatory process. Any uncontrolled production of these inflammatory cytokines may contribute to the pathogenesis of the disease. We have to bear in mind that inflammatory cytokines are important mediators in the protection against specific bacterial infections (O'Reilly et al. 1992), and that high production of these mediators are essential for clearing some bacterial infections. However, the same beneficial enhanced response has another edge, which is the possibility of tissue destruction as observed in periodontal disease.

Evidence that Genetics May Modify the Inflammatory Response

Diseases related to anomalies of leucocyte and neutrophil function are obvious, and excellent examples exist of how genetic disorders related to inflammatory cells can cause disease. Deficiency in neutrophil number (neutropenias), or neutrophil function may lead to increased susceptibility to infectious disease (Anderson & Springer 1987). For example, Infantile Genetic Agranulocytosis, a rare autosomal recessive disease where neutrophil numbers are very low, has also been associated with the expression of aggressive periodontal destruction (Saglam et al. 1995). Subjects affected by Papillon–Lefèvre syndrome, an autosomal recessive disorder characterized by mutations in the gene encoding the lysosomal protease cathepsin C, present clinical signs of severe aggressive periodontitis (Hart et al. 2000a, b, Cury et al. 2002, Noak et al. 2004). Cohen's syndrome is another autosomal recessive syndrome that is characterized (in addition to other symptoms) by neutropenia. Individuals with Cohen's syndrome show more frequent and extensive alveolar bone loss than age-, sex- and mental ability-matched controls (Alaluusua et al. 1997). Leucocyte adhesion deficiency (LAD), an autosomal recessive syndrome, is an example of leucocyte functional disorder. In this condition, circulating leucocytes express reduced or defective surface receptors and, as a result, do not adhere to vascular endothelial cells; thus, they cannot migrate and accumulate in sites of inflammation where they are needed. Reports of LAD indicate that although the blood vessels are full of neutrophils, the local disease sites lack sufficient leucocyte numbers and the infections spread rapidly. As a result, affected homozygotes suffer from acute recurrent infections that are commonly fatal in infancy. Those surviving will develop severe periodontitis that will begin as the deciduous dentition erupts (Waldrop et al. 1987). However, all the above monogenic disorders are rare, and, although indicating the importance of specific processes, they do not explain susceptibility to the periodontal diseases in the general population. However, they do emphasize the importance of neutrophil functions in the prevention of periodontal diseases, and how genetics may affect the process.

For many years, it was noted that not all individuals are equally affected by the accumulation of bacterial plaque. Some individuals might be very susceptible and might develop aggressive forms of periodontitis at a relatively young age, while others might be resistant and might never develop periodontitis (Kinane & Hart 2003). In some cases, the disease will progress slowly, and the risk for loss of function of the teeth during a lifetime will be minimal, while in others the rate will endanger the function of the dentition. The findings that high levels of inflammatory mediators such as IL-1, TNF and PGE_2 are correlated with periodontal destruction (Offenbacher et al. 1986, Honig et al. 1989, Stashenko et al. 1991a) and that these mediators are able to aggravate the inflammatory response (Gemmell & Seymour 2004) led to the hypothesis that some individuals may be ''high-responders'' and will respond to periodontal infection with the production of high levels of inflammatory mediators, which in turn will result in attachment loss. Molvig et al. (1988) had shown that there are stable inter-individual differences in the response of monocytes to LPS stimulation, and they point out that some individuals will respond consistently with high production of IL-1 and TNF, while others will not. They hypothesized that this pattern of response to environmental stimuli is genetically determined and may be the basis for chronic inflammatory disorders. Shapira et al. (1994a) and others support this hypothesis and suggest that inter-individual differences in monocyte/macrophage response can be found in periodontitis patients compared with subjects with no periodontitis (Garrison & Nichols 1989, Shapira et al. 1994a). Engebretson et al. (2002) have shown that probing depth and attachment levels are strongly correlated with gingival crevicular fluid (GCF) IL-1β levels. However, patients with severe disease had higher GCF levels of IL-1β in each probing depth category than those with mild/moderate disease. The differences between these two patient groups were more pronounced (around twofold) in shallow pockets, suggesting that high GCF IL-1β expression is in part a host trait, and not strictly a function of clinical parameters.

During experimental gingivitis studies, it was noted that there were individual variations in the rate of development of gingival inflammation. Wiedemann et al. (1979) reported that in a group of 62 subjects who were subject to a period of withdrawal from oral hygiene measures, eight were found to be ''resistant'' and did not develop gingivitis within 21 days, while another group of 25 subjects were found to be ''susceptible'' and exhibited substantial gingival inflammation within 14 days. The remaining subjects formed an intermediate group, which developed gingival inflammation by day 21. In a later study, Van der Weijden et al. (1994) described a group of subjects who consistently exhibited greater than average gingival inflammation, while another group were consistently below average, representing a ''resistant'' group. The difference in gingivitis susceptibility between the two groups could not be ascribed to qualitative plaque differences. From the data reported in the above experimental gingivitis studies, one can estimate that approximately 13% of subjects represent a ''resistant'' group (Tatakis & Trombelli 2004). In support of the earlier studies, Trombelli and associates (Tatakis & Trombelli 2004, Trombelli et al. 2004) have shown in a recent series of elegant experimental gingivitis studies that while all individuals will develop some degree of inflammation, there are inter-individual differences in response to dental plaque. Some individuals developed gingival inflammation more rapidly and/or in a more intense manner. These inter-individual differences may be explained by genetics or environmental effect. Using the ''twin study approach'', Michalowicz et al. (2000) could not demonstrate a strong association between gingival inflammation and genetics, perhaps because of the cross-sectional approach of the study. However, their

data support the major role of genetics in the development of periodontitis, in which gingival inflammation is considered a major part of the pathogenesis. In addition, preliminary genetic evidence supports the possibility that there are inter-individual differences in the ability to develop gingival inflammation and showed that specific genetic characteristics (for example carriage of certain IL-1 polymorphisms, ''composite genotype positivity'') may contribute to exacerbated gingival inflammation in response to plaque accumulation (Goodson et al. 2000). In summary, all of the above studies are consistent with the hypothesis of genetically based host modulation of gingival inflammation.

