Inflammation and Tooth Movement: The Role of Cytokines, Chemokines, and Growth Factors

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When an orthodontic force is applied, the periodontal tissues express extensive macroscopic and microscopic changes, leading to alterations in 5 microenvironments: extracellular matrix, cell membrane, cytoskeleton, nuclear protein matrix, and genome. Capability of adaptive reaction to applied mechanical loading relies in the DNA of periodontal ligament (PDL) and alveolar bone cells. However, an inflammatory process is a precondition for these modifications to occur, which will lead to orthodontic tooth movement (OTM). PDL’s vascularity and blood flow changes, as well as mechanical alterations in the cytoskeleton of PDL and bone cells, will result in local synthesis and release of various key mediators, such as chemokines, cytokines, and growth factors. These molecules will induce many cellular responses by various cell types in the periodontium, providing a favorable microenvironment for bone resorption or deposition and, consequently, for OTM. In these inflammation and tissue remodeling sites, cells may also communicate with one another through the interaction of cytokines and other related molecules. The aim of this review is to bring focus on the role of these important local inflammatory mediators that are closely related to the mechanotransduction involved in OTM. (Semin Orthod 2012;18:257-269.) © 2012 Elsevier Inc. All rights reserved.

Mechanotransduction induced by orthodontic force occurs when external strain induces mechanosensing (the cell senses structural changes in the extracellular matrix, caused by external mechanical loading), transduction, and cellular response in several paradental tissues. This process leads to vasculature and extracellular matrix remodeling in the periodontal ligament (PDL), gingiva, and alveolar bone. This remodeling is facilitated by proliferation, differentiation, and apoptosis of local periodontal cells, bone cell precursors, and leukocyte migration from the microvascular compartment.1,2 In this context, an aseptic acute inflammatory response is occurring in the early phase of orthodontic tooth movement (OTM), followed by an aseptic and transitory chronic inflammation. As orthodontic forces (continuous, interrupted, or intermittent) are not uniform throughout the applied region, areas of tension or compression are developed leading to varied inflammatory processes resulting in different tissue remodeling responses.1,3

This sequence of events leads to an increase in the number of monocytes and polymorphonuclear leukocytes, which exit from the micro-
vasculature into the extravascular space.\textsuperscript{1} These migratory cells produce various inflammatory mediators, which interact directly or indirectly with the entire population of native paradental cells. These molecules, such as chemokines, cytokines, and growth factors (GFs), mediate and maintain the vascular and cellular changes in an autocrine or paracrine way, stimulating or inhibiting cellular activity.\textsuperscript{1} Such mediators are believed to activate tissue remodeling, characterized by selective bone resorption or deposition in compression and tension sites of the PDL, respectively.\textsuperscript{1,3}

This article reviews the current biomedical literature on inflammation in OTM. It seeks to summarize the role of chemokines, cytokines, and GFs and to explore their clinical implications in routine orthodontic practice. It does not propose a complete picture (as the research is as yet incomplete in the current scenario), but orients the reader to the role that these mediators play in the inflammatory periodontal tissue reactions, in response to orthodontic force application.

**Cytokines and Tooth Movement**

Cytokines are extracellular signaling proteins directly involved in the bone remodeling and inflammatory process during OTM, which act directly or indirectly, to facilitate bone and PDL cells differentiation, activation, and apoptosis.\textsuperscript{1,5} Investigations of their mechanisms of action have identified their effector (proinflammatory) and suppressive (anti-inflammatory) functions during OTM.

The receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) expressed by osteoblast and apoptotic osteocyte are the most important proinflammatory cytokines responsible for recruitment, differentiation, activation, and survival of osteoclasts.\textsuperscript{5} These cytokines bind to their respective receptors, RANK and c-Fms, expressed in osteoclast precursors and mature osteoclasts, to produce these events through osteoclast–osteoblast communication.\textsuperscript{2,4} By contrast, osteoblasts also express osteoprotegerin (OPG), a decoy receptor of RANKL, which inhibits the RANK/RANKL interaction, preventing osteoclastogenesis and accelerating mature osteoclast apoptosis.\textsuperscript{1,4}

