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Immunological differences and similarities between chronic periodontitis and aggressive periodontitis

PAULINE J. FORD, JORGE GAMONAL & GREGORY J. SEYMOUR

Chronic periodontitis is an inflammatory response in the periodontal tissues, which is elicited by the microorganisms present in dental plaque. The clinical manifestation of the disease is dependent upon the nature of this response, which, in turn, is determined by the patient's innate susceptibility. Chronic periodontitis in adults typically follows a cyclical course, with some forms remaining stable over many years and other forms progressing with subsequent tooth loss despite extensive treatment (44, 49).

The initial immune response in chronic periodontitis occurs following colonization of the gingival sulcus by periodontopathic bacteria. The presence of the bacteria induces the production of cytokines and chemokines by the gingival epithelium. This results in the expression of adhesion molecules, increased permeability of gingival capillaries and chemotaxis of polymorphonuclear neutrophils through the junctional epithelium and into the gingival sulcus. The specific cytokines and chemokines produced by this initial response lead to a perivascular T-cell/macrophage dominated inflammatory infiltrate in the connective tissues. If this cell-mediated immune response does not control the bacterial challenge, progression to a B-cell/plasma-cell lesion occurs. The antibodies subsequently produced may be protective and control the infection, or may be nonprotective with resultant connective tissue destruction and bone loss (reviewed in 42,44). The effectiveness of this response varies among individuals and appears to be important in determining disease susceptibility.

Currently, however, there is little evidence that aggressive periodontitis, either localized or generalized, follows the typical cyclical course of chronic periodontitis. Additionally, aggressive periodontitis appears to differ from chronic periodontitis in that clinically the gingival lesion is often absent, suggesting that the lesion of aggressive periodontitis may not follow the same sequence of initiation and progression as chronic periodontitis (from gingival T-cell lesion to progressive B-cell lesion). While further investigation of the nature of the immune response in both chronic periodontitis and aggressive periodontitis is clearly required, this review is not a comprehensive analysis of the immunopathology of these conditions but rather will cover only those elements where a direct comparison of similarities or differences is possible or where further work may highlight possible differences.

Innate immunity: similarities and differences

Toll-like receptors

The innate immune system senses invading microbes through their pathogen-associated molecular patterns via pattern-recognition receptors, such as toll-like receptors. Toll-like receptors are structurally related to the *Drosophila* toll receptor and are expressed in human keratinocytes (63, 82). As effectors of innate immunity, antimicrobial peptides and proteins play a predominant role, providing the first line of defense in the skin against invading microbes (28). Antimicrobial peptides are phylogenetically the oldest innate immune responses (48, 81). Antimicrobial

peptides and proteins directly kill a broad spectrum of microbes, including Gram-positive and Gram-negative bacteria, fungi and certain viruses. They have been identified in resident cells, such as keratinocytes, as well as in infiltrating cells, and are more than simple antibiotics. Their additional functions, as proteinase inhibitors, chemokines and neuropeptides, are also important to skin biology. Typical examples of pathogen-associated molecular patterns include lipopolysaccharide from Gram-negative bacteria, lipoteichoic acids and peptidoglycan of Gram-positive bacteria, and mannans of yeasts/fungi. Among the 10 human toll-like receptors identified so far, toll-like receptor-2 and toll-like receptor-4 are the most defined members (114). Toll-like receptor-2 is mostly involved in the recognition of a variety of different bacterial cell components, such as peptidoglycan and lipoproteins (72). Toll-like receptor-4 has been shown to specifically recognize lipopolysaccharide of Gram-negative bacteria and acts in cooperation with several protein components, such as lipopolysaccharide-binding protein and CD14 (126).

Cell wall components of periodontopathic bacteria stimulate, via toll-like receptor-2 and toll-like receptor-4, the production of proinflammatory cytokines from the host, such as interleukin-1beta and tumor necrosis factor-alpha, which induce alveolar resorption and the production of matrix metalloproteinases (MMPs). A large body of evidence indicates that *Porphyromonas gingivalis* lipopolysaccharide stimulates toll-like receptor-2, and not toll-like receptor-4 (59, 91, 126). By contrast, Gram-negative enterobacteria can stimulate both toll-like receptor-2 and toll-like receptor-4 (77). Kikkert et al. (59) demonstrated that Gram-negative periodontal bacteria primarily interact with toll-like receptor-2. Only *Aggregatibacter actinomycetemcomitans* and *Veillonella parvula* were capable of stimulating both toll-like receptor-2 and toll-like receptor-4.