''Genetic Modifiers of Inflammation'' – Candidate Genes

Inflammatory and anti-inflammatory cytokines

The inter-individual differences in the inflammatory response (and the differences in periodontal disease susceptibility) are controlled by genetic and environmental factors as well as the interaction between the environment and the genotype. The genetic background may affect many functions, which in turn might affect inflammation and periodontal disease expression. This includes the inflammatory response, innate and adaptive immunity, bacterial colonization and other modifying factors (i.e. stress effect of smoking, etc.). The periodontal diseases, chronic periodontitis and aggressive periodontitis, are not considered as ''simple genetic diseases'', in which a mutation in a single gene causes some disruption of a specific protein, but as a ''complex disease'' in which many genes as well as other environmental factors contribute to the onset and severity of the disease. The genetic variants (polymorphisms) that contribute to complex diseases are prevalent in any population and are often reported to differ between diseased and healthy individuals. Because of the inflammatory nature of periodontal destruction, researchers are searching for a connection between periodontal diseases and variants in genes that are involved in the inflammatory and immune responses. A wide range of genes exist and the search is on for variation in genes whose products have been associated with periodontal destruction. Genetic polymorphisms may occur in any part of the gene, including the promoter region coding region, or 3′ UTR. Many of these polymorphisms may be functionally neutral, but others may have direct functional significance by affecting gene transcription, RNA stability or the coded protein itself. Interpretation of the biological significance of specific polymorphisms associated with altered activity or function is further complicated by the fact that a polymorphism that is associated with altered biological function may simply reflect a locus that is in linkage disequilibrium with a biologically significant polymorphism in an adjacent region of the genome. However, the demonstration of association of a polymorphism with a biological activity is of major importance in studies in this area, particularly as it offers insights into the pathogenic mechanisms involved. Most of the available data on gene–disease associations today are concentrated on polymorphisms of genes that have some role in the inflammatory response.

The central role of IL-1 in periodontal destruction is supported by a large body of data regarding its properties and its accumulation in gingival tissues and crevicular fluid (Honig et al. 1989). Furthermore, blockage of the activity of IL-1β (and TNF-α) was found to slow down the progression of experimental periodontitis in primates (Graves & Cochran 2003). Two single-nucleotide polymorphisms (SNPs) were identified in the IL-1 gene cluster. The first is a C to T transition at position -889 in the IL-1A promoter and the second is at position $+3954$ of the IL-1B gene, where allele 2 also results from a C to T transition (Kornman et al. 1997a). The transition from allele 1 to 2 in the IL-1B gene was also claimed to be correlated with increased secretion of the protein IL-1 (Kornman et al. 1997a).

TNF-α is another potent pro-inflammatory cytokine that induces tissue destruction and bone resorption and was suggested to be involved in the pathogenesis of periodontitis. A few variants (microsatellite and SNPs) were described for the TNF-α gene, but most of them were found to be non-functional. The single base transition polymorphism (G to A) at the -308 base site (TNF2) was found to result in a significantly higher transcription of the rare allele, TNF2, using a reporter gene construct (Wilson et al. 1997). Several other investigators have noted that stimulated peripheral blood mononuclear cells of carriers of the TNF2 genotype show a significantly higher release of TNF-α than cells from individuals with the G at position -308 (Pociot et al. 1993, Bouma et al. 1996). The structure–function correlation makes this specific gene variation a possible candidate for association studies.

IL-6 is another pro-inflammatory mediator and plays a role in B-cell differentiation and T-cell proliferation (Lotz et al. 1988). It also stimulates haematopoiesis (Revel 1989) and accelerates bone resorption (Ishimi et al. 1990). High levels of IL-6 in biological fluids and blood have been determined in infections and chronic inflammatory diseases (Hirano et al. 1988). This cytokine is involved in the pathogenesis of several inflammatory diseases (Hirano et al. 1988, Houssiau et al. 1988, Grossman et al. 1989), therefore constituting a major mediator of the host response to injury and infection. Some polymorphisms in cytokine gene promoter sequences are associated with transcriptional activity (Pociot et al. 1992, Wilson et al. 1997, Olomolaiye et al. 1998, Shirodaria et al. 2000). A G to C substitution at position -174 in the promoter of IL-6 is located immediately upstream of the multiresponsive element, located at positions -173 to -151 relative to the transcription start site (Morse et al. 1999). The C allele was shown to alter the IL-6 gene transcription response to stimuli such as LPS and IL-1 (Fishman et al. 1998) and is suggested to be involved in genetic susceptibility to inflammatory diseases.