When subjected to continuous (0.5-3.0 g/cm\textsuperscript{2}) or intermittent (2.0 or 5.0 g/cm\textsuperscript{2}) mechanical-compressive force, PDL cells induce osteoclastogenesis in vitro through downregulation of OPG expression and upregulation of RANKL expression, via prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and interleukin (IL)-1β synthesis.\textsuperscript{5,6} In accordance, mice research also demonstrated that osteoclastogenesis appears to be primarily regulated through M-CSF and RANKL signaling by PDL cells in the compression side in the first week of orthodontic force application.\textsuperscript{7,8} In the compression sites during human OTM (250 g), the same standard of RANKL and OPG expression is observed in gingival crevicular fluid (GCF) after 24 hours.\textsuperscript{9} By contrast, in vitro cyclic tensile strain increases OPG levels and decreases RANKL synthesis in cultured osteoblasts and PDL cells in a force magnitude-dependent way.\textsuperscript{10}

Furthermore, RANKL gene transfer or M-CSF administration to the periodontal tissue induces osteoclastogenesis and accelerates experimental OTM in rodents,\textsuperscript{11} whereas local OPG gene transfer has opposite results.\textsuperscript{12} Therefore, local MCSF/RANKL or OPG treatment might be a useful tool for shortening the duration of orthodontic treatment or improving orthodontic anchorage, respectively, but their clinical applications are still far off. Currently, alveolar decortication has been the most frequently applied clinical alternative to accelerate orthodontic treatment in humans.\textsuperscript{13} Animal studies have shown that alveolar corticotomy increases the expression of M-CSF and RANKL in PDL, enhancing the rate of OTM during the initial tooth-displacement phase.\textsuperscript{14} In addition, a previous study reported that OPG levels were greater than RANKL levels under physiological conditions in human GCF.\textsuperscript{15} However, an increased RANKL/OPG ratio was observed in patients with severe root resorption under orthodontic treatment that could be related to a greater bone resorption. Therefore, a local OPG treatment might be a future tool to prevent or paralyze root resorption during OTM.

Tumor necrosis factor (TNF)-α is another proinflammatory cytokine that has been investigated in OTM and is involved in bone resorption and acute as well as chronic inflammation. TNF-α is produced primarily by activated monocytes and macrophages, but also by osteoblasts, epithelial cells, and endothelial cells.\textsuperscript{16} In vitro
studies have demonstrated that in bone, TNF-α can directly and indirectly induce osteoclastogenesis by binding to its p55 receptor on osteoclast precursors and by upregulating expression of RANKL, M-CSF, and other chemokines on osteoblasts.17-19 TNF-α is also an apoptotic factor for osteocytes, which could be the signal for osteoclast recruitment to resorb bone in the PDL pressure side, at the same time inhibiting osteoblasts.20

The real role of TNF-α in bone resorption, upregulating and increasing the amount of OTM, was shown in rodent models with TNF-α receptor impairment.21,22 A recent in vitro study suggested that PDL fibroblasts secrete higher levels of TNF-α at the PDL compression side than at the tension side.23 This imbalance leads to RANKL expression by activating CD4+ T cells, thereby facilitating bone resorption during OTM. Taken together, local TNF-α treatment or stimulation of cells that produce this proinflammatory protein might be a future alternative to accelerate OTM. Moreover, local TNF-α-antibody injection might be a useful tool to improve the anchorage site during OTM, as a previous study has demonstrated its clinical application by inhibiting bone resorption in rheumatoid arthritis.24

Like TNF-α, IL-1 (alpha and beta) is a proinflammatory cytokine that is highly expressed on the PDL pressure side of humans and animals and the adjacent alveolar bone in the early stages of OTM.6,25-28 Its role in OTM has been the focus of previous human studies25,27,28 that demonstrated an increase in osteoclast activity and survival, while at the same time inducing bone marrow cells and osteoblasts to produce RANKL in the early phase of OTM.29,30 Moreover, IL-1 can induce osteoclast formation directly from osteoclast precursors under TNF stimulation in vitro.31

Other in vitro studies have also demonstrated that IL-1 strongly promotes osteoclast formation by increasing M-CSF and PGE2 production and decreased OPG production by osteoblasts.26,32 Under 24 hours of continuous compressive forces in vitro (3.0 g/cm2), osteoblastic cells respond by expressing IL-1β, IL-6, IL-11, TNF-α, and receptors for IL-1, IL-6, and IL-8, suggesting an osteoblastic autocrine mechanism induced by mechanical stress.33 Indeed, animal studies with absence of IL-1β and/or TNF-α signaling demonstrated impaired tooth movement,21,22 but the mechanisms behind this finding remain unknown.