Expression of both toll-like receptor-2 and toll-like receptor-4 has been demonstrated in healthy oral epithelium but the expression of both was markedly upregulated with inflammation (112). In chronic periodontitis tissues, both toll-like receptor-2 and toll-like receptor-4 were detected, whereas in healthy tissues, there was only weak expression of toll-like receptor-2 and no expression of toll-like receptor-4 (94). Similarly, toll-like receptor-2 and toll-like receptor-4 were expressed in periodontal tissues, and the ratio of toll-like receptor-2-positive cells to toll-like receptor-4-positive cells was highest overall in connective tissue subjacent to pocket epithelium in patients with severe periodontitis (84).

Recently, several studies have shown that mutations in toll-like receptor-4 (Asp299Gly and Thr399Ile) and in toll-like receptor-2 (Arg677Trp and Arg753Gly) are associated with endotoxin hyporesponsiveness in periodontal diseases. However, findings associating these mutations with periodontal disease have been inconsistent. In some studies, no association was found between chronic periodontitis and the mutations in toll-like receptor-2 and toll-like receptor-4 (25, 67). Others, however, have reported a strong association between toll-like receptor-4 mutations and chronic periodontitis, but not between toll-like receptor-4 mutations and aggressive periodontitis. No association between toll-like receptor-2 mutations and either chronic periodontitis or aggressive periodontitis was observed (102). The importance of toll-like receptor-4 function in periodontal disease susceptibility is further supported by others who showed that the toll-like receptor-4 polymorphism, Thr399Ile, was more frequent in a combined group of patients with aggressive periodontitis and chronic periodontitis than in healthy controls (10). Associations were not observed if the aggressive periodontitis and chronic periodontitis groups were analyzed separately. These specific missense toll-like receptor-2 and toll-like receptor-4 mutations were not found in a Japanese population; however, other toll-like receptor-2 and toll-like receptor-4 polymorphisms were associated with moderate and severe periodontitis in this group (26). Additionally, studies of Turkish (7) and Czech (50) populations failed to find an association between toll-like receptor-2 or toll-like receptor-4 polymorphisms and susceptibility to chronic periodontitis.

While, as discussed, some studies have found an association between toll-like receptor-4 polymorphisms and periodontitis susceptibility, they were not able to discriminate between chronic periodontitis and aggressive periodontitis on this basis (10, 102). In contrast, however, the toll-like receptor-4 mutation Asp299Gly has been associated with protection against aggressive periodontitis, but not against chronic periodontitis, in a group of young Caucasian adults (53). The authors suggest that this mutation modulates the toll-like receptor-mediated responses in these individuals, thereby damping down the inflammatory responses initiated by bacterial products in the periodontal tissues. Interestingly, this polymorphism has also been linked with protection against systemic inflammatory conditions such as atherosclerosis (2). Another study showed that toll-like receptor-2 responses were upregulated in the peripheral blood of patients with localized aggressive periodontitis com-

pared with controls (110). By contrast, a lower expression of toll-like receptor-2 was found in gingival mononuclear cells from subjects with chronic periodontitis (85). This finding was explained as endotoxin tolerance caused by the continual exposure of gingival cells to lipopolysaccharide resulting in the downregulation of toll-like receptor-2 and toll-like receptor-4. Therefore, both toll-like receptor polymorphisms and the ability to achieve tolerance to lipopolysaccharide may be points of differences between chronic periodontitis and aggressive periodontitis.

While toll-like receptor-2 and toll-like receptor-4 recognize lipids, nucleic acids (bacterial and viral) are recognized by other toll-like receptors (toll-like receptor-3, toll-like receptor-7, toll-like receptor-8 and toll-like receptor-9). Toll-like receptor-5 binds the protein flagellin. Toll-like receptor-7 and toll-like receptor-9, along with toll-like receptor-2 and toll-like receptor-4, were found to be expressed at higher levels in periodontitis lesions than in gingivitis lesions (57). Toll-like receptor-5 was expressed in both groups of tissues but there was no difference between periodontitis and gingivitis, perhaps because of the presence of similar levels of oral bacteria with flagellae in both disease states. A summary of the various studies analyzing the association between toll-like receptor-2 and toll-like receptor-4 with chronic periodontitis and aggressive periodontitis is shown in Table 1.

Many factors could account for the observed discrepancies among these studies, for example: smoking, ethnicity, number of subjects, use of blood donors with unknown age, gender and periodontal disease status as controls, as well as differences in the selection criteria of the studied population. Taken together, these results suggest that toll-like receptor-2 and toll-like receptor-4 are involved in the pathogenesis of both chronic periodontitis and aggressive periodontitis and that specific polymorphisms of these innate receptors may be associated with disease susceptibility. However, this is inconsistently observed across different ethnic groups.