Anti-inflammatory cytokines, such as IL-10 and IL-4, have a role in controlling the inflammatory response. Animal studies have shown that depletion of IL-10 caused aggravated alveolar bone resorption (Al-Rasheed et al. 2003), and, furthermore, human studies suggest that lack of IL-4 from gingival samples correlates with periodontitis (Yamamoto et al. 1996). Because of these reasons, IL-10 and IL-4 were also considered as candidate genes that encode susceptibility to periodontitis. IL-10 is produced by T-regulatory 1 cells, T-helper 2 cells, macrophages and B cells, and inhibits the synthesis of pro-inflammatory cytokines. IL-10 is also a B-cell stimulator, enhancing B-cell proliferation and differentiation (Hajeer et al. 1998). Three SNPs in the IL-10 gene were identified at positions -1087, -819 and -592, and have been associated with altered synthesis of IL-10 in response to inflam-

matory stimuli (Turner et al. 1997, Berglundh et al. 2003). The −1087 SNP is a G to A substitution, the −819 is a C to T substitution and the −592 SNP is a C to A substitution. The three polymorphisms are in strong linkage disequilibrium and appear in three preferred haplotypes: GCC, ACC and ATA. The haplotype GCC has been associated with a high production of IL-10 (Turner et al. 1997), suggesting that the other two gene variants may exhibit a stronger inflammatory response, compared with the GCC haplotype.

IL-4 is a cytokine with anti-inflammatory properties, produced by T cells, mast cells and basophils. It is an important factor in the clonal expansion of antigenic-specific B cells and modulates humoral responses to antigenic stimuli. Two polymorphisms in the IL-4 gene, a C to T polymorphism at position −590 in the promoter region (PP) and a 70 bp repeat polymorphism in intron 2 (PI) were detected in 27.8% of Caucasian patients with aggressive periodontitis (Michel et al. 2001). As IL-4 levels in the sera of these patients were not found to be significantly different compared with the other groups ($p < 0.01$), an association of the composite of these two polymorphisms with the ability of the host to express IL-4 was postulated (Michel et al. 2001).

Receptors involved in inflammation

Many cell surface receptors respond to host derived or external ligands and affect the physiologic events during inflammation. This group includes receptors to cytokines, Ig receptors and pattern-recognition receptors.

The leucocyte Fcγ receptor genes are found on chromosome 1 and encode three main receptor classes: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). These classes are further subdivided into subclasses: FcγRIa and b, FcγRIIa, b and c, and FcγRIIIa and b. FcγRIIa is found on all granulocytes, antigen-presenting cells, platelets, endothelial cells and a subset of T cells. FcγRIIIa is present on monocytes and macrophages, NK cells and a subset of T cells. The FcγRIIIb is the major IgG receptor of neutrophils (Loos et al. 2003). Structural and functional differences in FcγRIIa, IIIa and b have been described. A G-to-A transition polymorphism in the FcγRIIa gene results in the substitution of histidine (H) for arginine (R) at amino acid position 131 of the receptor. While the H131 variant was found to bind IgG2 immune complexes in an efficient manner, the R131 variant has a low affinity to IgG2 (Warmerdam et al. 1991). The G-to-T transition polymorphism in the FcγRIIIa gene results in an amino acid substitution in position 158, valine (V), substituted for phenylalanine (F). The F158 variant was found to bind IgG1 and IgG3 in lower affinity than the V158 variant and cannot bind IgG4, while V158 can. A biallelic polymorphism in the FcγRIIIb gene underlies the FcγRIIIb-neutrophil antigen (NA) 1 or NA2 allotype. This is caused by four amino acid substitutions in the Fc-binding region, resulting in differences in glycosylation. The NA2 type binds human IgG1 and IgG3 immune complexes less efficiently than FcγRIIIb-NA1 (Loos et al. 2003). All of these changes in the Fc receptor genes may alter the inflammatory response to infection, but their effect depends on the type of antibody response to the periodontal pathogen. If a specific periodontal pathogen induces an antibody response that is dominated by a specific IgG subclass and a polymorphism exists in the receptor that affects the binding of the specific IgG subclass, then the combined effect may modify the host response to the pathogen.

The host immune system detects invading pathogens primarily through an array of pattern-recognition receptors. These receptors recognize conserved pathogen-associated molecular patterns that are typically shared by large groups of microorganisms, i.e. bacterial LPS. LPS, a major component of the outer cell wall of Gram-negative periodontal pathogens, is a key factor in eliciting the inflammatory response that can lead to disease state (Suffredini & O'Grady 1999) and is considered as an important virulence factor during the pathogenesis of periodontal disease. The recognition of LPS by inflammatory cells and the transduction of LPS signal involve the TLRs and several additional molecules, particularly the CD14 receptor and LBP (Shapira et al. 1994b, Chow et al. 1999). Recently, base-pair changes in the genes for both CD14 and TLR4 have been described in humans. Arbour et al. (2000) demonstrated two co-segregating polymorphisms in the extracellular domain of the receptor of the human TLR4 gene. The first is an A-to-G substitution at position 896 from the start codon of the TLR4 gene, which results in an aspartic acid (Asp) to glycine (Gly) substitution at position 299 of the amino acid sequence. A co-segregating polymorphism that results in a threonine (Thr) to isoleucine (Ile) substitution at position 399 of the amino acid sequence was also identified. These mutations were found to be associated with decreased airway responsiveness after LPS stimulation, and support functional variability that may affect the host response to Gram-negative infection. A polymorphism in the promoter region of the CD14 gene has also been described, and involves a C-to-T substitution at −159 of the 5′ flanking region of the CD14 gene (Baldini et al. 1999). The TT homozygote genotype of this polymorphism has been associated with increased circulating soluble CD14 levels and a higher density of the monocyte CD14 receptor (Baldini et al. 1999, Hubacek et al. 1999). Agnese et al. (2002) have found a significantly higher incidence of Gram-negative infections among patients with the TLR4 polymorphism, but no association between CD14 polymorphism(s) and the incidence of infection. Periodontal infection is dominated by Gram-negative pathogens, and it reasonable to hypothesize that any functional polymorphism in LPS receptors may affect the inflammatory process and the clinical outcomes in the periodontium.