Polymorphisms in IL-1 gene, which determines the degree of alteration in the amount of cytokines secreted, have been studied in OTM.34 The allele 1 at the IL-1 gene, known to decrease the production of IL-1 cytokine in vivo, significantly increases the risk of external apical root resorption in patients during OTM. The clinical implication is that potential orthodontic patients can be screened for the IL-1β genotype by analyzing the DNA from a simple cheek swab or mouth wash taken during the initial examination, to identify those who carry 2 copies of the high-risk allele. It would then be possible to inform patients about their predispositions before starting treatment, and closely monitor those at risk, by periodic radiographs. Moreover, a recent study has shown that IL-1β (+3954) genotype was associated with faster tooth movement in humans.35

In addition, IL-1 receptor antagonist (IL-1Ra) competitively blocks the interactions of IL-1 with IL-1 receptors, inhibiting its activity. IL-1Ra has been used as a therapeutic tool in conditions related to bone resorption, such as rheumatoid arthritis.36 Moreover, a decreased physiological IL-1Ra expression in GCF has been shown to correlate with faster OTM in humans.37 We speculated that in the future, IL-1Ra can be clinically used to modulate the amount and side effects of OTM.

Other cytokines, such as IL-6, IL-8, and IL-11, also stimulate alveolar bone resorption during OTM by acting early in the inflammatory response.38-40 These cytokines can be enhanced by, or can act synergistically with, TNF-α and IL-1.41 By contrast, IL-11 can have anabolic effects, alone or in association with bone morphogenetic protein-2 (BMP-2), inducing osteoblastic differentiation in mouse mesenchymal cells.42 Different anti-inflammatory cytokines play inhibitory effects, controlling inflammation and bone resorption. IL-18 and IL-10 are also expressed in the PDL during OTM, and both inhibit osteoclastogenesis and bone resorption.43 Furthermore, IL-10 inhibits the production of IL-1, IL-6, and TNF-α, and its expression is higher in PDL tension than in compression sites.25,44

From a clinical standpoint, analysis of cytokine levels in GCF during OTM may, in the
future, reveal the rate of OTM and determine the optimum force level that should be applied by orthodontic devices. Analysis of cytokine levels in GCF may also be helpful in monitoring the biological activities in the periodontium during the retention period, which could provide information about possible relapse.

Chemokines and Tooth Movement

Chemokines belong to the superfamily of small heparin-binding cytokines. The ability to induce cell migration is the common feature that distinguishes this group of cytokines. Structurally, the chemokines are classified in 4 subfamilies based on the position of 2 highly conserved cysteine residues at the N-terminus: C, CC, CXC, and CX3C. To mediate their cellular effects, these molecules bind to selective 7-transmembrane domain receptors, which are coupled to heterotrimeric G proteins, differentiating also from other cytokines. The chemokine receptors are named according to their ligand family, such as CCR for receptors of CC ligands and CXCR for CXC ligands. The chemokine system is promiscuous or redundant, as different chemokines can bind to a given chemokine receptor, and a given chemokine may bind to different chemokine receptors. However, binding of chemokines to their respective receptors does not necessarily achieve the same functions in vivo.

Chemokines present different biological outcomes in different tissues, which are controlled by geography and timing. They play a central role in trafficking and homing of leukocytes, immune cells, and stromal cells, during physiological (homeostatic chemokines) and inflammatory conditions (inflammatory chemokines). In addition, chemokines induce other biological processes, such as angiogenesis, cell proliferation, and apoptosis.