Defensins

The epithelium is not only a physical barrier, but also has chemical defense mechanisms containing antimicrobial peptides, such as defensins (122). Defensins are endogenous, small, cysteine-rich antimicrobial peptides that are produced by leukocytes and epithelial cells. Substantial evidence has accumulated in recent years indicating that mammalian defensins are multifunctional and, by interacting with host cell receptor(s), participate in both the in-

Table 1. Summary of studies analyzing the association of toll-like receptor-2 (TLR2) and toll-like receptor-4 (TLR4) with periodontitis

Study	Periodontitis	Population	Toll-like receptor	Association
Schroder et al. 2005 (101)	Chronic aggressive	Germany	TLR4 TLR4	Yes No
James et al. 2007 (54)	Chronic aggressive	Great Britain	TLR4	No Yes
Brett et al. 2005 (10)	Chronic aggressive	Great Britain	TLR4 TLR4	No No
Folwaczny et al. 2004 (25)	Chronic	Germany	TLR2 TLR4	No No
Laine et al. 2005 (67)	Chronic	The Netherlands	TLR4	No
Ren L et al. 2005 (94)	Chronic	Chinese	TLR2 TLR4	Yes Yes
Holla et al. 2007 (50)	Chronic	Czech	TLR4	No
Berdeli et al. 2007 (7)	Chronic	Turkish	TLR4	No
Fukusaki et al. 2007 (26)	Chronic	Japan	TLR2 TLR4	No No
Kajita et al. 2007 (57)	Chronic	Japan	TLR4 TLR2	Yes Yes
Sorensen et al. 2008 (110)	Localized aggressive Generalized aggressive	Denmark	TLR2 TLR2	Yes No

nate and adaptive antimicrobial immunity of the host. Defensins contain cysteine residues that form characteristic disulfide bridges. Human defensins are classified into two subgroups: alpha-defensins and beta-defensins. Six alpha-defensins (hND-1 to hND-6) and four beta-defensins (hBD-1 to hBD-4) have been defined in humans. Polymorphonuclear leukocytes express alpha-defensins as part of their nonoxidative antimicrobial mechanisms, and beta-defensins are expressed by mucosal epithelial cells (17). Searches of the human genome have revealed almost 40 potential coding regions for hBDs (103); however, only the first four hBDs (hBD-1 to hBD-4) have been characterized in detail (90). The localization of hBD-1, hBD-2 and hBD-3 in stratified squamous epithelium (including oral mucosa and skin) has been confirmed at protein and messenger RNA (mRNA) levels (1, 16). In gingival tissues, hBD-1 and hBD-2 are localized in the sulcular epithelium, but not in the junctional epithelium (16). By contrast, hBD-3 was mainly localized in the basal layer of the gingival epithelium, not only in the keratinocytes but also in Langerhans' cells and Merkel cells, suggesting that hBD-3 facilitates cross-talk between the gingival epithelium and the connective tissues, serving as a link between the innate and adaptive immune responses (51, 74). Studies have also shown that hBDs are chemotactic for immature dendritic cells, memory T-cells and monocytes (31, 125). Expression of hBD-1, hBD-2 and hBD-3 was observed in inflamed and noninflamed gingival tissue samples, as detected by real-time polymerase chain reaction (PCR). In biopsy samples from periodontitis tissue, hBD-2 expression was significantly higher than that of hBD-1, while the expression levels of hBD-2 were comparable to those of hBD-3 (18). The expression of *hBD-2* mRNA by human gingival epithelial cells stimulated with *P. gingivalis* was upregulated compared with the expression of *hBD-2* mRNA in unstimulated cells. These results suggest that the expression of *hBD-2* mRNA in human gingival epithelial cells is induced in a *P. gingivalis*-dependent manner and is likely to occur in the initial stages of the inflammatory response after exposure to periodontal bacteria (113).

Some studies have suggested differences in the expression of hBD-1 and hBD-2 at different sites in the oral cavity (16, 80). While hBD-1 was expressed in most locations in the oral cavity, hBD-2 was only found in gingival tissues and especially in sites of inflammation. A basal level of mRNA expression was found to exist for hBD-1, hBD-2 and hBD-3 in both healthy patients and in patients with chronic periodontitis. Significantly higher expression of hBD-3, however, was seen in healthy tissues than in chronic periodontitis tissues, suggesting a protective role for this peptide (8). The gingival epithelium from periodontally healthy subjects exhibited significantly higher expression of hBD-2 than the clinically healthy tissues from patients with chronic periodontitis. When the expression levels of hBD-1 and hBD-2 in tissues from the same individual were assessed, both defensins were expressed at a higher level in pocket epithelia than in the adjacent healthy gingival epithelia (75). This suggests that higher baseline levels of hBD expression are protective for chronic periodontitis. In addition, a recent study found that expression of hBD-1 and hBD-2 was somewhat higher in human gingival tissues affected by chronic periodontitis or peri-implantitis compared with gingivally healthy tissues, and that hBD-1 was expressed more strongly than hBD-2 in the inflamed tissues (66).