Although TLR2 is primarily involved in the recognition of peptidoglycans and lipoteichoic acid of Gram-positive bacteria (Underhill et al. 1999, Arbour et al. 2000), it was found to be involved in the recognition of LPS or other cell wall components of *Porphyromonas gingivalis* (Ogawa et al. 2002, Yoshimura et al. 2002). Two SNP, position 677 Arg to Trp and position 753 Arg to Gln, have been identified in the TLR2 gene, and found to diminish the ability of TLR2 to mediate a response to bacterial components (Bochud et al. 2003). Because of its unique ability to respond to *P. gingivalis* antigens, the polymorphic changes in the TLR2 structure may affect the course of periodontitis associated with *P. gingivalis* infection.

Many other gene variations may affect inflammation in a less direct manner, such as hormone and hormone receptors that are not directly correlated to inflammation, but may modify any physiologic response of the host. Receptors proposed to be involved indirectly in the inflammatory process are the vitamin D receptor and oestrogen receptor (Kinane 2000), but their biologic effect on inflammation is not fully understood.

Effect of Genetic Variability on Gingival Inflammatory Parameters

Bleeding on probing (BOP) (Table 1)

IL-1 gene polymorphism

Gingival bleeding is considered one of the most sensitive clinical indicators of early gingival inflammation (Mombelli et al. 1991). Lang et al. (1986) reported that sites that bled on probing at several visits had a higher probability of losing attachment than those that bled at one visit or did not bleed. In their original paper regarding the connection between IL-1 polymorphism and periodontal disease, Kornman et al. (1997b) also investigated the correlation between genotype positive (C-to-T transition at position −889 in the IL-1A promoter and C-to-T transition at position +3954 of the IL-1B gene) and BOP in 99 non-smoking untreated Caucasians and found lack of significance between the two groups, 16.2% and 19.1% for the genotype negative and genotype positive group, respectively. Lang et al. (2000) conducted a prospective longitudinal study that investigated the association between the same IL-1 complex genotype and gingival inflammation as determined by BOP. All patients were at the maintenance stage following non-surgical or surgical periodontal treatment. The results for 139 non-smokers subjects indicated that IL-1-positive genotype patients were found to have a significantly higher chance of presenting BOP and twice the chance of having increased BOP (31% *versus* 15%) over a four-appointment recall. These results suggest that the specific composite genotype of IL-1 may determine susceptibility to develop gingival inflammation. In contrast, Papapanou et al. (2001) investigated 132 patients suffering from ''destructive periodontitis'' and 73 periodontally healthy controls and failed to find any association between full-mouth bleeding score (FMBS) and IL-1 composite genotype in the disease or control group. Similar results were reported by Feloutzis et al. (2003), which also could not find any correlation between IL-1 gene polymorphism and inflammatory parameters (measured by FMBS) in 89 patients with chronic periodontitis (smokers and non-smokers), at maintenance stage. In an intervention study, Ehmke et al. (1999) typed the IL-1 composite genotype of untreated periodontitis patients and also did not find any significant difference in FMBS between the genotype-positive and genotype-negative groups. Because of the known masking effect of smoking as reported by Kornman et al. (1997a), the inclusion of smokers in the two last studies makes the interpretation of the results difficult.

De Sanctis & Zucchelli (2000) evaluated chronic periodontitis patients who had been treated by guided tissue regeneration surgery and who complied with supportive periodontal therapy ($n = 40$, smokers and non-smokers). The authors could not demonstrate any significant difference in FMBS between IL-1 genotype-positive and genotype-negative patients at baseline, 1 year and 4 years after the surgery. In a similar study design, but only with non-smoking patients, Christgau et al. (2003) evaluated the influence of IL-1 gene polymorphism on the healing outcomes following guided tissue regeneration surgery in patients with chronic periodontitis. The papillary bleeding index score at the surgical site did not reveal statistically significant differences between the genotype-positive and genotype-negative patients at baseline and 1 year after the surgery. Cutler et al. (2000) evaluated the effect of non-surgical treatment (water irrigations and prophylaxis therapy for 14 days) on clinical signs among 52 genotype-positive and genotype-negative patients with mild-to-moderate chronic periodontitis and could not demonstrate any effect of the genotype on any of the baseline and post-treatment clinical parameters, including BOP.

Because of the reported inter-individual differences in the gingival response to plaque accumulation, several investigators tested the association between IL-1 genotype and the severity of gingival inflammation. Cullinan et al. (2001) evaluated 295 unselected patients (smokers and non-smokers) and did not find significant differences in FMBS for the IL-1 composite genotype-positive and genotype-negative patients (13.3% and 14.1%, respectively). Using the experimental gingivitis model, Jepsen et al. (2003) evaluated the relationship of IL-1 genotype and the susceptibility to develop gingivitis in 10 genotype-positive and 10 genotype-negative young volunteers with healthy gingiva. They could not find any association between BOP and IL-1 genotype after 21 days of no plaque control. In contrast, in another preliminary report, Goodson et al. (2000) found significant differences in BOP between two similar groups of healthy volunteers who were subject to a 10-day experimental gingivitis trial. Unfortunately, this report was also based on the comparison of small groups (seven IL-1 genotype-positive subjects compared with 13 IL-1 genotype-negative subjects) and was published only in abstract form.