In inflammatory sites, cellular recruitment begins when local cytokines, pathogens, GFs, chemokines themselves, and mechanical stress trigger off the production of inflammatory chemokines by several cell types. These locally produced chemokines bind to the cell surface of the vascular endothelium and/or to the extracellular matrix, formatting gradients of chemokines, and, consequently, directing cell recruitment to inflammation sites. Therefore, recruited and activated macrophages, neutrophils, and lymphocytes will respond to the tissue factors and secrete several mediators that will act together, leading to bone and PDL remodeling during OTM.

In addition to leukocytes, the chemokines provide key signals for trafficking, differentiation, and activity of bone cells, playing an important role in bone remodeling and bone inflammatory disease. In this context, some researchers have investigated how a given chemokine or chemokine receptor can regulate this process. Previous studies in vitro have demonstrated that CC-chemokine ligand 3 (CCL3), CCL2, CCL5, and CXC-chemokine ligand (CXCL9) chemokines promote chemotaxis of osteoclasts when binding to their respective CC receptors (CCR1, CCR2, CCR3, CCR5, and CXCR3), which are expressed by osteoclast precursors. Others have shown that CCL5, CCL7, CCL2, CCL3, CXCL12, and IL-8 (CXCL8) promote RANKL-induced differentiation of osteoclast precursors. Chemokines also stimulate activity of osteoclasts, such as CCL2, CCL3, and IL-8, and prolong osteoclast survival, such as CCL3 and CCL9 (ligands CCR1). Moreover, RANKL induces osteoclast production of CCL2, CCL3, and CCL5, which suggests an autocrine and paracrine signalization during osteoclastogenesis, and an increase of bone resorption.

Chemokines can also induce recruitment, proliferation, and survival of osteoblasts. Osteoblasts express chemokine receptors, such as CXCR1, CXCR2, CXCR3, CXCR5, CCR1, CCR3, CCR4, and CCR5. CCL5, a ligand of CCR1, CCR3, CCR5, and CCR4, can induce osteoblast recruitment and avoid apoptosis of this cell. The chemokine CXCL10 induces osteoblast proliferation and release of alkaline phosphatase and β-acetylhexosaminidase, while CXCL12 and CXCL13 induce both proliferation and collagen type I mRNA expression in osteoblasts.

The osteoblast–osteoclast communication through RANK/RANKL/OPG system is essential in the regulation of bone remodeling, as previously described. This interaction between osteoclast and osteoblast can also be mediated by chemokines through paracrine signalization. In inflammation sites, this process is initiated when the proinflammatory cytokines IL-1 and TNF-α promote the production of CCL2, CCL3, and CCL5 by the osteoblast.
chemokines by osteoblasts can significantly contribute to recruitment and development of osteoclasts at osteolysis sites, thereby exacerbating bone loss.17,48 Furthermore, CCL3 is also indirectly involved with osteoclast differentiation, as this chemokine stimulates increased expression of RANKL by osteoblasts53 and induces cell–cell adhesion between osteoclasts and osteoblasts.52

Because chemokines play an important role in bone remodeling, several studies have investigated the levels of these proteins in OTM. CCL2, CCL3, CCL5, IL-8 (CXCL8), and CXCL12 expression were greatly increased in periodontal tissues of murines and humans submitted to orthodontic force.22,56-58 A recent study in Wistar rats has demonstrated that IL-8 and CCL2 may facilitate the process of root resorption after 7 days of excessive mechanical loading (50 g).58 Although the expression of these chemokines has been observed in periodontium submitted to orthodontic force, their functional role in OTM is yet to be known. The authors of the present article demonstrated that the axis TNF-α/p55 induces expression of CCL5 after 12 hours of continuous force (10 g) in the PDL of mice during OTM.22 As CCL5 is a ligand of CCR5 receptor, we have investigated the role of this chemokine receptor after the application of orthodontic force.22 The results demonstrated that the amount of tooth movement and the number of osteoclasts were increased in CCR5-deficient mice (CCR5<sup>−/−</sup>) after 12 days of mechanical loading (10 g). The data also revealed that the mRNA levels of osteoclastogenesis and osteoclast activity markers (Cathepsin K, RANKL, and matrix metalloproteinase-13(MMP-13)) were higher in CCR5<sup>−/−</sup> mice after 3 days. Moreover, osteoblast differentiation markers (runt-related transcription factor 2 [RUNX2] and osteocalcin [OCN]), and negative regulators of bone resorption (OPG and IL-10) were decreased in CCR5<sup>−/−</sup> mice after 3 and 7 days. Taken together, these results suggested that diminished osteoblast differentiation in CCR5<sup>−/−</sup> led to a reduction of inhibitory signals for osteoclasts, resulting in increased bone resorption and greater OTM. Therefore, CCR5 might be a downregulator of bone resorption during OTM, as this receptor indirectly inhibits osteoclast recruitment and reduces this cell activity.56 Further studies are now required to confirm the role of other chemokines/chemokine receptors involved in osteoclast and osteoblast recruitment, differentiation and activity during OTM, and their possible impact in clinical orthodontics.