The expression of hBD-1 and hBD-2 in the gingival tissues of patients with gingivitis, aggressive periodontitis and chronic periodontitis was investigated (118). The results showed the differential expression of *hBD-1* and *hBD-2* genes in these groups of patients. Expression of hBD-1 and hBD-2 was lower in gingival tissues from patients with gingivitis than in the tissues from healthy controls, perhaps indicating an enhanced susceptibility of patients with gingivitis to periodontal infections. The expression of hBD-1 was decreased, and that of hBD-2 was increased, in patients with aggressive periodontitis compared with healthy controls. By contrast, patients with chronic periodontitis expressed higher levels of hBD-1 compared with healthy controls, and the authors suggest that although these higher levels of hBD-1 may have offered a protective effect, they were not sufficient to prevent periodontal infection and destruction. The increased hBD-2 expression in aggressive periodontitis may be caused by the stimulation of this antimicrobial peptide by *A. actinomycetemcomitans*. This organism has been shown to stimulate *hBD-2* mRNA expression in human oral keratinocytes *in vitro* (12).

P. gingivalis, one of the most pathogenic bacteria in chronic periodontitis, stimulates hBD-2 expression in gingival epithelial cells, and this is markedly upregulated by the secretion of proteases by the organism through a protease-activated receptor pathway (13). It is interesting that the challenge, by *A. actinomycetemcomitans*, of gingival epithelial cells from a patient with localized aggressive periodontitis resulted in little to no induction of hBD-2 or interleukin-8 compared with the challenge of cells derived from a healthy individual (69). As the transcription of

hBD is dependent upon recognition by toll-like receptors and other pattern-recognition receptors, this may be explained by the reduced basal levels of toll-like receptor-2 in the patient with localized aggressive periodontitis. However, no variation in the toll-like receptor-2 sequence was observed in the patient with localized aggressive periodontitis. These results suggest that a differential expression of innate immune response genes to *A. actinomycetemcomitans* in the gingival epithelium could be an underlying factor of susceptibility to localized aggressive periodontitis. A reduction in the toll-like receptor-2-mediated recognition of periodontal pathogens, such as *A. actinomycetemcomitans*, could lead to an increased susceptibility to colonization. Similarly, toll-like receptor-4 polymorphisms have been shown to modulate the ability of gingival epithelial cells to produce hBD-2 upon exposure to *P. gingivalis* and this has been suggested to contribute to susceptibility to colonization with this organism (62).

Adaptive immunity: similarities and differences

It is now well established that the progression of chronic periodontitis requires a number of factors: periodontopathic bacteria; high levels of proinflammatory cytokines, MMPs and prostaglandin E_2; and low levels of inflammation inhibitory cytokines such as interleukin-10, transforming growth factor-beta and tissue inhibitors of metalloproteinase. The susceptibility and extent of tissue destruction therefore appear to be determined by the complex balance of cytokines induced by the presence of many possible combinations of periodontal pathogens (reviewed in ref. 44). Whether or not the same is also true for aggressive periodontitis is unknown, but the fact that both diseases have a similar immunohistological profile (109) suggests that similar immunopathological mechanisms are involved.

Dendritic cells / Langerhans' cells

Epithelial cells play an important role in the initiation of adaptive immune responses and can trigger and modify the activation and differentiation of dendritic cells, B-cells and T-cells (101). Langerhans' cells are the best characterized immature dendritic cells, located above the basal layer of epithelial cells in the skin, oral, nasal, esophageal, pulmonary, vaginal and rectal mucosa (45). In their immature state, dendritic cells are efficient antigen-capture cells, but as they mature, they undergo phenotypic changes that facilitate their migration towards lymphoid organs and their unique ability to prime naive T-cells (111). The studies of Langerhans' cells in the gingiva showed varying results, with increased numbers (97), decreased numbers (104) and no quantitative change (41) with periodontal disease. Other studies have described a marked change in dendritic cells from health to chronic periodontitis, with the numbers of immature $CD1a^+$ dendritic cells (by definition antigen-capture cells) being increased significantly in the diseased epithelium. The numbers of $CD83^+$ mature dendritic cells (by definition, antigen-presenting cells) were increased within the lymphoid-rich diseased lamina propria (55). Previous studies, however, have shown that during chronic periodontitis the number of gingival Langerhans' cells decreases according to the severity of the periodontal disease (104) and that in chronic periodontitis the major antigen-presenting cell is a $CD83^+$ B-cell (41).

Morphological changes of Langerhans' cells may reflect a cellular adaptation during their epithelial transmigration (104) towards connective tissue in response to the presence of bacterial elements (14) that penetrate the gingival epithelium. Dermal dendritic cells are present in the lamina propria of gingival tissues from healthy subjects and from those with chronic periodontitis and contribute to the $CD83^+$ populations observed in inflamed gingivae (56). These data suggest that multiple dendritic cell subsets may be present in the gingival tissues and engaged in antigen presentation in the pathogenesis (56) and maintenance (9) of chronic periodontitis. It remains to be determined whether the same is also true for aggressive periodontitis.

Immune regulation in periodontitis

The immune response in periodontal disease is governed by the net effect of T-helper 1 (Th1) and T-helper 2 (Th2) cytokines. Th1 cytokines include interleukin-2 and interferon-gamma and promote cell-mediated immunity, while the Th2 cytokine, interleukin-4, suppresses cell-mediated responses and enhances humoral immunity (reviewed in ref. 27). Recently, a new subset of T-helper cells, Th17 cells, characterized by the production of interleukin-17, has been described. This subset may have both destructive and protective effects in periodontal diseases.

The early / stable lesion of chronic periodontitis is dominated by macrophages and T-cells, suggesting that Th1 cytokines are important in the development of this response, while the advanced / progressive

lesion of chronic periodontitis, which is characterized by B-cells and plasma cells, is dependent upon Th2 cytokines (36, 105, 33, 38, 92, 123). Interferon-gamma, produced by the Th1 cells in the early lesion, would act to limit the infection by enhancing the phagocytic activity of neutrophils and macrophages. Owing to the persistence of the bacterial antigens in the plaque biofilm, however, the lesion cannot resolve (33). Progression from a stable lesion to progressive periodontitis is characterized by a change in the nature of the inflammatory infiltrate and an increase in the number of B-cells and plasma cells. This may occur as a result of the continued presence of pathogens and the existence of an ineffective Th1 response. The production of interleukin-4, possibly as a result of mast cell stimulation, causes a Th2 response with B-cell activation and the production of antibodies. Protective antibodies are likely to be effective in containing the infection, but nonprotective antibodies may also be produced, leading to persistence of the infection and high levels of interleukin-1 with resultant tissue destruction (33, 34, 40, 42, 105). A reduced Th1 response has been shown in chronic periodontitis, where peripheral blood mononuclear cells obtained from patients with chronic periodontitis and then stimulated with mitogens (106), *P. gingivalis* and *Fusobacterium nucleatum* (33) showed lower levels of Th1 cytokines. Additionally, increased levels of Th2 cytokines have been reported in the gingival crevicular fluid (93), gingival tissue (68, 78 116, 124) and peripheral blood (3, 5, 123) of patients with chronic periodontitis. These studies support the concept that in chronic periodontitis the early / stable lesion is characterized by a Th1 response and that the advanced / progressive lesion is associated with a Th2 response. Because of the B-cell / plasma-cell nature of the aggressive periodontitis lesion it is likely that aggressive periodontitis is also a Th2-mediated lesion.

Interleukin-10 has been implicated in the pathogenesis of chronic periodontitis. By stimulating B-cell immunity, while at the same time suppressing innate immunity and antigen-specific T-cell responses, in particular Th1-mediated responses, the role of interleukin-10 in human chronic infections is both complex and critical. Indeed, interleukin-10 may be critical in controlling the balance between Th1 cells and Th2 cells in chronic periodontitis, whereby an excess of interleukin-10 may shift the balance in favour of a Th2 response and progressive disease, whereas a shortage of interleukin-10 may lead to increased interleukin-1 production and increased tissue destruction (15).

By contrast, high levels of interleukin-10 may even inhibit B-cell activation and proliferation (79), hence further illustrating the complex role of this cytokine. Low levels of interleukin-10 have been demonstrated in chronic periodontitis lesions compared with gingivitis (35), which may allow continued polyclonal B-cell activation to occur in chronic periodontitis.

Even though it is now generally agreed that chronic periodontitis is a Th2 response (61), the role of Th1 / Th2 responses in both chronic periodontitis (19, 99, 117) and aggressive periodontitis, however, remains unresolved.