TNF-α gene polymorphism

Shimada et al. (2004) investigated whether (TNFR2)+587 T/G gene polymorphism were associated with chronic periodontitis. Shimada and coworkers found that in 196 non-smoking Japanese with different severities of chronic periodontitis, the FMBS between TNFR2 +587 allele G-positive group ($n = 46$) and allele G-negative group ($n = 150$) were 21.1% and 17.9%, respectively, without significant difference between the groups.

IL-4 gene polymorphism

Michel et al. (2001) investigated the relationship between two IL-4 polymorphisms PI and PP to FMBS in 21 healthy control and 18 patients diagnosed as aggressive periodontitis patients (early-onset periodontitis). The FMBS of the diseased IL-4-negative group ($n = 13$) and diseased positive group ($n = 5$) were not different (44% *versus* 46.6%, respectively).

Pontes et al. (2004) investigated the correlation between the IL-4 SNPs, PP and PI, to periodontal disease in a non-smoking African–American–Brazilian population. The results showed no significant association between FMBS and any of the IL-4 SNPs.

FcγR polymorphism

Kobayashi et al. (2001) evaluated 89 non-smoking Japanese patients with different severities of chronic periodontitis and found that patients who were positive for the composite genotype (FcγRIIIb-158V plus FcγRIIIb-NA2) showed significant higher FMBS in comparison with the negative group, 28.6% and 17.8%, respectively.

Conclusions

It is difficult to conclude from the above studies whether the composite IL-1 polymorphism and other ''inflammatory'' SNPs have an effect on gingival bleeding, as a major and early sign of inflammation, and it seems that the

results are highly dependent on the study design. Only a few studies were able to demonstrate an effect of a particular SNP on gingival bleeding, while most of the studies failed to do so. The problem is mainly because of small sample size, the fact that most of the studies use the inflammatory parameters as the secondary outcome variable and the strong effect of environmental factors on gingival bleeding. For example, because of the known effect of smoking on gingival bleeding, the inclusion of smokers may mask the results and prevent the researchers from showing differences between genotype-positive and genotype-negative subjects. Large amounts of dental plaque may also have a ''masking effect'', and only short-term experimental gingivitis studies may prove the relationship between genetic variability and inflammation. On the other hand, the lack of correlation between specific polymorphism and bleeding in short-term maintenance studies following surgical therapy may also be because of superior compliance achieved in these patients. Large-scale experimental gingivitis and maintenance studies are needed to show a correlation, if it exists at all, between selected genetic variability and gingival inflammation.

Gingival index (GI)

Caffesse et al. (2002) investigated periodontally healthy Hispanic patients who underwent recession coverage surgery for the association between IL-1 genotype and the results of the mucogingival surgery. Caffesse and coworkers found that 3 years after surgery, the mean GI for the IL-1 genotype-positive and genotype-negative groups was 1.13 and 1.06, respectively, with no significant differences between the groups. When the subjects were divided according to genotype and smoking habits, the GI values remained similar. Similar results were briefly mentioned in an intervention study of Cutler et al. (2000) as mentioned in detail above.

Conclusions

There is not enough data regarding the correlation between any genetic variation and GI to draw any meaningful conclusions.

Inflammatory Mediator Levels in GCF (Table 2)

Levels of IL-1β have been strongly associated with periodontal tissue destruction experience. IL-1β potently stimulates bone resorption, protease production and arachidonic acid metabolite synthesis, and these activities are directly correlated to periodontal disease (Birkedal-Hansen 1993). Several studies indicate that increased levels of IL-1β are detected in diseased periodontal tissues (Stashenko et al. 1991a) and GCF (Masada et al. 1990, Wilton et al. 1992, Hou et al. 1995). In addition, tissue levels of IL-1β correlate with episodes of active periodontal destruction (Stashenko et al. 1991a). Therefore, the relationship between genotype status and IL-1β production can help us to understand how genotype status influences periodontal health. Shirodaria et al. (2000) investigated the correlation between IL-1 genotype and the quantity of IL-1α and IL-1β in the GCF of severe chronic periodontitis patients. The results showed that allele 2 of the IL-1A -889 gene was associated with a fourfold increase of IL-1α in the GCF. Engebretson et al. (1999) evaluated whether GCF levels of IL-1β and TNF-α, and gingival tissue levels of IL-1α, IL-1β and TNF-α correlated with genotype positivity before and after non-surgical periodontal treatment. All patients ($n = 24$) were >35 years old, healthy and non-smokers with moderate-to-severe periodontitis. The results demonstrated that in shallow sites (<4 mm), the total IL-1β levels in the GCF were 2.5 times higher for genotype-positive patients prior to treatment and 2.2 times higher after treatment. A reduction in IL-1β concentration in the GCF was seen for genotype-negative but not for genotype-positive patients after treatment. There were no differences between the different genotypes and TNF-α concentrations before or after treatment. The differences between the two genotype groups in IL-1α and IL-1β levels in the gingival tissue were

Table 1. Association between genetic polymorphisms and bleeding on probing (BOP)

Gene	Polymorphism	Disease	Index	Pattern	References
IL-1	Composite genotype	ChP	FMBS	NS	Kornman et al. (1997b)
		ChP (SPT)	FMBS	↑	Lang et al. (2000)
		ChP	PBI	NS	Christgau et al. (2003)
		ChP or Perio-healthy	FMBS	NS	Papapanou et al. (2001)
		ChP	FMBS	NS	Ehmke et al. (1999)
		ChP (SPT)	FMBS	NS	Feloutzis et al. (2003)
		Mild-ChP/gingivitis	FMBS	NS	Cullinan et al. (2001)
		Gingivitis	FMBS	NS	Jepsen et al. (2003)
		ChP	FMBS/GI	NS	Cutler et al. (2000)
		ChP	FMBS	NS	De Sanctis & Zucchelli (2000)
		Perio-healthy	GI	NS	Caffesse et al. (2002)
TNF-α	TNFR2+587	ChP	FMBS	NS	Shimada et al. (2004)
IL-4	PP+PI	AgP	FMBS	NS	Michel et al. (2001)
	PP	ChP+perio-healthy	FMBS	NS	Pontes et al. (2004)
	PI				
	PP+PI				
FcγR	FcγRIIIa -158V+FcγRIIIb-NA2	ChP	FMBS	↑	Kobayashi et al. 2001

