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INFLAMMATORIC MEDIATORS IN PERIAPICAL BONE LESION

GV Suyatna, EI Auerkari

Departement of Oral Biology Faculty of Dentistry University of Indonesia

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Abstract

The cytokines affecting periapical bone lesions have been reviewed for current understanding of the mechanisms and mediators involved. Periapical bone lesions can result from dental disease that spreads into the root canal and expands to the surrounding periapical tissue. This will create periradicular tissue inflammation, where cells like monocytes, PMNs and lymphocytes regulate and are regulated by cytokines responsible for inflammation. Periapical bone resorption can be caused by inflammation, by cytokines including IL-1, MMP-8, GM-CSF. Measuring of level MMP-8s from root-canal exudates during endodontic treatment could be used as a biochemical indicator of the inflammatory status of the periapical tissue. In addition, various cytokines such as TGF-β_1, IL-6, IL-10 promote bone proliferation. The variance in cytokine receptor expressions may explain the selective recruitment in the infiltration of inflammatory cells at the local inflammation site. Cytokines, each having their own duty, are key mediators of periapical bone resorption that still needs to be investigated in detail.

Keywords: Periapical lesion, cytokines

Introduction

In spite of a remarkably high degree of success, endodontic treatment can also fail. The failure of endodontic treatment may contribute to the etio-pathogenesis of periapical lesions.

Periapical lesion is most often developed as a reaction to pulp inflammation resulting from dental caries. Besides caries, endodontic procedures such as pulp extirpation, mechanical cleaning and shaping of the root canal, chemical root canal irrigating agents, antimicrobial agents, and root canal filling materials often generate localized periradicular tissue inflammation.[1,2] Periapical lesions are considered to be the result of a local inflammatory responses mediated by production of inflammatory mediators. However, the pathogenesis of this lesion is not clearly understood. Cytokines are thought to be key mediators that result in bone-resorption in periapical lesion. It is the purpose of the present study to discuss about the role of cytokines on the genesis of periapical lesions. This review will focus on how these inflammation spread and how their cytokines products affecting and contributing to the pathogenesis of periapical bone lesions.

Spread of inflammation from the pulp

Extension of pulp infection to the periapical tissue may begin soon after an area of necrosis has developed in the coronal pulp. Periapical immune

response may be viewed as the second line of defense, and the objective is to localize the infection within the confines of the root canal system and prevent its spread and systemization.[1,2]

The type of inflammatory lesion that develops in the periapical tissue resulting from pulp disease depends greatly on the virulence of the bacteria in the root canal and the status of the host defensive system. If the bacteria draw out a cell-mediated response, a granuloma will develop. On the other hand, if pyogenic bacteria are dominant, a large build up of neutrophils will cause suppuration, which will progress into an abscess.

Periapical lesion is also the most frequent lesion of the jaw, especially osteolytic lesion. It is thought to be caused by continuous antigenic stimulation from infected pulp. The granuloma lesion undergoes circumferential enlargement that destroys the surrounding bone, manifesting the pattern of bone resorption.[1,3]

Periapical granuloma

As soon as infection sets in the root canal, the causative bacterial populations will develop further, setting up an area that can be referred to as a "privileged sanctuary". Host defense cannot attack the bacteria, because the root canal lacks the blood vessels that are needed to transport the antibacterial agents. The body will try to establish a barricade at the opening of the apical foramen to fight the toxic substances produced by bacteria and dead pulp. In doing so, the body tries to prevent bacteria and toxic substances from crossing the root canal into the periapical tissue.