A third helper T-cell subset, defined as Th17, has been recently identified, and these cells appear to add a further dimension to the Th1 / Th2 paradigm of immune regulation in periodontal disease. Th17 cells are characterized by the production of interleukin-17, a cytokine that has been associated with a number of systemic chronic inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, Crohn's disease and psoriasis (83). These diseases have features in common with chronic periodontitis – local chronic inflammation with production of inflammatory cytokines with resultant tissue destruction and ineffective repair processes. Th17 cell development is dependent upon the presence of interleukin-23 (a monocyte product) and also interleukin-6 and transforming growth factor-beta. Th17 cells express genes associated with chronic inflammation (121) and may lead to the production of metalloproteinases and the upregulation of receptor activator of nuclear factor-κB ligand (RANKL) expression on osteoblasts and resultant osteoclast formation and bone destruction. In this context, Th17 cells may play a role in the tissue destruction seen in chronic periodontitis. The development of Th17 cells in the presence of interleukin-23 is a negative regulator of Th1-mediated responses (130) and hence may be important in the development of progressive chronic periodontitis. Interleukin-17 expression has, in fact, been demonstrated to be higher in chronic periodontitis tissues than in healthy tissues (6, 87), and, along with interleukin-1beta and tumor necrosis factor-alpha, interleukin-17 has been shown to induce the production of pro-MMP-1 and MMP-3 by gingival fibroblasts (6). By contrast, interleukin-17 receptor knockout mice showed increased *P. gingivalis*-induced periodontal bone loss (129), suggesting a possible protective role for interleukin-17 in *P. gingivalis*-induced tissue destruction. It has been shown that Th17 cells can be converted to Th1 cells or Th2 cells under the influence of interleukin-12 or interleukin-4 respectively (71), while $CD4^+$, $CD25^+$,

forkhead box P3 (Foxp3$^+$) regulatory T-cells can be converted to an interleukin-17-producing cell when co-cultured with dendritic cells selectively activated via dectin-1 (89). These latter findings highlight the complex regulatory networks that are probably operating in both chronic periodontitis and aggressive periodontitis (Fig. 1). At this stage, however, it is not possible to differentiate between these two diseases on the basis of their immunoregulatory profiles.

CD4$^+$CD25$^+$ regulatory T-cells, along with increased expression of Foxp3 were found more frequently in chronic periodontitis tissues than in gingivitis tissues (11, 86), and more than 90% of T-cell clones derived from chronic periodontitis lesions expressed Foxp3 (52). In addition, the expression of Foxp3 and CD25 mRNA was found to be greater in active periodontal lesions than in inactive lesions (20). We have recently demonstrated that CD4$^+$CD25$^+$Foxp3$^+$ regulatory T-cells were present in inflammatory lesions of both human peri-implantitis and peri-implant mucositis; however, the significantly higher levels of CD4$^+$CD25$^+$Foxp3$^+$ regulatory T-cells in the peri-implantitis lesions suggest that regulatory T-cells may play a role in the pathogenesis of peri-implantitis (unpublished results). However, the role of these cells in chronic periodontitis is still uncertain because others have demonstrated reduced numbers of CD25$^+$Foxp3$^+$ cells in chronic periodontitis tissues compared to tissues with gingivitis (23).

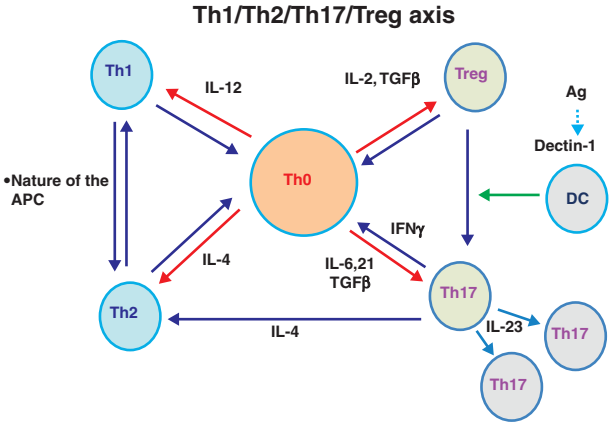

Fig. 1. Showing a putative Th1/Th2/Th17/Treg regulatory axis. Th17 cells can be converted to Th1 or Th2 cells under the influence of interleukin (IL)-12 or IL-4 respectively (71), while CD4+, CD25+, forkhead box P3 (Foxp3+) regulatory T cells (Treg) can be converted to an IL-17 producing cell when co-cultured with dendritic cells selectively activated via dectin-1. Th1 cells can be converted to Th2 cells depending upon the nature of the antigen (Ag) presenting cell (APC) (43) highlighting the complex regulatory networks which are likely to be operating in both chronic and aggressive periodontitis. TGFβ, transforming growth factor-β; IFNγ, interferon-γ.