↑, significantly higher BOP; NS, non-significant; ChP, chronic periodontitis; AgP, aggressive periodontitis; SPT, supportive periodontal treatment; GI, gingival index; PBI, papillary bleeding index; FMBS, full-mouth bleeding score; PP, IL-4 promoter polymorphism at -590; PI, IL-4 70 bp repeat polymorphism in intron 2.

Table 2. Association between genetic polymorphisms and cytokine concentration in the GCF

Gene	Polymorphism	Cytokine	Disease	Significance	References
IL-1	Composite genotype	TNF-α	ChP	NS	Engebretson et al. (1999)
		IL-1β	ChP	NS	Engebretson et al. (1999)
	A −889	IL-1α	ChP	↑	Shirodaria et al. (2000)

↑, significantly higher cytokine concentration; NS, non-significant; ChP, chronic periodontitis.

not significant at baseline and after treatment, while TNF-α was not detectable in the tissue. Cutler et al. (2000) evaluated cytokine levels in GCF in 52 IL-1 composite genotype-positive and genotype-negative patients with mild-to-moderate adult periodontitis following 14 days of no oral hygiene, routine oral hygiene or oral hygiene+water irrigation. At baseline, no notable differences were demonstrated in the GCF levels of IL-1β, PGE_2, IL-10 and interferon (IFN)-γ between genotype-positive or genotype-negative patients. Following treatment, the authors describe that IL-1 genotype-positive subjects showed a marginal but non-significant increase in the levels of IL-1β, but significant increased levels of IL-10, and no notable effects of any genotype on PGE_2 or IFN-γ levels. However, the post-treatment results were described by genotype for the pooled groups, so because of differences in treatment for these groups, the conclusions should be evaluated with caution.

Conclusions

The great variation between studies with respect to the level of IL-1β in GCF suggests that other parameters in addition to IL-1 gene polymorphism are involved in the complex regulation of cytokine cascades that occur in inflammation. These results prevent us from establishing the correlation between high IL-1β levels and IL-1 gene polymorphism. The question arises as to whether there is a real functional effect of the polymorphism and indeed whether clinical trials are the best model system for such verification.

Inflammatory mediator levels in blood

Patients with severe chronic periodontitis were found to express high serum levels of inflammatory mediators (Kweider et al. 1993, Ebersole et al. 1997, Loos et al. 2000, Noack et al. 2001, Amar et al. 2003, D'Aiuto et al. 2004). Furthermore, genetic variations in cytokine genes, such as IL-1A, IL-6 and TNF-A, have been linked to enhanced systemic inflammatory responses as evaluated by serum levels of specific mediators (Fishman et al. 1998, Vickers et al. 2002, Gonzalez et al. 2003). These findings led to the hypothesis that periodontal infections can influence the systemic inflammatory response of the individual.

Michel et al. (2001) investigated the relationship between two IL-4 polymorphisms (as mentioned above) and the IL-4 serum levels in 21 healthy control and 18 patients diagnosed as suffering from aggressive periodontitis. IL-4 could not be detected by enzyme-linked immunosorbent assay in the serum of diseased patients who were positive for the two polymorphisms, whereas in patients who were negative to the polymorphisms as well as in the age-matched healthy controls, low concentrations of IL-4 were measured. These results were statistically significant and suggest that the polymorphism might affect the production of IL-4. D'Aiuto et al. (2004) investigated 94 subjects with severe chronic periodontitis for systemic inflammatory response, estimated as serum levels of C-reactive protein (CRP) and IL-6. The results showed that increased IL-6 was associated with carrying allele 2 for ILA (−899), TNF-A (−308) and IL-6 (−174), while serum CRP was associated with allele 2 for IL-A (−899) and IL-6 (−174) after correcting for age, body mass index, gender, ethnicity and cigarette smoking.

Conclusions

The above preliminary data suggest that the genetic variability of individuals with periodontal infection may affect the systemic levels of inflammatory mediators. These findings suggest that genetic polymorphisms may confer susceptibility to systemic inflammatory disorders in the presence of periodontal infection.

Cytokine production by inflammatory cells (Table 3)

Cytokine polymorphisms

Pociot et al. (1992) investigated the relationship between IL-1B genotype and IL-1β secretion by cultured peripheral blood monocytes of healthy and diabetic subjects after LPS stimulation. Monocytes from individuals homozygous for allele 2 secreted significantly higher levels of IL-1β than heterozygous individuals and heterozygous individuals more than allele 1 homozygous individuals ($p = 0.024$). A significant difference was observed between the groups, indicating an allele–dosage effect on the IL-1β response. In contrast, while evaluating the association between IL-1 genotype patterns with IL-1β production by monocytes harvested from periodontitis patients, in response to LPS of periodontal pathogens, Mark et al. (2000) did not find any significant difference between the two polymorphism groups. They concluded that genetic loci other than those defined by Kornman et al. (1997a) as being genotype positive may be the important regulators of monocytes IL-1 responses. The differences between the two studies may be because of patient selection, number of subjects included, and the presence of periodontal infection may mask the genetic variability effect.