The predominant classes of antibodies produced by plasma cells in periapical granuloma are IgG (70%) followed by IgA (14%), IgM (4%), and IgE (10%). With so much IgG in the lesion, antigen-antibody complexes are undoubtedly formed. The role these immune complexes play in the development of periapical granulomas is unclear.[1]

As infection occurs at the periapical part of root canal, chemotactic stimuli attract neutrophils to migrate from neighboring vessels and gather around the foramen of the infected root canal. This event can cause an acute inflammatory development within a granuloma. If the bacteria in the root canal become virulent, there could be an increase of suppurative response, resulting in a growth of abscess within the granuloma.[1]

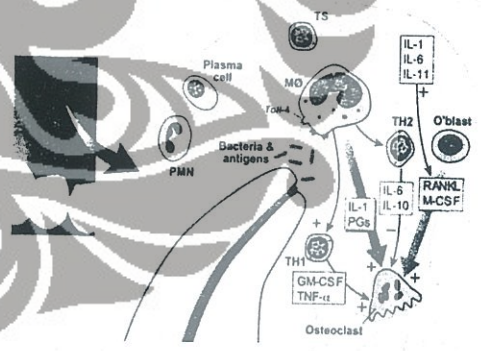
Pro-inflammatory cytokines

Antigenic substances continually spread from the infected root canal system into the surrounding periapical tissues. Once activated by bacterial components, macrophages and PMNs express a cascade of pro-inflammatory cytokines.[1,2]

The earliest periapical response, up to three days after pulpal exposure, shows an influx of PMNs and monocytes. About five days after lesion induction, the coronal pulp tissue may show periapical inflammation and bone resorption that is already detectable at this time. This indicates that:

1. Immune reactions in the pulp are insufficient to eliminate the continuous bacterial challenge.
2. Bacterial toxic substances, such as lipopolysaccharides, leak through the root apex and quickly affect the surrounding periapical tissue. Moreover, the periapical tissue reacts readily in pulpal infection and serves as a second line of defense to eradicate poisonous substances from the infected pulp.

Chemokines, including interleukin-8 (IL-8) and monocyte attractant peptide-∝ (MCP-1), are produced locally and are suspected to be involved in regulating PMN and monocyte infiltration. IL-8 also helps to mature PMNs for an elevated oxidative burst that is important in bacterial killing. Once activated, macrophages and PMNs express a cascade of pro-inflammatory cytokines, as shown in Fig 1.



PG : prostaglandin
GM-CSF : granulocyte-macrophage colony-stimulating factor
M-CSF : macrophage colony-stimulating factor
RANKL : receptor activator of NF-κβ-ligand
MO : macrophage

Fig 1. The role of pro-inflammatory cytokines in the root canal (Selzer & Bender) [2]

As a response to the presence of bacteria and antigens, macrophages induce Th1 and Th2 to produce cytokines[13]. Macrophage itself is a source of IL-1 and PGEs. IL-1 causes activation of mature osteoclasts by a PGE-independent mechanism, while stimulating osteoclast differentiation by a PGE-dependent mechanism[12].

Th1 produces TNF-∝ and GM-CSF. TNF-∝ is a potent stimulator of PGE production in bone cells, while GM-CSF induces osteoclast formation in the bone marrow. IL-6 is secreted in response to IL-1. IL-11, produced by stromal cells, and induces acute phase proteins.[13] IL-1, IL-6 and IL-11 induce RANKL and M-CSF, that can also be produced by osteoblasts. RANKL and M-CSF induce osteoclast formation in the bone marrow. In contrast, Th2 that produces IL-6 and IL-10, actually acts in roles suppressing bone inflammation and resorption.[2,12,13] The proximal members of this cascade include IL-1∝, IL-1β and TNF-∝. IL-1∝ and TNF-∝ may further stimulate each other's expression in an autoregulatory fashion. Downstream, cytokines IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are induced as secondary mediators. GM-CSF stimulates bone marrow for production of PMNs and monocytes, and also maturates PMN activation.[1]

Macrophages have been found in all phases of induced rat periapical lesions. Macrophages increased considerably during the active bone resorption but decreased when the growth of the lesion stopped. These results suggest that infiltrated macrophages play important roles in the initiation and development of periapical lesions.[3]

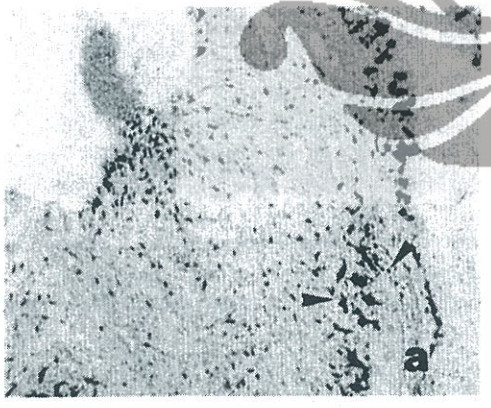


Fig 2. Immunolocalization of macrophages in the peri-apical lesion at day 20 after induction; macrophages aggregate around the root apex and in the areas with active bone resorption (arrowheads).[3]

IL-1∝, IL-β, TNF-∝, and TNF-β stimulate bone resorption by osteoclast

Bone resorption in human periapical lesions is largely mediated by the pro-inflammatory cytokines[1,4] such as IL-1∝,IL-1β,TNF-∝,TNF-β lymphotoxin), and prostaglandin E_2. Together these mediators form the activity previously called osteoclast-activating factor (OAF), or so called bone-resorbing activity. In humans, IL-1β provides most of the OAF activity. IL-1β has been shown to be almost 500-fold more potent than TNF in stimulating bone resorption.

IL-1 appears to play a crucial function in stimulating periapical bone resorption by osteoclasts.[2,5,12] The expression and activity of IL-1 is in turn modulated by a network of Th1 and Th2 regulatory cytokines.[5] The highest activity is during the active phase of lesion expansion (3 to 14 days after pulp exposure). This is further emphasized by results from research on periodontal disease. Treatment of monkeys with soluble IL-1 receptors (which block IL-1 activity) reduces periodontal bone loss by about 70%.[2,12] Surprisingly, mice deficient in the primary receptor for IL-1 or both receptors for TNF-∝, p55-p75, were reported to have greater periapical bone resorption than wild-type controls

This means that IL-1 and TNF-∝ may actually mediate functions that play a part in the protection of pulpal and periapical tissue, especially at early stages of infection. The reason is that both IL-1 and TNF-∝ can stimulate PMN migration to the area of infection, and mature PMN to heighten its bactericidal reaction. However, after the lesion grows, the effect of IL-1 is obviously destructive. Nonetheless, an increase of infiltration of PMNs and monocytes has been found in animals deficient in both IL-1 and TNF receptors.[2]

Pro-inflammatory cytokines, particularly IL-1 and TNF-∝, are potent inducers of MMPs, and often synergistic

Activated macrophages, fibroblasts, neutrophils, and epithelial cells produce a family of enzymes known as metalloproteinases that are dependent on zinc for their activation. IL-1 appears to play a crucial function in stimulating periapical bone resorption by osteoclasts.[2,5,12] The expression and activity of IL-1 is in turn modulated by a network of Th1 and Th2 regulatory cytokines. They

are present through both normal and pathologic remodeling phases of tissue, such as during embryonic development, tumor invasion, etc. MMPs form a group of structurally related but genetically distinct endopeptidases expressed at low levels in normal tissues, but up-regulated during inflammation.[4,6]

MMPs are also important in other inflammatory lesions such as periodontitis and possibly pulpitis. In normal situation, fibroblast produces and maintains collagen and extracellular proteins. At the onset of periodontitis, the genes for collagen production are switched off while the genes for metalloproteinase are switched on. This condition will be reversed if the periodontitis is successfully treated.[4,6]

When early opinions of connective tissue remodeling were developed 30 years ago, only one collagen type (type 1) and one collagenase (MMP-1) were known. By 1998, it has grown to 17 identified MMPs. This clearly shows that microbial colonization could cause tissue destruction by at least four markedly different mechanisms. These include:

1. Release of matrix-degraded enzymes (including collagenases);
2. Initiating an immune response that results in activation of the T-cell/ macrophage, release of cytokines and up-regulation of MMP expression by local and transient cells;
3. