CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Chemokines in Oral Inflammatory Diseases: Apical Periodontitis and Periodontal Disease

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ABSTRACT

The inflammatory oral diseases are characterized by the persistent migration of polymorphonuclear leukocytes, monocytes, lymphocytes, plasma and mast cells, and osteoblasts and osteoclasts. In the last decade, there has been a great interest in the mediators responsible for the selective recruitment and activation of these cell types at inflammatory sites. Of these mediators, the chemokines have received particular attention in recent years. Chemokine messages are decoded by specific receptors that initiate signal transduction events, leading to a multitude of cellular responses, including chemotaxis and activation of inflammatory and bone cells. However, little is known about their role in the pathogenesis of inflammatory oral diseases. The purpose of this review is to summarize the findings regarding the role of chemokines in periapical and periodontal tissue inflammation, and the integration, into experimental models, of the information about the role of chemokines in human diseases.

KEY WORDS: chemokines, inflammation, oral diseases, periodontitis.

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CHEMOKINES AS SELECTIVE RECRUITERS OF INFLAMMATORY CELLS

To mediate an effective response, leukocytes must find their way to sites of infection or inflammation. Although Elias Metchnikoff, at the end of the 19th century, recognized diapedesis as a fundamental mechanism of host defense, progress in uncovering the molecular events involved in the process of leukocyte emigration has been made only during the last three decades. Leukocyte invasion of tissues can be induced by several substances—including interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and bacterial lipopolysaccharide (LPS)—that cause leukocyte emigration when injected *in vivo*. All such compounds induce the production of chemoattractants, which in turn cause leukocyte migration. Therefore, chemotactic activity includes the receptor-mediated gradient perception and must be measured by the ability of a chemoattractant to induce directed leukocyte migration *in vitro*. The development of methods for the study of leukocyte migration *in vitro* (Boyden, 1962) facilitated the discovery of several chemoattractants, such as complement fragments C3a and C5a, arachidonic acid derivatives such as leukotriene B_4 (LTB_4) and 12-hydroxy-eicosanotetraenoic acid (12-HETE), and platelet-activating factor (PAF) (Schroder, 2000). The first cytokine identified to have chemotactic activity was interleukin-8 (IL-8), which proved to be a selective neutrophil chemoattractant (Yoshimura *et al.*, 1987). Subsequently, there has been considerable interest in the mediators responsible for the selective recruitment and activation of leukocyte subsets. Of these mediators, chemokines (from chemotactic cytokines) have been of great interest since 1996, and increasing knowledge is now available regarding the chemokine system, cellular distribution of individual chemokines, and chemokine receptors. Chemokines are a large family of small (from 7 to 15 kDa, from 67 to 127 amino acids in length), structurally related heparin-binding proteins, which are classified into 4 subfamilies according to the configuration of cysteine residues near the N-terminus, depending on whether the first 2 cysteines are separated (CXC, CX3C) or not (CC, C) by an intervening amino acid (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000). Chemokine receptors are named according to the family of their ligands, and the two major subfamilies are designated CCR and CXCR (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000). Recently, the nomenclature for chemokines was revised, utilizing the receptor nomenclature system, *e.g.*, CCL1, CXCL1 (Murphy *et al.*, 2000; Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000; Bacon *et al.*, 2002; Rot and von Andrian, 2004). While the old designations have also been retained, chemokines are identified by the old name followed by the new chemokine nomenclature, *e.g.*, I-309/CCL1. Interestingly, in addition to the crucial role of chemokines in cell trafficking, chemokine messages initiate signal transduction events leading to other biological processes, such as angiogenesis, cell proliferation, apoptosis, tumor metastasis, and host defense (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000; Bacon *et al.*, 2002; Rot and von Andrian, 2004;

Moser *et al.*, 2004; Esche *et al.*, 2005).

Some of the most important chemokines and receptors expressed in oral diseases are depicted in Fig. 1. The chemokine receptors CCR1, CCR2, and CCR5 are expressed on monocytes/macrophages (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000), and their ligands include MIP-1α/CCL3 and RANTES/CCL5 (ligands of CCR1 and CCR5) and MCP-1/CCL2 (a CCR2 ligand), which can be produced by fibroblasts, endothelial cells, monocytes/macrophages, osteoblasts, and mast cells (Gemmell *et al.*, 2001; Kabashima *et al.*, 2001; Park *et al.*, 2004; Wright and Friedland, 2004). CCR1 is also expressed in precursors of mature osteoclasts (Votta *et al.*, 2000; Yu *et al.*, 2004). Moreover, CCR5 and CXCR3 are expressed preferentially on Th1 lymphocytes. CXCR3 ligands consist of Mig/CXCL9, IP-10/CXCL10, and I-TAC/CXCL11 (Kaplan *et al.*, 1987; Bonecchi *et al.*, 1998; Loetscher *et al.*, 1998, 2001; Sallusto *et al.*, 1998a,b). In contrast, CCR3, CCR4, and CCR8 are expressed on Th2 cells (Bonecchi *et al.*, 1998; D'Ambrosio *et al.*, 1998; Sallusto *et al.*, 1998a,b; Gu *et al.*, 2000). The Th2 cell chemoattractants include: RANTES/CCL5, MCP-3/CCL7, Eotaxin/CCL11, MCP-4/CCL13, and HCC-2/CCL15 (CCR3 ligands); MDC/CCL22 and TARC/CCL17 (CCR4 ligands); and I-309/CCL1 (CCR8 ligand). B-lymphocytes characteristically express CXCR5, which binds BCA-1/CXCL13 (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000). Neutrophils express CXCR1 and CXCR2, which bind IL-8/CXCL8, GCP-2/CXCL6, and GROα/CXCL1 (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000). Furthermore, neutrophils can also express CC receptors (Menzies-Gow *et al.*, 2002).

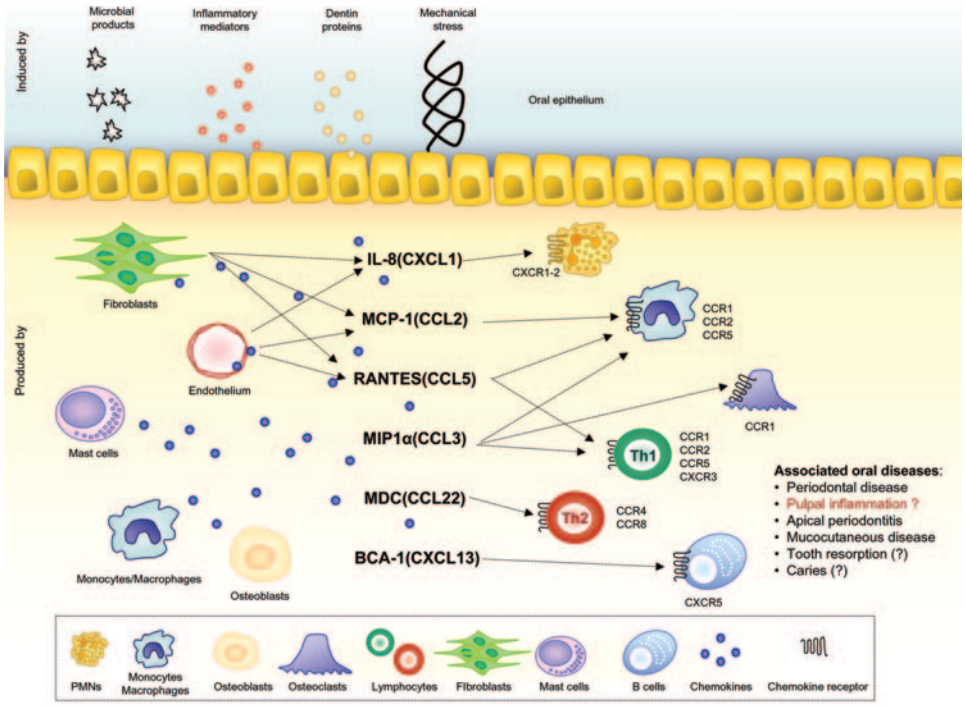

Figure 1. Chemokines and chemokine receptors in oral tissues: potential involvement in the induction and maintenance of inflammatory reactions. A schematic representation of chemokine and chemokine receptor networks in oral tissues. Chemokine expression can be triggered by microbial components, inflammatory mediators, host factors such as dentin proteins, or even by mechanical stress. Both resident (such as osteoblasts, fibroblasts, mast, epithelial, and endothelial cells) and inflammatory cells (polymorphonuclear leukocytes [PMNs], lymphocytes, monocytes/macrophages) can be sources of chemokines in the oral environment. The chemokines produced in oral tissues selectively attract different cell types to the tissues, such as PMNs, lymphocyte subsets, monocytes/macrophages, and osteoclasts, and, consequently, can determine the course of inflammatory reactions and the clinical outcome of potentially associated diseases.

How do chemokines mediate these effects on leukocyte migration? Engagement of chemokine receptors with their respective ligands regulates cytoskeletal re-arrangement, integrin-dependent adhesion, as well as the binding and detachment of cells from their substrate. Chemokines target all types of leukocytes, including hematopoietic precursors and mature leukocytes of the innate immune system, as well as naïve, memory, and effector lymphocytes through their binding to selective seven-transmembrane G-protein-coupled receptors. Chemokine receptor signaling activates the heterotrimeric G-proteins coupled to the chemokine receptors, and triggers several signaling pathways involving small GTPases (RAP1, RhoA, Rac) and kinases (PI_3K, atypical PKC, Pyk2) (Terricabras *et al.*, 2004). In addition, intracellular events induced by chemokines include inhibition of adenylate cyclase, activation of phospholipase C, calcium flux and inositol trisphosphate generation by G-protein-dependent mechanisms, mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK), increase of nuclear factor (NFκB), as well as the Janus-activated kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, or by activating protein 1 (AP-1)-mediated transcription (Darnell *et al.*, 1994; Ihle *et al.*, 1994; Bowie and O'Neill, 2000). These intracellular signals result in re-organization of the cytoskeleton and cell adhesion, causing the cells to send out pseudopodia and 'crawl up' the chemoattractant gradient (Terricabras *et al.*, 2004).

The chemokine-receptor axis interaction ensures proper tissue distribution of distinct leukocyte subsets under physiologic and inflammatory conditions. Critical determinants of the *in vivo* activities of chemokines in the immune system include their presentation by glycosaminoglycans in endothelial cells and the extracellular matrix, as well as their cellular uptake *via* "silent" chemokine receptors (interceptors), leading either to their endocytosis or to their degradation (Rot and von Andrian, 2004).

Chemokines are synthesized by several cell types, including endothelial, epithelial, and stromal cells, such as

fibroblasts, mast and bone cells, as well as leukocytes (Fig. 1). Functionally, chemokines can be divided into homeostatic and inflammatory molecules (Moser *et al.*, 2004). Homeostatic chemokines are expressed in bone marrow and lymphoid tissues and are important for hematopoiesis, immune surveillance, and adaptive immune responses (Murphy *et al.*, 2000; Moser *et al.*, 2004; Esche *et al.*, 2005). While the expression of some homeostatic chemokines seems to be constitutive, the so-called 'inflammatory' chemokines can be induced by stimuli such as cytokines, pathogens, and growth factors, by chemokines themselves, or by cell-cell contact (Campbell and Butcher, 2000; Sallusto *et al.*, 2000; Moser and Loetscher, 2001; Moser *et al.*, 2004). Therefore, under some circumstances, the expression and effects of chemokines may be influenced by other inflammatory molecules, such as IL-1, TNF-α, and interferon-γ (IFN-γ) (Tessier *et al.*, 1997; Zhang *et al.*, 2001). In addition to changes in chemokine production, inflammatory mediators control chemokine actions by the modulation of chemokine receptor expression (Lloyd *et al.*, 1995; Sica *et al.*, 1997; Sozzani *et al.*, 1998). Thus, the locally produced cytokines may control both chemokine production and chemokine receptor expression, which in turn regulate the kinetics and the composition of leukocyte infiltration.

Current knowledge regarding the roles of chemokines in infectious and inflammatory sites came from observations in different inflammatory models (Silva *et al.*, 2004b; Garlet *et al.*, 2005, 2006) and diseases, including periodontal diseases (Garlet *et al.*, 2003), apical periodontitis (Silva *et al.*, 2005), and mucocutaneous oral diseases, such as candidiasis (Schofield *et al.*, 2005) and lichen planus (Rhodus *et al.*, 2005). However, not much is known regarding the expression of chemokines and their receptors and their involvement in the pathways associated with inflammatory cell recruitment in oral tissues. Thus, the purpose of this review is to explore the effects of chemokines in oral sites. By using published data regarding chemokine expression in dental and periodontal tissues, as well as analogies of chemokines' participation in events related to both the repair and destruction of soft and mineralized tissues, we intend to construct a scenario for the participation of chemokines in oral diseases, particularly apical periodontitis and periodontal diseases.

LESSONS FROM TRANSGENIC AND KNOCKOUT MICE

For better definition of the spectrum of *in vivo* activities mediated by chemokines and their receptors, several studies have used chemokine transgene expression or chemokine and chemokine receptor disruption. These studies have confirmed the importance of these molecules in physiological and pathological conditions. The monocyte and T-cell chemoattractant MIP-1α/CCL3 was the first chemokine to be knocked out in these studies. Phenotypically, mice with the homozygous MIP-1α/CCL3 null mutation develop normally, with no apparent lymphoid or myeloid defect. These mice, however, are resistant to coxsackie-virus-induced myocarditis. Furthermore, their pulmonary inflammatory response to influenza virus is attenuated, and clearance of virus is delayed (Cook *et al.*, 1995). Moreover, MIP-1α/CCL3$^{-/-}$ mice were resistant to zymosan-induced multiple organ dysfunction syndrome (Miller *et al.*, 1996).

Although both MCP-1/CCL2- and CCR2-deficient mice have been reported to show defects in monocyte recruitment, the two types of animals differ in their effects on T-cell differentiation. Animals that lack MCP-1/CCL2 show diminished T-cell responses, with stronger effects on Th2-type responses (Lu *et al.*, 1998; Gu *et al.*, 2000). This includes increased resistance to *Leishmania* infection, which is indicative of a shift from a Th2 to a Th1 response (Gu *et al.*, 2000). In contrast, CCR2$^{-/-}$ mice have markedly reduced T-cell IFN-γ responses, diminished type 1 granuloma responses, defects in clearance of intracellular pathogens, and increased resistance to experimental autoimmune encephalomyelitis (Boring *et al.*, 1997; Kurihara *et al.*, 1997; Izikson *et al.*, 2000). Interestingly, CCR2$^{-/-}$ mice did not exhibit differences in arthritis development when compared with wild-type mice (Brown *et al.*, 2003).

A mouse generated by gene targeting to lack CXCR2 had pronounced neutrophilia, an abnormal production of myeloid stem cells, B-lymphocytosis (Cacalano *et al.*, 1994), and significant reduction in mast cell progenitors homing to the small intestine (Abonia *et al.*, 2005). CXCR2$^{-/-}$ mice also demonstrated defective neutrophil recruitment to the peritoneal cavity in response to thioglycolate (Cacalano *et al.*, 1994), and a significant decrease in tissue damage and disease severity in experimental models of arthritis (Brown *et al.*, 2003), acute pyelonephritis (Frendeus *et al.*, 2000), and hepatitis B virus infection (Sitia *et al.*, 2002).

A substantial defect in B-cell lymphopoiesis and myelopoiesis has been observed in SDF-1/CXCL12 mutant mice. In contrast, these mice have shown normal T-cell development (Nagasawa *et al.* 1996). Likewise, mice harboring a null mutation for CXCR5, which is expressed in B-lymphocytes and is activated by BCA-1/CXCL13, lack inguinal lymph nodes and possess few, if any, Peyer's patches. The migration of B-lymphocytes into splenic follicles is also impaired (Forster *et al.*, 1996).