The functional activity of regulatory T-cells in chronic and aggressive periodontitis needs further investigation because it has been suggested that T-cells exist that transiently express Foxp3$^+$ but lack a regulatory role, and that these cells may be involved in periodontal tissue destruction through the down-regulation of transforming growth factor-beta1 and interleukin-10 (20, 88). However, the role of these cells in aggressive periodontitis remains to be determined but is essential in terms of understanding the immunoregulation of aggressive periodontitis and hence identifying possible differences between chronic periodontitis and aggressive periodontitis.

Chemokines

Migration of inflammatory cells into the tissues occurs as a result of the expression, in tissues, of adhesion molecules by endothelial cells and keratinocytes and of cytokines that have chemotactic properties, namely chemokines. The particular chemokines present determine the subsets of leukocytes that are attracted into the tissue and therefore regulate the type of response which occurs (108). Chemokines themselves are, in turn, regulated by other cytokines.

Chemokines attract and activate leukocyte populations through interacting with specific receptors that are members of the seven-transmembrane-spanning G-protein-coupled proteins which are selectively expressed in these cells (96). The chemokines are a large family of small molecules (from 7 to 15 kDa, and from 67 to 127 amino acids in length), structurally related to heparin-binding proteins, which are classified into four subfamilies according to the configuration of cysteine residues near the N-terminus, depending on whether the first two cysteines are separated (CXC, CX3C) or not (CC, C) by an intervening amino acid (4, 96).

Besides their chemoattractant activity, chemokines are also implicated in the polarization of the immune response, in macrophage activation and in the pathogenesis of several diseases (76). These chemoattractant cytokines are responsible for the migration and subsequent activation of specific types of leukocyte populations into inflamed periodontal tissues (46). Chemokines are relevant in the inflammatory process, not only for their role in regulating leukocyte recruitment, but also for other physiological and pathological activities, such as lymphoid trafficking, Th1/Th2 development and wound healing (4, 96). They exert their effects on target cells by binding to specific receptors on the surface of a variety of cell types (98).

Monocyte chemoattractant protein-1 is a member of the CC chemokines (95). It shows significant chemotactic activity to cells of the monocyte/macrophage lineage, and is different from other chemoattractants in that it is relatively specific for monocytes (95). It has been previously shown that monocyte chemoattractant protein-1 is synthesized in inflamed gingival tissues by endothelial cells and mononuclear phagocytes of patients with chronic periodontitis (127, 128). The levels of monocyte chemoattractant protein-1 are influenced by interleukin-10 such that reduced levels of interleukin-10 in periodontitis may result in reduced levels of monocyte chemoattractant protein-1 with resultant impairment of cell-mediated immunity (119). However, monocyte chemoattractant protein-1 is also involved in the chemoattraction of Th2 cells into the lesion (47). The net effect that this chemokine exerts is therefore unclear. There is evidence for a role of monocyte chemoattractant protein-1 in the activation and recruitment of inflammatory and immune cells in periodontal disease. The gingival crevicular fluid levels of monocyte chemoattractant protein-1 and tumor necrosis factor-alpha were found to be elevated both in patients with aggressive periodontitis and chronic periodontitis compared to controls. Although a positive correlation between these levels and clinical parameters was found, the levels appeared to be comparable between the groups with aggressive periodontitis and chronic periodontitis (65).

Macrophage inflammatory protein-1alpha also appears to play a role in the recruitment of both Th1 and Th2 cells (60, 108). Both monocyte chemoattractant protein-1 (54) and macrophage inflammatory protein-1alpha (70) have been shown to attract neutrophils into the tissues, whereas interferon-gamma-inducible protein 10 chemoattracts Th1 cells (24). Macrophage inflammatory protein-1alpha and macrophage inflammatory protein-1beta are both acidic proteins belonging to the CC subgroup of chemokines (4) and have regulatory roles during cell-mediated immune responses (108), causing the selective migration of monocytes and lymphocytes (100). While macrophage inflammatory protein-1alpha is preferentially chemotactic for the $CD8^+$ T-cell subset, macrophage inflammatory protein-1beta is involved in the migration of the $CD4^+$ T-cell subset (100). In gingival tissues, low levels of monocyte chemoattractant protein-1 were expressed; however, the proportion of macrophage inflammatory protein-$1alpha^+$ cells increased in inflamed tissues with a greater CD8 and B-cell infiltrate and also in tissues with low levels of inflammation and higher numbers of macrophages (39).