In summary, the chemokines interfere directly and indirectly in osteoclast and osteoblast recruitment, differentiation, and consequent bone resorption and formation. Therefore, the development of strategies that are capable to locally and selectively modulate the chemokine pathways might contribute to a better control of the rate of OTM, as well as the stabilization of the orthodontic results. Drugs such as met-RANTES and P8A are currently used for blockage of specific chemokine receptors, leading to a diminished bone resorption in rheumatoid arthritis.59,60 In the future, the local administration of these present and upcoming drugs could be useful to increase biological anchorage at specific sites and stability of the final results.

**Growth Factors and Tooth Movement**

GF are substances that bind to specific receptors on the surface of their target cells, stimulating cell proliferation, migration, and differentiation. Moreover, they display important roles in hematopoiesis, the inflammatory process, angiogenesis, and tissue healing.61 GF may also act locally to modulate bone remodeling, and consequently, OTM.27,61

Vascular endothelial growth factor (VEGF) is an essential mediator of angiogenesis and increased vascular permeability.61 As osteoblast and osteoclast express VEGF receptor-1, some studies have investigated the effect of VEGF on bone remodeling under mechanical loading.62,63 In vitro studies have shown that PDL cells and apoptotic osteocytes increase VEGF production after compressive force application.62,63 VEGF can modulate the recruitment, differentiation, and activation of osteoclast precursors, increasing bone resorption.64 Moreover, VEGF can also indirectly induce bone resorption, as it promotes angiogenesis in vitro, allowing new capillaries to assist the recruitment of osteoclast precursors to the bone surface close to the resorption site.53 In accordance, VEGF enhances osteoclast recruitment and increases the rate of experimental OTM in vivo,65 which are both inhibited by anti-VEGF polyclonal antibody treatment.66 The expression of VEGF was
also detected in osteoblasts, osteocytes, and fibroblasts in PDL tension sites after 10 days of OTM in C57BL/6j mice.\textsuperscript{65} Taken together, it is reasonable to conclude that VEGF plays an important role in bone remodeling at both compression and tension sites of the PDL during OTM.

The transforming growth factor (TGF)-β superfamily (TGF-β1 to -β3) is another important GF related to bone and PDL tissue remodeling during OTM. Under mechanical loading, the cyclic tensile force upregulates TGF-β expression in osteoblasts and also in PDL cells in vitro.\textsuperscript{67} Furthermore, TGF-β stimulates OPG production and downregulates IL-6 expression, which inhibits the osteoclastogenesis-supporting activity of these cells.\textsuperscript{67} In accordance, increased levels of OPG and TGF-β1 mRNA are observed in osteoblasts and other PDL cells in the tension sites after 2 days of OTM in Wistar rats, simultaneously with a decreased number of osteoclasts in this area.\textsuperscript{68} In humans, the level of TGF-β1 is enhanced in GCF and in PDL tissue a few days after continuous orthodontic force application for rapid maxillary expansion and canine distal movement (force of 2-2.5 N), respectively.\textsuperscript{25} However, there are no differences in the level of this GF between tension and pressure sites.\textsuperscript{25} As it is clear that TGF-β plays an important role in the tension site during OTM, further studies should now investigate the TGF-β effect on pressure sites after orthodontic force application because it is an essential factor for RANKL-induced osteoclastogenesis and, consequently, OTM.