Gore et al. (1998) investigated the association of IL-1B +3954 polymorphism with cytokine production by oral and peripheral blood PMN cells in Caucasian patients affected by periodontal diseases and 32 controls. A strong, but not significant, trend towards increased IL-1β production by peripheral PMN cells was found in patients with advanced periodontitis who carried the IL-1B +3954 allele 2; yet, a similar increase in production by oral PMN was not detected.

Galbraith et al. (1998) investigated the association between three SNPs (TNF-α −308, TNF-α −238, TNF-β +252) and the level of TNF-α production by oral PMNs in 32 Caucasian patients with chronic periodontitis and 32 healthy adult Caucasians. No correlation was found between TNF-α production and TNF-α −238 or TNF-β +252 genotypes in either patients or controls. However, the TNF-α −308 1,2 genotype was significantly associated with increased TNF-α production by oral PMNs only in the patient group.

Soga et al. (2003) examined the association between TNF-α and IL-1β

Table 3. Cytokine production by inflammatory cells

Gene	Cytokine	Polymorphism	Disease	Cell	Significance	References
IL-1	IL-1β	B +3954	Healthy or diabetes	Peripheral blood monocyte	↑	Pociot et al. (1992)
			ChP	Peripheral blood PMNs or oral PMNs	NS	Gore et al. (1998)
		B −511/−31	Perio-healthy	Peripheral blood monocyte/macrophage	NS	Soga et al. (2003)
		Composite genotype	ChP	Peripheral blood monocyte	NS	Mark et al. (2000)
TNF-α	TNF-α	−1031/−863	Perio-healthy	Peripheral blood monocyte/macrophage	NS	Soga et al. (2003)
		−238	ChP or perio-healthy	Oral PMNs	NS	Galbraith et al. (1998)
		−308	ChP		↑	
			Perio-healthy		NS	
TNF-β		+252	ChP or perio-healthy		NS	
		−857	ChP	Peripheral blood monocyte/macrophage	NS	Soga et al. (2003)
CD14		−159	Perio-healthy	Peripheral blood monocyte	↑	Yamazaki et al. (2003)

↑, significantly higher cytokine production; NS, non-significant; ChP, chronic periodontitis.

production by monocytes from periodontally healthy Japanese subjects and the SNPs in the TNF-α gene promoter (−1031, −863, −857, −308, −238) and in the IL-1β gene (−31, −511, −3954). TNF-α levels in variants −1031C/−863A or −857 T allele carriers tended to be elevated in comparison with the dominant genotypes (−1031T/−863C and −857C carriers), but with no significant difference. IL-1β levels were similar between the variants (−511C/−31T) and dominants (−511T/−31C). It was concluded that in subjects carrying the variants of the TNF-α gene, TNF-α production by monocytes/macrophages was elevated compared with the dominant carriers. However, this conclusion was not based on significant statistical evidence.

CD14 receptor

Yamazaki et al. (2003) investigated the association between an SNP at position −159 in the promoter region of the CD14 gene with CD14 expression and TNF-α production by monocytes. Transfection studies using monocyte cell lines have demonstrated that the polymorphism C-159-T resulted in an 80% increase in constitutive gene expression. Different results were obtained using monocytes harvested from periodontally healthy subjects with the C-159-T transition. While no differences were found in CD14 expression between subjects with CC, CT or TT genotype, LPS-induced TNF-α secretion by monocytes from subjects with the TT genotype was elevated compared with the other two genotyps. The discrepancy between the findings of the two experiments might be because of the low number of subjects used ($n = 4$ for each genotype).

Table 4. Cytokine polymorphism and chronic periodontitis

Cytokine	Polymorphism	Association	References
IL-2	−330	+	Scarel-Caminaga et al. (2002)
IL-4	−590	−	Pontes et al. (2004)
	−590	−	Kang et al. (2003)
	−590	−	Scarel-Caminaga et al. (2003)
IL-6	−572	+	Holla et al. (2004)
	−174	+	Trevilatto et al. (2003)
IL-10	ATA haplotype	+	Scarel-Caminaga et al. (2004)
	−1087	+	Berglundh et al. (2003)
	−597, −824	−	Gonzales et al. (2002)
	Gene promoter region	−	Yamazaki et al. (2001)
TNF-α	−308	−	Folwaczny et al. (2004)
	−308	−	Fassmann et al. (2003)
	−1031, −863, −857	+	Soga et al. (2003)
	−376, −308, −238, +489	+	Craandijk et al. (2002)
	−238, −308, +252	−	Galbraith et al. (1998)

+, related to susceptibility to periodontitis; −, not related to susceptibility to periodontitis; Assocn (resist), associated with resistance to periodontitis.

Conclusions

Data from the medical and dental literature suggest that there may be genetic traits that affect cell functions and plasma levels of cytokines, suggesting a potential for excessive inflammatory response to external stimuli. Large-scale studies in which the genetic variant is studied in both in vitro as well as in vivo models will be the only approach that will be able to correlate between functional genetic variants and parameters of gingival inflammation.

Effect of Genetic Variability on Periodontal Disease Expression

Periodontal diseases are complex diseases and not Mendelian-inherited disorders.