Another chemokine that may be involved is regulated on activation normal T-cell expressed and secreted (RANTES), which, like monocyte chemoattractant protein-1 and interferon-gamma-inducible protein 10, has been demonstrated to be expressed by gingival keratinocytes at lower levels than those of macrophage inflammatory protein-1alpha. Expression of these chemokines, but not of macrophage inflammatory protein-1alpha, is reduced with increasing inflammation, suggesting a role for this chemokine in later, as well as in early, stages of inflammation (37). The RANTES levels have been demonstrated to become lower with periodontal therapy (30), thereby implicating it in the inflammatory process. RANTES also belongs to the CC chemokine subfamily and has a unique spectrum of chemotactic activity (46). It is a potent chemoattractant for eosinophils, monocytes, natural killer cells and Th1 cells; however, it is not effective on Th2 cells (108, 120). The presence of RANTES in the gingival crevicular fluid of patients with chronic periodontitis has previously been demonstrated (29). In another study, patients with aggressive periodontitis had significantly higher levels of gingival crevicular fluid monocyte chemoattractant protein-1 and RANTES compared with the healthy group. The levels of these chemokines also positively correlated with both probing depth and clinical attachment loss, suggesting that they could play a role in the pathogenesis of generalized aggressive periodontitis (21).

Interleukin-8 is a chemoattractant for neutrophils expressing the receptor CXCR1. Enhanced accumulation of neutrophils in the pocket epithelium and adjacent connective tissue of patients with chronic periodontitis and with generalized aggressive periodontitis was associated with the upregulation of interleukin-8, intercellular adhesion molecule-1, interleukin-1beta and tumor necrosis factor-alpha expression, which related to the severity and activity of generalized aggressive periodontitis (73). Using reverse transcription-PCR techniques to evaluate the pattern of mRNA expression in gingival biopsies from patients with aggressive periodontitis and chronic periodontitis, the prevalence of expression of interleukin-8 and its receptor, CXCR1, was similar in the tissues of control subjects and patients. However, the intensity of expression was greater in patients with periodontitis. Gingival biopsies from patients with aggressive periodontitis demonstrated more frequent and higher expression of the chemokines macrophage inflammatory protein-1alpha and interferon-gamma-

inducible protein 10 and their respective receptors, CCR5 and CXCR3. These tissues were also associated with higher expression of interferon-gamma and lower expression of interleukin-10. By contrast, patients with chronic periodontitis exhibited a more frequent and intense expression of monocyte chemoattractant protein-1 and its receptor, CCR4, as well as interleukin-10 (32). However, the levels of macrophage inflammatory protein-1alpha and macrophage inflammatory protein-1beta in the gingival crevicular fluid were found to be similar in chronic periodontitis and generalized aggressive periodontitis groups of patients (22). These data suggest that there is a tendency towards the development of a predominantly Th2 response in chronic periodontitis, whereas the observed expression of specific chemokines and their receptors in aggressive periodontitis may suggest the chemoattraction of Th1 cells. This, however, does not equate with the overwhelming finding that aggressive periodontitis is predominantly a B-cell / plasma-cell Th2-mediated lesion.

Endothelial-monocyte activating polypeptide-II, a novel tumor-derived mediator, was originally purified from the supernatant of cultured murine fibrosarcoma cells based on its ability to induce tissue factor on the surface on endothelial cells *in vitro* (58). This cytokine-like molecule has a wide range of biological properties on the activity and function of mononuclear and polymorphonuclear phagocytes (64). Stimulation of monocytes in culture with endothelial-monocyte activating polypeptide-II resulted in the production of interleukin-8, tumor necrosis factor-alpha and tissue factor (115). The levels of endothelial-monocyte activating polypeptide-II in the gingival crevicular fluid were significantly elevated in patients with generalized aggressive periodontitis compared to patients with chronic periodontitis (22). Considering the role of endothelial-monocyte activating polypeptide-II in both the activation and recruitment of inflammatory cells, it can be speculated that the rapid and severe periodontal destruction in generalized aggressive periodontitis could be enhanced by the production of endothelial-monocyte activating polypeptide-II by mononuclear cells in the periodontium (22). In summary, the selective production of chemokines is important in determining the spatial localization of the inflammatory cells in periodontal tissues for optimizing host defenses and in directing leukocyte infiltration into the area (107). The recruited cells may act protectively to limit tissue damage or may result in ineffective inflammatory responses that cause destruction of the host tissue. Further understanding of the role played by chemokines in the various periodontal disease states will contribute to a clearer picture of the differences between chronic periodontitis and aggressive periodontitis.

Conclusions

This review has looked at some aspects of the innate and adaptive immune systems where either differences between chronic periodontitis and aggressive periodontitis have been shown to exist or where further studies may be able to highlight significant differences between the two diseases. As stated elsewhere in this volume of *Periodontology 2000*, variations and changes in the definitions, and hence the identification, of patients with aggressive periodontitis over the past four decades has meant that it is difficult to draw any firm conclusions. At present it is not possible to identify real differences in the immunopathology of the two diseases. This may be because there are no differences, or because the differences only reflect variations in the degree of severity or susceptibility rather than actual different immunopathologies.

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