Chemokine and chemokine receptor gene disruption indicates that a single chemokine or receptor has a partial effect on inflammatory and immunological responses. The partial effect may indicate overlapping functions among several closely related chemokines. Furthermore, some chemokines appear to have a broader spectrum of immune functions, ensuring that leukocytes arrive in the proper environments and undergo appropriate maturation. This is evident in chemokine and chemokine receptor knockout mice, which, in addition to deficiencies in leukocyte recruitment, have been shown to have alterations in the Th1/Th2 balance and lymphoid and myeloid development.

CHEMOKINES IN THE BONE ENVIRONMENT

The integrity of bone tissues depends on the maintenance of a delicate equilibrium between bone resorption by osteoclasts and bone deposition by osteoblasts. The major regulatory mechanism of osteoclast activity is driven by members of the TNF family of receptors, RANK (receptor activator of nuclear factor-κB) and osteoprotegerin (OPG), and the ligand RANKL (Boyle *et al.*, 2003). RANK is expressed on osteoclastic precursors and on mature osteoclasts, while its ligand, RANKL, a transmembrane protein, is expressed particularly on osteoblasts in homeostatic conditions. Interaction between RANK and RANKL is required for the differentiation and activation of osteoclasts, an event regulated by OPG, a decoy

receptor of RANKL that strongly inhibits bone resorption by preventing RANK-RANKL engagement (Boyle *et al.*, 2003). Imbalances in this system are pivotal to the etiology of some bone disorders, since excessive resorptive activity causes bone loss (as seen in periodontal and periapical diseases), whereas defective resorptive activity can block tooth eruption (Rodan and Martin, 2000; Romas *et al.*, 2002).

However, factors other than the RANKL system, such as chemokines, are involved in both the physiology and pathology of bone tissue. Chemokines have been recognized as essential signals for the trafficking of osteoblast and osteoclast precursors, and consequently as potential modulators of bone homeostasis (Bendre *et al.*, 2003; Wright *et al.*, 2005). Chemokine effects on bone metabolism are illustrated in Fig. 2.

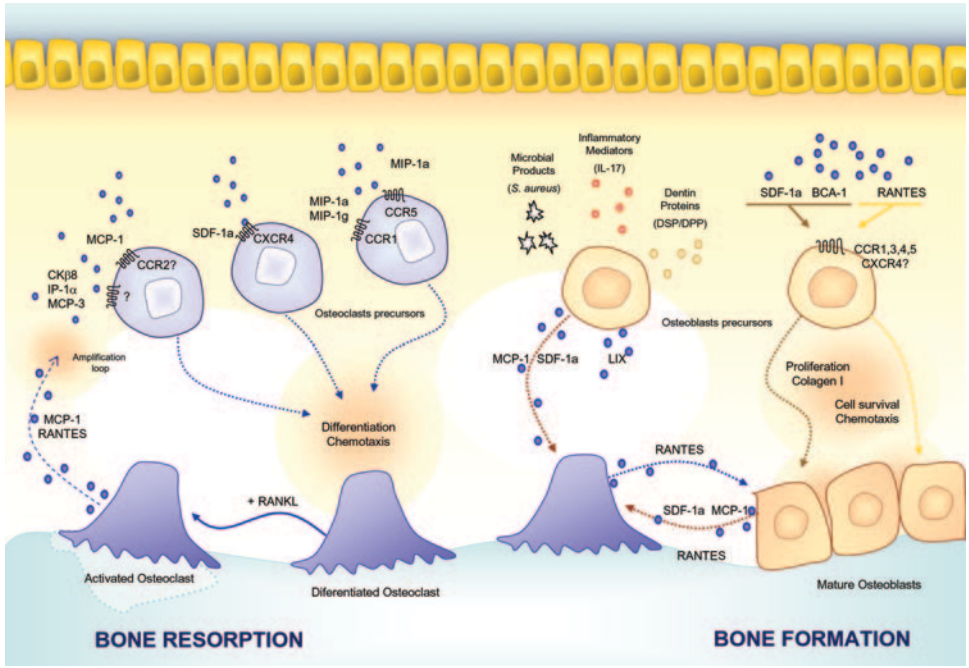

Figure 2. Chemokines and bone tissue. A schematic representation of chemokine networks in bone tissue, in which both osteoclasts and osteoblasts can be targets of chemokines. Chemokines such as MCP-1/CCL2, SDF-1α/CXCL12, MIP-1α/CCL3, and MIP-1γ/CCL9 can induce the chemotaxis and differentiation of osteoclast precursors into osteoclasts. Other chemokines, such as IL-8/CXCL1, MCP-3/CCL7, CKβ8/CCL23, and IP-10/CXCL10, also act in osteoclasts. However, the activation of osteoclasts is achieved only with RANKL, which also induces chemokine production, generating an amplification loop to potentiate bone resorption. Chemokines such as SDF-1α/CXCL12, BCA-1/CXCL13, and RANTES/CCL5 act on osteoblast precursors, driving their proliferation and cell survival, chemotaxis, and the production of type-I collagen, which can result in increased bone formation. The osteoblasts are also an important source of chemokines, produced in response to a wide range of stimuli, such as microbial products, inflammatory mediators, or dentin proteins. While osteoblast-derived MCP-1/CCL2 and SDF-1α/CXCL12 participate in an interesting chemokine cross-talk between osteoblasts and osteoclasts, chemokines such as LIX/CXCL5 and BCA-1/CXCL13 can also attract different leukocyte subsets, suggesting an important role for osteoblasts in the inflammatory-immune reaction.

Osteoclasts originate from hematopoietic precursors of the monocyte-macrophage lineage that reside within the bone marrow and, guided by chemokines, emigrate from the peripheral circulation into bone. In addition, some chemokines are able to induce their differentiation into osteoclasts. IL-8/CXCL8 has a direct effect on osteoclast differentiation and activity by signaling through the specific receptor, CXCR1 (Bendre *et al.*, 2003). The interaction of SDF-1/CXCL12 with the receptor, CXCR4, which is expressed in human osteoclast precursors, induces chemotaxis and differentiation into osteoclasts (Wright *et al.*, 2005). The chemokine receptor CCR1, also expressed by osteoclast precursors, is able to bind to chemokines such as MIP-1α/CCL3, RANTES/CCL5, MIP-1γ/CCL9, MCP-3/CCL7, and CKβ8/CCL23, thereby stimulating osteoclast precursor chemotaxis and presumably guiding them to sites where they will fuse (Votta *et al.*, 2000; Lean *et al.*, 2002; Okamatsu *et al.*, 2004; Yu *et al.*, 2004; Yano *et al.*, 2005; Yang *et al.*, 2006), and also stimulating their differentiation (Scheven *et al.*, 1999; Choi *et al.*, 2000; Han *et al.*, 2001; Okamatsu *et al.*, 2004; Yu *et al.*, 2004; Yang *et al.*, 2006). Osteoclast precursors have also been found to express CXCR3, which makes them responsive to the chemokine MIG/CXCL9 and results in their migration and the adhesion of osteoclast precursors (Kwak *et al.*, 2005). In addition, MCP-1/CCL2 is associated with osteoclast chemotaxis and differentiation, probably through the interaction with the receptor CCR2 (Kim *et al.*, 2006a,b). *In vivo*, MCP-1/CCL2 mediates the recruitment of monocytes in osseous inflammation (Okamatsu *et al.*, 2004), bone remodeling (Graves *et al.*, 1999), and tooth eruption (Wise *et al.*, 1999).

The chemokine-driven osteoclast differentiation was found to occur through pathways dependent on (Yu *et al.*, 2004) or independent of (Han *et al.*, 2001) RANKL. However, although the differentiation of osteoclasts can be achieved by chemokine-chemokine receptor interaction, their activation seems to be dependent on RANKL (Wright *et al.*, 2005; Kim *et al.*, 2006b). Interestingly, RANKL also induces the production of MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5, and MIG/CXCL9 by osteoclasts, suggesting an amplification loop composed of autocrine and paracrine signals during osteoclast differentiation, which could contribute to bone resorption (Kim *et al.*, 2006a).