Because of the complexity of periodontal disease and the large number of host derived and environmental factors involved in disease pathogenesis, it is logical to assume that multiple genetic variants (SNPs) at different genes may contribute to overall disease susceptibility. As such, a simple cause and effect relationship between a particular genetic allele and a disease is not possible. An association between periodontitis and gene variants is based on the statistically significant association of specific genetic polymorphisms in individuals with periodontal disease compared with periodontally healthy individuals. Such statistical associations are not always based on functional changes caused by the polymorphism. Association studies should evaluate polymorphisms with biological effect

Table 5. Cytokine polymorphism and aggressive periodontitis

Cytokine	Polymorphism	Association	References
IL-4	PP and IP	–	Gonzales et al. (2004)
	PP and IP	+	Michel et al. (2001)
IL-10	– 597, – 824	–	Gonzales et al. (2002)
	(R and G microsatelites)	–	Kinane et al. (1999)
TNF-α	– 1031, – 863, – 857, – 308, – 238	–	Endo et al. (2001)
	– 308	–	Shapira et al. (2001)
	Microsatellite polymorphisms	–	Kinane et al. (1999)

+, related to susceptibility to periodontitis; –, not related to susceptibility to periodontitis; Assocn (resist), associated with resistance to periodontitis.

Table 6. Genetic polymorphism of inflammatory receptors and chronic periodontitis

Receptor	Polymorphism	Association	References
TNF receptor 2	+587	+	Shimada et al. (2004)
TLR-2	Arg753Gln, Arg677Trp	+	Folwaczny et al. (2004)
TLR-4	Asp299Gly, Thr399Ile	+	Folwaczny et al. (2004)
FcγRIIa	H/H (in smokers)	+	Yamamoto et al. (2004)
	R/R	+	Tang et al. (2004)
	131R	–	Chung et al. (2003)
	H/H	–	Loos et al. (2003)
	131R	–	Colombo et al. (1998)
FcγRIIb	646 – 184, intron 4	+	Yasuda et al. (2003)
FcγRIIIa	158V	+	Kobayashi et al. (2001)
	158V	+	Loos et al. (2003)
	V/V genotype	+	Meisel et al. (2001)
FcγRIIIb	NA1	+ with resistance	Sugita et al. (2001)
	NA2	+	Kang et al. (2003)
	NA2	–	Colombo et al. (1998)
	NA2	+	Kobayashi et al. (1997)
CD14	– 1359	+	Holla et al. (2002)
	– 159	–	Yamazaki et al. (2003)

+, related to susceptibility to periodontitis; –, not related to susceptibility to periodontitis; Assocn (resist), associated with resistance to periodontitis.

Table 7. Genetic polymorphism of inflammatory receptors and aggressive periodontitis

Receptor	Polymorphism	Association	References
FcγRIIa	131R	–	Chung et al. (2003)
	H/H	+	Loos et al. (2003)
FcγRIIb	695	+	Yasuda et al. (2003)
FcγRIIIa	158F	+	Kobayashi et al. (2000)
	158V	+	Loos et al. (2003)
FcγRIIIb	NA2	+	Kobayashi et al. (2000)
FMLP receptor	329, 378	+	Zhang et al. (2003)

+, related to susceptibility to periodontitis; –, not related to susceptibility to periodontitis; Assocn (resist), associated with resistance to periodontitis.

and a large number of individuals in population-based studies, which may have the power to detect a significant association. This review has not attempted to summarize the plethora of studies regarding the association between genetic polymorphisms and chronic or aggressive periodontitis, but summarizes these data in tabular form (Tables 4–7). It is important to realize that most of these studies are underpowered to draw any definitive conclusions or to be incorporated into meta-analyses and systematic reviews. The powering of studies to reveal associations between multiple SNPs and periodontal disease will involve thousands of subjects rather than, at best, the hundreds currently used.

General Conclusions

(1) Clinical inflammation is a crucial aspect of the periodontal diseases. It is accepted that bacteria cause infection and the subsequent inflammation is the main part of the disease process. The fact that genetics as well as environmental factors influence the inflammatory process is an important concept in our understanding of the disease process.

(2) The distribution and frequency of gene polymorphisms may vary considerably between different cohorts, particularly those of differing racial origins. Consequently, gene association studies may require the investigation of large homogenous cohorts and data obtained from one study may not be readily generalized to other patient cohorts. In addition, the use of the ''candidate gene'' approach risks overlooking other genes involved in the regulation of inflammation and consequently missing genetic loci that could be strongly associated with disease risk. With the development of newer technologies such as genome-wide ''SNP Chips'', it is likely to be possible to carry out more systematic studies to identify gene loci associated with a risk of disease in the near future.

(3) The ''candidate gene'' approach is limited in exploring a genetic influence on gingival inflammation because of the large number of genes that may be involved in the inflammatory and disease processes.

(4) The powering of studies to reveal associations between single or multiple SNPs and inflammatory parameters as well as disease expression is complex and will need to involve much larger number of subjects than used previously.

(5) To date, the available SNP data (including the IL-1 composite genotype) do not support the utility of such tests in the diagnosis and prognostic assessment of periodontal diseases.

(6) In recent years, many studies investigated the role of cytokine and

receptor polymorphisms in periodontal disease. Many of these reports evaluated the inflammatory parameters in the investigated subjects; however, none of them analysed the association between the investigated polymorphisms and the inflammatory parameters. It will be beneficial to re-examine the data for these associations as there is little evidence on this subject (Colombo et al. 1998, Diehl et al. 1999, Armitage et al. 2000, Kobayashi et al. 2000, Shirodaria et al. 2000, Socransky et al. 2000, Endo et al. 2001, Meisel et al. 2001, Gonzales et al. 2002, Scarel-Caminaga et al. 2002, Anusaksathien et al. 2003, Berglundh et al. 2003, Chung et al. 2003, de Souza et al. 2003a, b, Gonzales et al. 2003, Scarel-Caminaga et al. 2003, 2004, Trevilatto et al. 2003, Folwaczny et al. 2004, Gruica et al. 2004, Yamamoto et al. 2004).

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