Bone morphogenetic proteins (BMPs) are multifunctional GFs that belong to the TGF-β superfamily and play an important role in upregulating various transcription factors involved in osteoblastic differentiation, and consequently, in bone formation.\textsuperscript{69} To date, more than 20 BMPs have been discovered, but BMP-2, BMP-6, BMP-7, and BMP-9 seem to have the most potent osteogenic activity.\textsuperscript{69,70} Studies have shown that under tensile strain, human PDL cells in culture increase BMP-2 and BMP-6 expression, suggesting that these BMPs might play an important role in PDL tensile sites during OTM.\textsuperscript{70,71} However, there is a lack of information on the actual role of BMPs in OTM.

Insulin-like growth factors (IGFs) are involved in bone formation by inducing proliferation, differentiation, and apoptosis of osteoblasts.\textsuperscript{72} The IGFs effect is regulated by growth hormone, parathyroid hormone, vitamin D3, corticosteroids, TGF-β, IL-1, and platelet-derived growth factor. Studies have shown that under continuous tensile mechanical loading, rat tibiae osteocytes and calvaria osteoblasts increase IGF-I synthesis, which stimulates bone formation.\textsuperscript{73,74} In PDL tissues, IGF also acts as an antiapoptotic and proliferative factor for fibroblasts and osteoblasts in vitro.\textsuperscript{75} Accordingly, an in vivo study using Wistar rats demonstrated that 4 hours of a continuous tensile force (0.1-0.5 N) applied to a tooth induces increased expression of IGF-I and IGF-I receptor in PDL cells in tension sites, but a decreased expression in compression sites.\textsuperscript{76} Therefore, a local increase of IGF-I appears to provide a link between the mechanical loading and tissue remodeling in the tensile site during OTM.

Fibroblast growth factors (FGFs) belong to a family of 23 members that bind to 4 structurally related high-affinity receptors.\textsuperscript{77} Among FGFs, FGF-2 can regulate bone remodeling by stimulating osteoblast-like cell proliferation and differentiation in vitro, and by increasing osteoclast formation and activity.\textsuperscript{78} An in vitro study demonstrated that compressive forces induce production of FGF-2 by human PDL cells, which stimulates RANKL expression.\textsuperscript{79} Moreover, there is an increased expression of this GF in the compression site of PDL tissues after 1 day of mechanical loading in humans in vivo.\textsuperscript{39} The results suggest that FGF-2 can be involved in bone resorption during OTM.\textsuperscript{39,79} However, whether FGF-2 plays an important role in the tensile site after mechanical loading is yet to be elucidated. As FGF-6, FGF-8, FGF-9, FGF-18, and FGF-23 are also known as regulators of bone cell functions, they should be tested following application of the orthodontic force.\textsuperscript{39,77,79}

A further GF showing increased levels in GCF after the first 24 hours of OTM in humans is the epidermal growth factor (EGF).\textsuperscript{27} A recent study has shown that the administration of EGF–liposome in the mucosa adjacent to the tooth in Holtzman rats was able to stimulate the expression of RANKL, leading to an increased number of osteoclasts in the PDL compression sites, and consequently, to enhancement of tooth movement under a continuous force (20 g).\textsuperscript{80} In addition, the expression of both EGF and EGFR in osteoblasts and PDL fibroblasts is increased at
PDL tensile sites in rats and cats.\textsuperscript{80,81} Taken together, these data suggest that EGF is involved in remodeling of mineralized and nonmineralized extracellular matrix in both tensile and compressive sites during OTM.

Taken together, the current knowledge indicates that mechanical loading stimulates local expression of many GF involved in bone and PDL remodeling in the early stages of OTM in both tensile and compressive sites. In the future, local injection of a single GF or a combination of multiple GFs in the periodontal tissues might modulate the rate of OTM or help to improve the stability of the orthodontic results.