In addition to its role in osteoclastogenesis, chemokines also affect osteoclast functions/properties through their interactions with CXCR4 or CCR1. The CXCR4 ligand, SDF-1α/CXCL12, was found to increase MMP-9 activity in human osteoclasts, resulting in increased bone resorption activity (Grassi *et al.*, 2004). MIP-1γ/CCL9 plays an important role in the survival of osteoclasts, and part of the RANKL effect on osteoclast survival is dependent on its ability to induce MIP-1γ/CCL9 production (Okamatsu *et al.*, 2004). Another CCR1

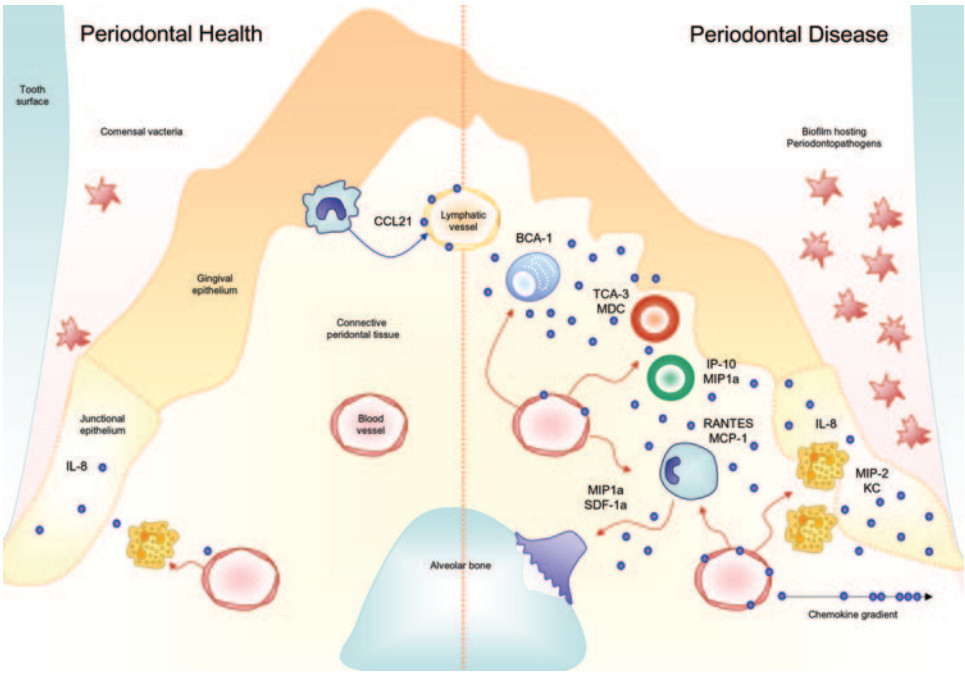

Figure 3. Chemokines in healthy and diseased periodontal tissues. A schematic representation of chemokine networks in periodontal tissues, in which the bacterial biofilm hosting periodontopathogens is thought to trigger the expression of chemokines and other inflammatory mediators in gingival tissues. In healthy conditions (left), a slight expression of IL-8/CXCL8 in junctional epithelium is correlated with the subclinical inflammatory infiltrate, composed basically of polymorphonuclear leukocytes (PMNs). In addition, 6Ckine/CCL21 expression in lymphatic vessels is exclusively found in healthy periodontal tissue. In contrast, in periodontitis tissues, an intense expression of several chemokines can be observed. An increased expression of IL-8/CXCL8 is found in junctional epithelium, and directs an intense PMN influx to these areas. In periodontal connective tissues, chemokines such as MCP-1/CCL2 and RANTES/CCL5 can drive the migration of macrophages, IP-10/CXCL10 and MIP-1α/CCL3 are chemoattractants of Th1-type lymphocytes, and chemokines such as MDC/CCL22 and TCA-3/I-309/CCL1 can attract Th2-type lymphocytes. The accumulation of B-cells can be driven by BCA-1/CXCL13, while chemokines such as MIP-1α/CCL3 and SDF-1α/CXCL12 can also be involved in the migration and activation of osteoclasts, thereby contributing to disease severity.

ligand, MIP-1α/CCL3, also induces adhesion of osteoclasts to primary osteoblasts, thereby suggesting a function for this chemokine in the regulation of the interaction between these two cell types (Watanabe *et al.*, 2004). In contrast, controversial results point to inactivities of MIP-1β/CCL4, MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MCP-4/CCL13, HCC-1/CCL14, Eotaxin-2/CCL24, PARC/CCL18, IL-8/CXCL8, GROα/CXCL1, and SDF-1/CXCL12 in osteoblast and osteoclast chemotaxis/behavior (Votta *et al.*, 2000).

Osteoblasts are found to express several chemokine receptors, including CXCR1, CXCR3, CXCR4, and CXCR5, and the CC receptors 1, 3, 4, and 5 (Yano *et al.*, 2005), which can modulate their function through the binding of chemokines. The chemokine IP-10/CXCL10 induces osteoblast proliferation and alkaline phosphatase and beta-N-acetylhexosaminidase release (Lisignoli *et al.*, 2003, 2004), while SDF-1α/CXCL12 and BCA-1/CXCL13 induce both proliferation and collagen type I mRNA expression in osteoblasts (Lisignoli *et al.*, 2006). There is some evidence that RANTES/CCL5 can also act on osteoblasts, resulting in chemotaxis and promoting cell survival (Yano *et al.*, 2005). Taken together, these studies suggest that chemokines can effectively contribute to the bone remodeling process by driving osteoblast migration and activation.

In the chemokine crosstalk between bone cells, the osteoblasts also seem to be an important source of chemokines. Chemokine production by osteoblasts can be induced by microbial products, inflammatory mediators, dentin proteins, and even by particulate wear debris (Rahimi *et al.*, 1995; Lisignoli *et al.*, 2004; Ruddy *et al.*, 2004; Silva *et al.*, 2004a; Wright and Friedland, 2004; Fritz *et al.*, 2005; Marriott *et al.*, 2005). These chemokines include MCP-1/CCL2 and SDF-1α/CXCL12, whose effects on osteoclasts have been previously described. Osteoblasts are also able to produce the other chemokines, such as KC/CXCL1, CINC-1/CXCL1, LIX/CXCL5, and BCA-1/CXCL13, which are involved in the recruitment of neutrophils and of different lymphocyte subsets, suggesting an interesting role for osteoblasts in inflammatory-immune reaction development (Lisignoli *et al.*, 2004; Marriott, 2004; Ruddy *et al.*, 2004; Bischoff *et al.*, 2005). Furthermore, the production of chemokines, with the consequent chemoattraction of inflammatory cells in the bone environment, may contribute to the disruption of bone homeostasis, resulting in tissue destruction, as discussed in the next sections.

CHEMOKINES IN PERIODONTAL DISEASES: FROM HOST PROTECTION TO TISSUE DESTRUCTION

Periodontal disease (PD), a chronic inflammatory disease of the attachment structures of the teeth, is one of the most significant causes of tooth loss in adults and the most prevalent form of bone pathology in humans, besides being a modifying factor of the individuals' systemic health. The bacterial biofilm attached to the surface of the tooth, close to the periodontal tissues, is the etiologic factor for this disease. The inflammatory and immune responses, initiated by periodontopathogens, are thought to protect the host against infection. However, the persistence of a local chronic host response may alter the protective roles of inflammatory cells and produce deleterious effects in these tissues (Graves and Cochran, 2003; Berglundh and Donati, 2005; Kinane *et al.*, 2005). In fact, the development of the periodontal diseases seems to be related to the progression of the inflammatory cell infiltrate into the deeper periodontal tissues (Graves *et al.*, 1998). In this situation, chemokines, found in both gingival tissue and crevicular fluid, are thought to play important roles in the

immunopathogenesis of periodontal diseases (Fig. 3).

IL-8/CXCL8, a chemoattractant of polymorphonuclear leukocytes (PMNs), is detectable in healthy periodontal tissues and has been associated with low subclinical inflammation, basically comprised of PMNs (Payne *et al.*, 1993; Mathur *et al.*, 1996). After cessation of toothbrushing, a rapid increase in the levels of IL-8/CXCL8 in gingival crevicular fluid (GCF) precedes the clinical signs of disease (Zhang *et al.*, 2002). In fact, PMNs are the first cell type found in high numbers in early periodontal lesions (Garlet *et al.*, 2005). In persons with periodontitis, the levels of IL-8/CXCL8 in both periodontal tissue and GCF are drastically increased, and have been correlated with disease severity (Tsai *et al.*, 1995). *In vitro* studies have demonstrated that IL-8/CXCL8 can be produced by gingival fibroblasts, gingival epithelial cells, and endothelial cells (Takashiba *et al.*, 1992; Takigawa *et al.*, 1994; Huang *et al.*, 1998; Yumoto *et al.*, 1999). However, the expression of IL-8/CXCL8 *in situ* is preferentially found in junctional epithelium (Tonetti *et al.*, 1994; Fitzgerald and Kreutzer, 1995). This preferential expression, in a tissue characterized by its high permeability, effectively directs polymorphonuclear phagocyte migration toward the infecting micro-organisms, and allows PMNs access to the periodontal pocket (Tonetti *et al.*, 1994; Fitzgerald and Kreutzer, 1995). Analysis of the chemokines KC/CXCL1 and MIP-2/CXCL2 (the rodent analogues of IL-8/CXCL8), in experimental models of periodontal diseases in mice and rats, revealed their expression in diseased tissues, preferentially in the junctional epithelium, and their correlation with the migration of PMNs (Miyauchi *et al.*, 2004; Garlet *et al.*, 2005). Neutrophils, representing the first line of the host defense mechanism against microbial infection, are thought to play important roles in maintaining periodontal health. In fact, defective function of PMNs is associated with severe forms of periodontal diseases. In contrast, the hyperactivity of this cell type is associated with periodontal tissue destruction (Attström, 1975; Del Fabbro *et al.*, 2000; Waddington *et al.*, 2000).

In contrast to IL-8/CXCL8, the chemokine MCP-1/CCL2 was found to be preferentially expressed in diseased periodontal sites, and presents a differential spatial distribution in the periodontal tissues, since it is expressed along the basal layer of the oral epithelium and by endothelial cells, fibroblasts, and mononuclear phagocytes in the inflammatory infiltrate (Tonetti *et al.*, 1994; Yu and Graves, 1995). MCP-1/CCL2 is supposed to be the major chemoattractant of macrophages in periodontal diseases (Hanazawa *et al.*, 1993). Macrophages are found in large numbers in inflamed gingival tissues and are thought to play a significant role in the killing of pathogens and in the release of pro-inflammatory mediators, such as TNF-α, IL-1, and nitric oxide (Yamamoto *et al.*, 1996; Baker, 2000; Kinane and Lappin, 2001; Graves and Cochran, 2003). These mediators also enhance the cellular immune response, which may be useful in the control of invasive periodontopathogens. In contrast, the inflammatory products widely produced by macrophages are known to induce bone resorption by promoting the differentiation and maturation of osteoclasts (Yamamoto *et al.*, 1996; Graves and Cochran, 2003). Thus, the chemoattraction of macrophages by MCP-1/CCL2 could contribute to enhanced severity of periodontal diseases, a hypothesis supported by analysis of data showing that greater numbers of macrophages were found in active sites of periodontitis (Gamonal *et al.*, 2000), and that MCP-1/CCL2 activity in GCF increased with severity of the disease (Hanazawa *et al.*, 1993).

Besides being attracted by MCP-1/CCL2, through the binding of CCR2, macrophages can also express CCR1 and CCR5. Thus, chemokines such as RANTES/CCL5 and MIP-1α/CCL3 may also be involved in the migration of macrophages to periodontal tissues (Gemmell *et al.*, 2001). RANTES/CCL5 has been detected in both the periodontal tissue and the GCF of persons with periodontitis, and in higher amounts in active sites *vs.* inactive periodontitis sites (Gamonal *et al.*, 2000; Gemmell *et al.*, 2001; Emingil *et al.*, 2004). Cell cultures of whole blood from persons with periodontitis stimulated with LPS produce higher levels of RANTES/CCL5 than do cultures from control individuals. In addition, persons with periodontitis were found to continue producing high levels of RANTES/CCL5, even after periodontal therapy, suggesting an intrinsic susceptibility of these individuals to periodontitis development (Fokkema *et al.*, 2003). MIP-1α/CCL3 was found to be the most abundantly expressed chemokine in periodontitis tissues, with its expression localized in the connective tissue subjacent to the pocket epithelium of inflamed gingival tissues (Gemmell *et al.*, 2001; Kabashima *et al.*, 2002). It has also been shown that MIP-1α/CCL3-positive cells increase in number with increasing severity of periodontal disease (Kabashima *et al.*, 2002), and are associated with augmented proportions of lymphocytes in tissues with increasing inflammation (Gemmell *et al.*, 2001). However, MIP-1α/CCL3 levels in GCF were similar in healthy and diseased sites (Gemmell *et al.*, 2001; Kabashima *et al.*, 2002; Emingil *et al.*, 2005). The receptor of RANTES/CCL5 and MIP-1α/CCL3, CCR5, was found to be exclusively expressed in diseased tissues, mainly in cells located in connective tissue subjacent to the pocket epithelium (Gamonal *et al.*, 2001; Kabashima *et al.*, 2002). As previously described, CCR5 as well CXCR3 are characteristically expressed by Th1-type lymphocytes (Sallusto *et al.*, 1998a).

In addition to CCR5 and its ligands, CXCR3 and its ligand IP-10/CXCL10 are also expressed in diseased periodontal tissues (Kabashima *et al.*, 2002; Garlet *et al.*, 2003), and are associated with higher levels of IFN-γ in these tissues (Garlet *et al.*, 2003). Since IFN-γ-producing Th1 cells are classically involved in the activation of macrophages (Baker *et al.*, 1999; Burger and Dayer, 2002; Ma *et al.*, 2003), their chemoattraction could contribute to disease progression. This possibility is compatible with the evidence that the adoptive transfer of Th1 cells results in alveolar bone resorption in mice (Kawai *et al.*, 2000). In agreement with this finding, we have previously demonstrated a preferential expression of Th1-type cytokines and chemokines in aggressive *vs.* chronic periodontitis (Garlet *et al.*, 2003, 2004), and the predominance of such mediators in the early phase of experimental periodontal disease, characterized by an intense inflammatory reaction and bone loss (Garlet *et al.*, 2005, 2006). Conversely, Th2-type lymphocytes, which can produce the anti-inflammatory cytokines IL-4, IL-10, and IL-13, could attenuate the periodontal tissue destruction (Onoe *et al.*, 1996; Wiebe *et al.*, 1996; Sasaki *et al.*, 2000; Pestka *et al.*, 2004).

Chemokines such as MDC/CCL22, TARC/CCL17, and I-309/CCL1 (or their murine analogue, TCA-3/CCL1) are able to attract T-cells with a Th2 phenotype that characteristically

expresses CCR4 and CCR8 (D'Ambrosio *et al.*, 1998; Sallusto *et al.*, 1998a; Gu *et al.*, 2000). Accordingly, we have demonstrated the expression of MDC/CCL22 (unpublished data), TCA-3/CCL1 and its receptor CCR4 (Garlet *et al.*, 2003, 2005), in periodontitis tissues. CCR4 is found expressed at higher levels in chronic periodontitis, and it is associated with higher levels of IL-4 and IL-10 messages in the tissues (Garlet *et al.*, 2003, 2004). In experimental periodontal diseases, TCA-3/CCL1 and CCR4 were associated with an increase in Th2 cytokine expression and with the attenuation of disease progression (Garlet *et al.*, 2005, 2006). Furthermore, these chemokines and chemokine receptors are also involved in the migration of $CD4^+CD25^+FOXp3^+$ regulatory T-cells (Tregs), recently identified in periodontal lesions (Nakajima *et al.*, 2005), and are potentially involved in the control of disease severity (Iellem *et al.*, 2001). Therefore, the expression of Th2 and Treg chemoattractants (MDC/CCL22, TARC/CCL17, and I-309/TCA-3/CCL1) could attenuate periodontal disease severity. Other chemokines, such as fractalkine/CX3CL1 and MIP-3α/CCL20, are also found in diseased tissues, and, through the binding to CX3CR1 and CCR6, may be involved in the migration of T-cell subsets, such as memory cells, characteristically found at these sites (Hosokawa *et al.*, 2002, 2005b).

While T-lymphocytes predominate in the chronic periodontal lesion and are mainly located subjacent to the pocket epithelium, B-cells and plasma cells predominate in the central portion of the lamina propria, and their proportion increases with the progression of the disease (Seymour *et al.*, 1979; Reinhardt *et al.*, 1988; Nakajima *et al.*, 2005). The B-cell chemoattractant, BCA-1/CXCL13, is expressed in diseased tissues (unpublished data) and may account for the attraction and accumulation of these cells in the periodontium. Therefore, the presence of B-cells in the periodontium contributes to the local production of antibodies that are supposed to provide a protective role against infection (Klausen *et al.*, 1989), suggesting that the expression of BCA-1/CXCL13 may be important to the local response against periodontopathogens. In contrast, since B-cells may be an important source of inflammatory cytokines in the periodontium (Gemmell *et al.*, 2001), their chemoattraction may contribute to increased disease severity. In addition, the accumulation of B-cells in an inflammatory environment may result in inappropriate activation, leading to autoantibody production and disease aggravation (Bick *et al.*, 1981; Yoshie *et al.*, 1985; Berglundh *et al.*, 2002).

As previously discussed, chemokines can also exert important effects on bone cells, inducing the migration and activation of osteoclasts. MIP-1α/CCL3, described as an osteoclast differentiation factor (Scheven *et al.*, 1999; Choi *et al.*, 2000; Han *et al.*, 2001), and RANTES/CCL5, a chemotactic factor for such cells (Votta *et al.*, 2000), are found in periodontitis tissues. In addition, SDF1α/CXCL12 has also been described as a positive regulator of osteoclast function, and was recently identified in diseased periodontium (Hosokawa *et al.*, 2005a; unpublished data). Therefore, the presence of these osteclast chemoattractants in the periodontal environment may be involved in the exacerbation of disease severity.

The selective production of chemokines may be involved in the determination of the spatial localization of the inflammatory cells in periodontal tissues for optimization of host defenses, and may contribute to leukocyte infiltration into the infected and inflamed area, thus limiting tissue damage. Several cell types present in the periodontium, such as fibroblasts, epithelial cells, and endothelial cells, are able to produce chemokines in response to bacterial products or inflammatory molecules (Berglundh and Donati, 2005; Kinane *et al.*, 2005; Madianos *et al.*, 2005). However, the virulence factors of periodontopathogens such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* are able to interfere with this process (Madianos *et al.*, 1997; Darveau *et al.*, 1998; Kobayashi-Sakamoto *et al.*, 2003; Ohguchi *et al.*, 2003).

In agreement with the hypothesis that chemokines may be related to periodontitis severity, some studies have demonstrated that their levels in GCF decrease after periodontal therapy (Gamonal *et al.*, 2000, 2001; Jin *et al.*, 2000). Thus, chemokines seem to be interesting therapeutic targets for periodontal disease management.

PULPAL AND PERIAPICAL SCENARIOS ORCHESTRATED BY CHEMOKINES

Dental pulp is protected from micro-organisms of the oral cavity by enamel and dentin. The exposure of dental pulp to micro-organisms and their products, as a consequence of dental caries, fractures, or operative procedures, triggers a pulpal inflammatory response. Generally, severe pulpitis, resulting from dental caries, is characterized by a marked inflammatory infiltrate. However, little is known about the recruitment of these cells in inflamed dental pulp lesions. It is becoming accepted that pulp cells are able to respond to micro-organisms and toxic products through chemokine production. In a recent study, MIP-3α/CCL20 expression in human inflamed pulp was observed distributed mostly in macrophages that had accumulated in the area adjacent to caries lesions. Moreover, CCR6 (which binds MIP-3α/CCL20) expression was mostly associated with infiltrating lymphocytes. Both MIP-3α/CCL20 and CCR6 are rarely detected in normal pulp (Nakanishi *et al.*, 2005). Furthermore, a higher concentration of IL-8/CXCL8, the major chemoattractant of polymorphonuclear cells, has been detected in pulps diagnosed with irreversible pulpitis. This chemokine was predominantly expressed in areas with a heavy infiltration of inflammatory cells, demonstrating its possible contribution to the local inflammatory process. In contrast, normal pulps showed negative or weak IL-8/CXCL8 immunoreactivity (Huang *et al.*, 1999).

Human odontoblasts from intact third molars constitutively expressed low levels of IL-8/CXCL8, which increased in response to *Escherichia coli* LPS exposure (Levin *et al.*, 1999). Additionally, *Prevotella intermedia* LPS, IL-1α, IL-1β, and TNF-α are capable of stimulating pulpal fibroblast cultures to express IL-8/CXCL8 (Nagaoka *et al.*, 1996). Remarkably, IL-8/CXCL8 and MCP-1/CCL2 production by pulp cells, pulp tissue, and endothelial cells *in vitro* is modulated by neuropeptides, such as substance P and calcitonin gene-related peptide (Patel *et al.*, 2003; Park *et al.*, 2004). The study of chemokine-dependent cellular infiltration in pulp may provide important information concerning leukocyte migration in the periapical region, considering the close relationship between these tissues. Moreover, periapical remnants of pulp tissue might account for chemokine levels in early events of periapical inflammatory disease.

The progression of pulpal inflammation to the periapical

region and micro-organism colonization of the root canal system lead to innate and adaptive immune responses, and, as a result, to periapical alveolar bone destruction and periapical lesion formation (Fig. 4). Concomitantly, resorption of the tooth's hard structures, cementum and dentin, may occur, resulting in considerable reduction of tooth stability (Nair, 1997, 2004).

Chronic apical periodontitis is sometimes referred to as periapical granuloma and can evolve to form a periapical cyst. Granulomas consist of a granulomatous inflammatory tissue that is heavily infiltrated by cells and circumscribed by a fibrous capsule, while cysts are characterized by an epithelium-lined cavity (Nair, 1997, 2004). The key elements of these lesions are polymorphonuclear leukocytes, macrophages, T- and B-lymphocytes, mast cells, osteoclasts, osteoblasts, fibroblasts, and epithelial cell rests (Nair, 1997, 2004; Rodini and Lara, 2001; de Oliveira Rodini *et al.*, 2004). Although periapical granulomas do not exhibit the typical morphologic organization of classic immunogenic tuberculoid granuloma, their mechanism of formation is believed to be quite similar. In this setting, chemokines have been considered as key host elements involved in granuloma formation (Segovia-Juarez *et al.*, 2004).