**Inflammation in OTM**

When an orthodontic force is applied to a tooth, immediate changes are observed in periodontal tissues (Fig 1). Compression sites

![Diagram](image)

**Figure 1.** In compression sites, the orthodontic force induces local hypoxia and mechanotransduction. The local hypoxia increases the expression of interleukin (IL)-1\(\beta\), IL-6, IL-8, tumor necrosis factor (TNF)-\(\alpha\), and vascular endothelial growth factor (VEGF) in periodontal ligament (PDL) fibroblasts. The physical strain also stimulates the PDL cells to produce growth factors, prostaglandin E\(_2\) (PGE\(_2\)), and chemokines (mechanotransduction). Moreover, stressed PDL peripheral nerve fibers release vasoactive neurotransmitters, such as calcitonin gene–related peptide and substance P. The cytokines IL-1\(\beta\), IL-6, IL-8, and TNF-\(\alpha\) lead to increased expression of adhesion molecules (VCAM-1 and ICAM-1) and chemokines, which in turn promote leukocyte adhesion and migration. Furthermore, PGE\(_2\) and VEGF increase vascular flow and permeability, leading to plasma extravasation and leukocyte diapedesis. These alterations trigger an acute inflammatory response, which is replaced by a chronic process that allows leukocytes and osteoclast precursors to continue their migration into the strained paradental tissues, modulating this remodeling process. (Color version of figure is available online.)
are characterized by tissue and cell damage, reduction in the number of patent capillaries, occlusion, and partial disintegration of blood vessels, leading to ischemia and hypoxia. These alterations trigger an acute inflammatory response, featured by vasodilatation and migration of leukocytes out of capillaries. This process is initiated when local hypoxia increases the expression of IL-1β, IL-6, IL-8, TNF-α, and VEGF in PDL fibroblasts. At the same time, the physical strain stimulates the PDL production of these cytokines, as well as of GFs and chemokines, through a process called mechanotransduction. In this context, IL-1 and TNF-α activate microvascular endothelial cells, leading to increased expression of adhesion molecules (VCAM-1 and ICAM-1), inducing local chemokine expression, which in turn promotes leukocyte adhesion and migration.

Moreover, stressed PDL peripheral nerve fibers release vasoactive neurotransmitters, such as calcitonin gene–related peptide and substance P. These neuropeptides, together with VEGF and PGE₂, increase vascular flow and permeability, leading to plasma extravasation and leukocyte diapedesis.

These recruited leukocytes interact directly or indirectly with the entire population of native periodontal cells, increasing the production of specific chemokines, cytokines, and GFs involved in bone resorption. In this way, the acute phase of inflammation is replaced by a chronic process that allows leukocytes and osteoclast precursors to continue their migration into the strained periodontal tissues, modulating this remodeling process.

**Bone Resorption**

Under mechanical loading, fibroblasts, osteoblasts, and other PDL cells, located at the pressure site (Fig 2), release signaling molecules, such as PGE₂, IL-1, IL-6, TNF-α, and IL-11. Among these mediators, IL-1 and TNF-α stimulate osteoblasts to produce chemokines, such as CCL3, CCL2, and CCL5. These chemotactic proteins, joined by others, such as CXCL12, and cytokines (RANKL and TNF-α) induce chemotactic recruitment of osteoclast precursors to osteolysis sites, where these cells differentiate into mature osteoclasts through osteoblast–osteoclast communications.

For the differentiation to occur, PGE₂ and cytokines, such as IL-1, IL-6, IL-8, and TNF-α, stimulate, directly or indirectly, osteoblast/stromal cells to produce the main regulators of osteoclast differentiation: M-CSF and RANKL. This process is achieved when M-CSF and RANKL bind to their respective specific receptors, c-Fms and RANK, which are both expressed on osteoclast precursors. However, osteoclastogenesis can be downregulated when OPG, a RANKL decoy receptor produced by osteoblastic and PDL cells, binds to RANKL, inhibiting the RANK/RANKL interaction. Therefore, OPG level is decreased in compression sites during OTM, enhancing osteoclastogenesis in this area.

The mechanical loading-induced hypoxia promotes the expression of hypoxia inducible factor 1-α, which increases the expression of RANKL by human PDL fibroblasts at pressure sites, enhancing osteoclastogenesis. In addition to osteoblast and PDL cells, damaged osteocytes are also a source of RANKL and M-CSF. In this context, orthodontic force causes microdamages in alveolar bone near PDL pressure sites that compromise osteocyte integrity, physically damaging the cells by oxidative stress, or by disruption of the blood flow and fluid flow in the lacunar–canalicular system.

This damaged tissue, together with local TNF-α and IL-1, can induce osteocyte apoptosis, which initiates bone resorption close to the microdamaged sites, as it upregulates the expression of RANKL, VEGF, and M-CSF and, consequently, modulates the osteoclast precursor recruitment and differentiation. Moreover, VEGF also indirectly induces bone resorption, as it promotes angiogenesis, allowing new capillaries to augment osteoclast precursors recruitment to the bone surface close to the resorption sites.

Not only RANKL, but also other cytokines (IL-β, TNF-α, IL-6, IL-11), GFs (FGF-2, EGF), and chemokines (CCL2, CCL3, CCL5, CCL7, CCL9, IL-8) can, directly or indirectly, increase osteoclast differentiation, survival, and activity. Moreover, in an amplified loop, CCL3 increases the expression of RANKL by osteoblasts. In parallel, RANKL induces production of CCL2, CCL3, and CCL5 by osteoclasts, suggesting an autocrine and paracrine signalization during osteoclastogenesis, and an increased bone resorption.
Bone Formation

In orthodontics, bone formation (Fig 3) begins 40-48 hours after force application in the PDL tension sites. Osteocytes participate in the process of osteogenesis, being acutely sensitive and responsive to applied tensile orthodontic forces. Their cellular projections facilitate communications with neighboring osteocytes, as well as with alveolar bone surface-lining cells and bone marrow cavity cells. Osteoblasts, which maintain direct contact with osteocytes, respond to these signals and initiate bone apposition.

Moreover, stretched PDL fiber bundles stimulate cell replication. Stem-cells (pericytes), migrated from blood vessel walls, and mesenchymal stem-cells differentiate into preosteoblastic cells 10 hours after force application. Chemokines, cytokines, and GFs are directly involved in this process. CCL3, CCL5, CXCL10, CXCL12, and CXCL13 induce osteoblast precursor recruitment, proliferation, differentiation, and survival. Local osteoblasts and osteocytes express GFs, such as TGF-β and IGF-1, that promote osteoblast precursors proliferation and differentiation, and mineralization of new bone by mature osteoblasts. In addition, BMP, EGF, and IL-11 upregulate osteoblast differentiation and function. In PDL tension sites, osteoblasts and PDL fibroblasts express VEGF, which stimulates angiogenesis, an important
Moreover, anti-inflammatory cytokines involved in bone formation, such as IL-10 and OPG, are produced by osteoblasts and inhibit osteoclastogenesis. To maintain the integrity of the PDL apparatus simultaneously with bone formation, TGF-β and IGF-1 stimulate proliferation and differentiation of osteoblasts and PDL cells, as well as collagen synthesis.

**Conclusions**

Chemokines, cytokines, and GFs are the main molecules involved in bone cell recruitment, activation, proliferation, differentiation, and survival. These molecules stimulate PDL and bone cells to orchestrate an inflammatory response, followed by osteoclastogenesis and bone resorption in compression sites, and by osteoblast and new bone formation at PDL tension sites. The research trend is now directed toward elucidating the molecular mechanisms involved in these events. Although several studies have investigated chemokines, cytokines, and GFs in OTM, it is difficult to establish a global vision that illustrates the specific role and the exact moment that these molecules participate in PDL formation.
and bone remodeling. Different experimental conditions, such as animal species, time and force magnitude, appliance design, and methods to detect the expression of these molecules, restrain the general comprehension of the cellular and molecular mechanisms involved in OTM. Nevertheless, current knowledge raises the possibility of local administration of substances that are able to act on specific cytokines, chemokines, and GF. These molecules can modulate the outcome of the application of orthodontic force, accelerating OTM, enhancing biological anchorage at specific sites, possibly decreasing the rebound effect, and assisting in the prevention of root resorption.

References

56. Andrade Jr, Taddei, and Souza