Classically, chemical and mechanical preparation of the root canal and local medication, followed by filling of the root canal system, results in elimination of the infection and healing of the periapical tissues. However, in some cases, apical periodontitis does not respond favorably. The lack of success is mostly attributed to the anatomical complexity of root canals, which makes the satisfactory elimination of micro-organisms impossible. Moreover, host factors must function satisfactorily for the control of infection as well as for repair. In this regard, appropriate migration

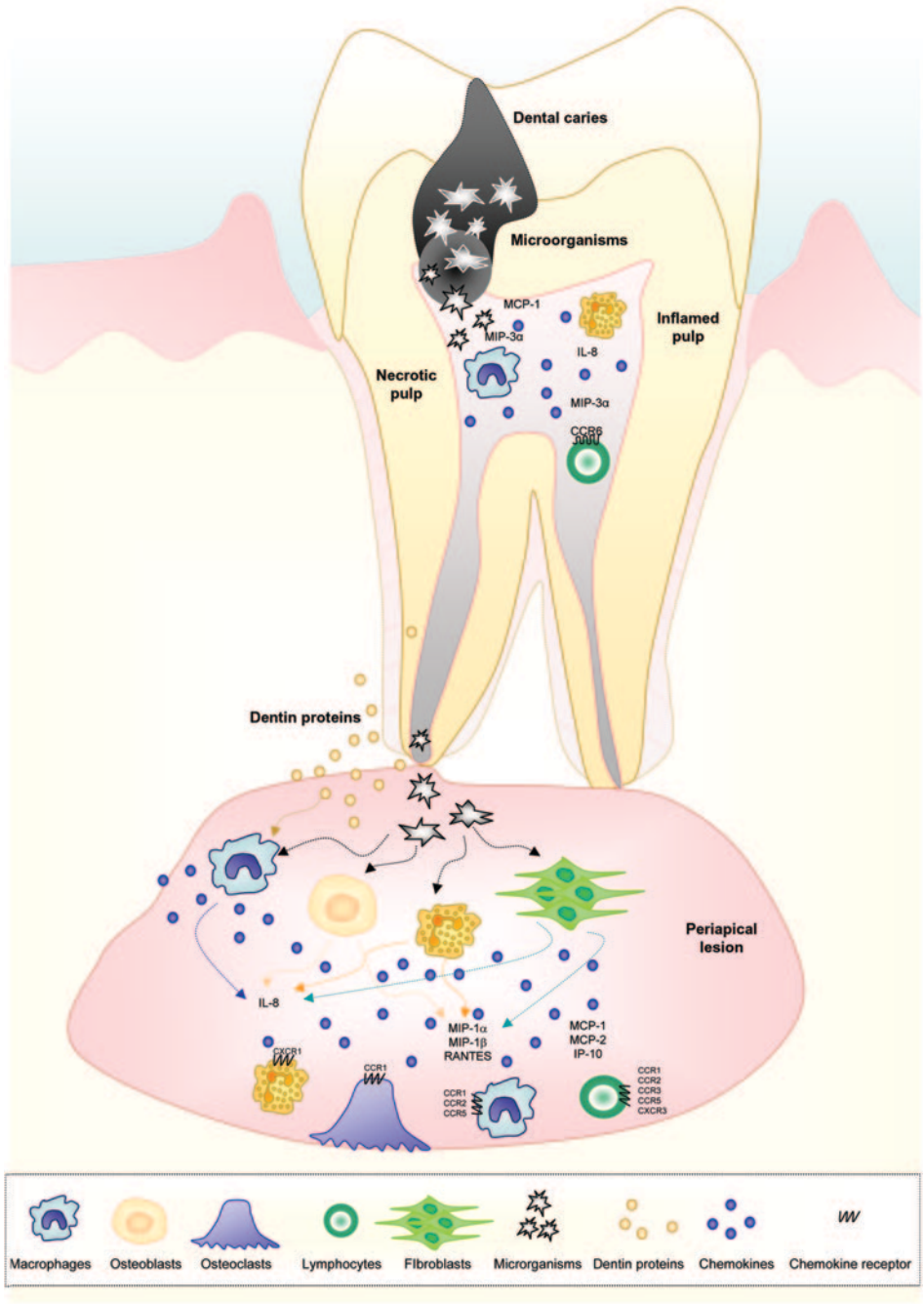

Figure 4. Pulpal and periapical scenarios orchestrated by chemokines. A schematic representation of periapical lesion formation, supported by an inflammatory and immune response against micro-organisms that invade and destroy the dental pulp. In inflamed pulp, the chemokines MIP-3α/CCL20 (a CCR6 ligand), IL-8/CXCL-8, and MCP-1/CCL-2 expression contribute to inflammatory cell infiltration. The progression of pulpal inflammation to the periapex and colonization of the root canal system by micro-organisms leads to soft- and hard-tissue destruction. In the periapex, chemokine production, which can be invoked by micro-organisms, dentin proteins, and dental materials, supports migration of leukocyte subsets, lymphocytes, and bone cells. Gram-negative flora is able to induce the production of IL-8/CXCL8 by pulp fibroblasts, osteoblasts, and the production of MIP-1α/CCL3 and MIP-1β/CCL4 by neutrophils. CXCR1 expression was detected in neutrophils, which are attracted by IL-8/CXCL8. Chemokines and receptors expressed in cysts and granulomas comprise, CCR1—expressed in monocytes/macrophages, lymphocytes and osteoclasts—and its ligands MIP-1α/CCL3 and RANTES/CCL5. CCR2 and CCR5 are found in monocytes/macrophages and lymphocytes, and their ligands are MCP-1/CCL2 (CCR2), MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5 (CCR5 ligands). CXCR3 and CCR3 are expressed in lymphocytes, and their ligands are MCP-2/CCL8 (CCR3) and IP-10/CXCL10 (CXCR3). Chemokine expression in apical periodontitis contributes to persistent inflammatory cell infiltration and the chronicity of apical lesions.

of T- and B-lymphocytes (Teles *et al.*, 1997; Hou *et al.*, 2000), neutrophils (Yamasaki *et al.*, 1994; Kawashima *et al.*, 1999), and mononuclear cells (Chae *et al.*, 2002) is essential for the periapical tissue response. It is noteworthy that MCP-1/CCL2 plays a critical role in mononuclear cell migration to the periapical sites, as shown in the MCP-1/CCL2-deficient mice that are susceptible to the spreading of endodontic infection, due to the significant impairment of monocyte recruitment (Chae *et al.*, 2002). These results reinforce the role of mononuclear cells in the control of micro-organism dissemination and, consequently, in the prevention of infection-induced bone loss in apical periodontitis.

Chemokine production in periapical sites may be elicited by micro-organisms such as bacteria, fungi, and viruses, and their products, by other inflammatory molecules, such as IL-1, TNF-α, and IFN-γ, by chemokines themselves, and by molecules released from the dissolution of mineralized tissues, such as bone, dentin, and cemmentum. As previously demonstrated, dentin constituents stimulate the release of chemotactic factors by osteoblasts *in vitro* (Silva *et al.*, 2004a), and specific dentin proteins are capable of stimulating neutrophil migration *via* the induction of KC/CXCL1 and MIP-2/CXCL2 release (Silva *et al.*, 2004b).

The predominantly anaerobic Gram-negative flora of the infected root canals, *i.e.*, *Porphyromonas endodontalis, P. gingivalis,* and *P. intermedia,* are able to induce the production of IL-8/CXCL8 by pulp fibroblasts, osteoblasts (Yang *et al.*, 2003), human whole-blood cultures (Matsushita *et al.*, 1999), MIP-1α/CCL3 and MIP-1β/CCL4 by neutrophils (Ko and Lim, 2002), and KC/CXCL1 by mouse macrophages (Murakami *et al.*, 2001). Another likely source for chemokine production in periapical lesions is trauma, injury from instrumentation, or irritation from chemical and endodontic materials, which might evoke a chemokine-dependent host response (Schmalz *et al.*, 2000; Tuncer *et al.*, 2005).

In human periapical granulomas, the presence of IL-8/CXCL8, MIP-1-α/CCL3, MIP-1β/CCL4, IP-10/CXCL10, MCP-1/CCL2, RANTES/CCL5, and the receptors CCR5, CXCR3, and CCR3 has been previously demonstrated by immunohistochemical methods (Marton *et al.*, 2000; Kabashima *et al.*, 2001, 2004; Shimauchi *et al.*, 2001). Detectable levels of IL-8/CXCL8 were found in approximately 95% of periapical exudates collected from root canals during routine endodontic treatment of human periapical lesions, suggesting a pivotal role for IL-8/CXCL8 in neutrophil migration in acute phases of apical disease. IL-8/CXCL8 also has a direct effect on osteoclast recruitment and activity (Bendre *et al.*, 2003), which may account for the significant osteolysis associated with apical abscess. In fact, neutrophils are active in periapical tissue damage, since neutropenic animals demonstrate a considerable decrease in peripical lesion formation (Yamasaki *et al.*, 1994). In addition, a significantly positive association between IL-8 levels and painful symptoms has been observed, indicating a role for IL-8 in the occurrence of the symptoms of periapical disease (Shimauchi *et al.*, 2001). In a recent study, we found increased levels of CCR1, CCR2, CCR3, CCR5, CXCR1, and CXCR3 in cysts and granulomas (Silva *et al.*, 2005). However, cysts exhibited a higher expression of RANTES/CCL5, IP-10/CXCL10, MCP-1/CCL2, CCR3, CCR5, CXCR1, and CXCR3 compared with granulomas. As previously demonstrated, RANTES/CCL5, IP-10/CXCL10, MCP-1/CCL2, CCR3, CCR5, CXCR1, and CXCR3 have important effects on chemotaxis and the differentiation of bone cells (Lisignoli *et al.*, 2003, 2004; Okamatsu *et al.*, 2004; Kwak *et al.*, 2005; Yano *et al.*, 2005; Kim *et al.*, 2006a,b), and might be responsible for the bone and root resorption seen in chronic periapical lesions.

Although we have reported an increased expression of these chemokines and receptors in cysts and granulomas, the exact role of each chemokine in the progression of the lesion has not yet been clarified. In granulomas, the analysis of chemokines *vs.* infiltrating cells suggests a relationship between RANTES/CCL5 and the recruitment of $CD4^+$ and $CD68^+$ cells, while MIP-1β/CCL4, MIP-1-α/CCL3, and IP-10/CXCL10 were associated with the $CD8^+$ population. In addition, MIP-1β/CCL4 and MIP-1-α/CCL3 expression was associated with $CD45RO^+$ cell infiltration. Moreover, in cysts, $CD4^+$ and $CD8^+$ populations were found to be related to CCR2 (Silva *et al.*, 2005). These results, apparently, suggest a redundancy of pathways to guarantee the appropriate migration of lymphocytes to periapical sites, given that the pivotal role of these cells is to prevent dissemination of micro-organisms from periapical lesions (Teles *et al.*, 1997; Hou *et al.*, 2000).

The expression of chemokines and their receptors in cells of pulp and periapical tissues is represented in Fig. 4. The difference in the chemokine and chemokine receptor expression in cysts and granulomas may affect the immune patterns of response, given that Th1 and Th2 cells migrate to different tissues through the expression of different sets of chemokine receptors (Bonecchi *et al.*, 1998; Sallusto *et al.*, 1998a,b, 2000). As previously mentioned, Th1 cells express CCR5 and CXCR3 (Kaplan *et al.*, 1987; Bonecchi *et al.*, 1998; Loetscher *et al.*, 1998, 2001; Sallusto *et al.*, 1998a,b), while CCR3 is expressed on Th2 cells (Bonecchi *et al.*, 1998; Sallusto *et al.*, 1998a,b; Gu *et al.*, 2000). Although the overall role of Th1 and Th2 responses in inflammatory periapical diseases has not been fully determined, the Th1 response appears to be predominant in early lesions (Kawashima and Stashenko, 1999), while the Th2 response is dominant in chronic granulomas (Kabashima and Nagata, 2001). However, in humans, the observation that Th1 type (CCR1, CCR5, and CXCR3) and Th2 type (CCR2 and CCR3) receptors are increased in cysts and granulomas (Silva *et al.*, 2005) may indicate the concomitant occurrence of both responses in periapical lesions.

Despite data concerning the function of chemokines and their receptors in the innate and immune responses (Zlotnik and Yoshie, 2000), bone resorption (Wise *et al.*, 1999), repair (DiPietro *et al.*, 2001), and angiogenesis (Rosenkilde and Schwartz, 2004), the importance of these effects in the repair or maintenance of these processes in the periapical region remains unclear. To date, the evidence regarding the role of chemokines in the maintenance of periapical lesions, or in the conversion of granulomas to cysts, is speculative. Corroborating this hypothesis, the demonstration of IL-8/CXCL8 expression in the epithelial rests of Malassez, the putative source of cyst-lining (Marton *et al.*, 2000), may indicate that this chemokine could serve as an inducer of rests of Malassez proliferation to form the cyst epithelium lining. Moreover, chemokines are continuously produced and bind to the extracellular matrix, thereby forming an immobilized gradient in periapical diseased sites (Marton *et al.*, 2000). Furthermore, the higher expression of chemokines and receptors—particularly RANTES/CCL5 and MCP-1/CCL2 and CCR3, CCR5, and CXCR1—in cysts

compared with granulomas (Silva *et al.*, 2005) may have some importance in the evolution of granulomas to cysts. Therefore, chemokines may be useful as diagnostic tools for evaluating the progression and exacerbation of lesions, and for assessing whether the lesions are active or healing by sampling *via* the root canal prior to obturation. Studies assessing the kinetics of chemokine production and using animals with genetic deletions of chemokines and receptors will be helpful to elucidate the role of chemokines and receptors in periapical sites. This knowledge may provide additional means of treating apical periodontitis and also other bone-destructive diseases, given the pivotal role of chemokines in the pathogenesis of these lesions.

THE OTHER SIDE: CHEMOKINES AS INDUCERS OF REPAIR AND ANGIOGENESIS

Although chemokine biology was originally considered to be restricted to the recruitment of subpopulations of leukocytes, it has become increasingly clear that these cytokines can display pleiotropic effects in mediating repair and angiogenesis. There is some evidence to support these effects, such as the expression of MIP-3β/CCL19, MIP-3α/CCL20, TECK/CCL25, and CCR5 in gingival wounds in mice (McGrory *et al.*, 2004). MIP-1α/CCL3 has been linked to enhanced macrophage influx, angiogenic activity, and collagen production in dermal punch wounds in mice (DiPietro *et al.*, 1998). However, in the absence of this chemokine, the wound re-epithelialization was not significantly affected (DiPietro *et al.*, 2001; Low *et al.*, 2001). In contrast, MCP-1/CCL2-deficient mice demonstrate drastically delayed wound re-epithelialization (DiPietro *et al.*, 2001; Low *et al.*, 2001).

Chronic inflammation is generally associated with chronic fibroproliferation that, microscopically, appears as a granulation-like tissue, such as that observed in inflamed periapical and periodontal diseases. CXC chemokines are unique in that they may exhibit either angiogenic or angiostatic activity and, consequently, influence the pathogenesis of chronic inflammatory disorders (Strieter *et al.*, 1995; Rosenkilde and Schwartz, 2004). The CXC chemokine family members that promote angiogenesis are GROα/CXCL1, GROβ/CXCL2, GROγ/CXCL3, ENA-78/CXCL5, GCP-2/CXCL6, NAP-2/CXCL7, and IL-8/CXCL8. Conversely, the angiostatic members of the CXC chemokine family include PF4/CXCL4, Mig/CXCL9, IP-10/CXCL10, I-TAC/CXCL11, and BRAK/CXCL14 (Strieter *et al.*, 1995; Rosenkilde and Schwartz, 2004).

CONCLUDING REMARKS

This review demonstrates that chemokines represent a highly sophisticated and finely tuned system, able to elicit responses that could favor the regeneration or destruction of dental and periodontal tissues. The balance of cytokines, chemokines, and molecules released from injured tissues is likely to play a key role in regulating cell functions such as migration, proliferation, and matrix synthesis during the process of oral inflammation. Bearing in mind that the understanding of these complex cell-chemokine-receptor interactions is still in its infancy, we present evidence that chemokines orchestrate a large proportion of the cellular and molecular events in oral diseases. The findings presented herein, concerning the variety of process affected by chemokines, support the notion that therapy directed at chemokines may be a novel approach in the treatment of a variety of disorders affecting oral tissues.

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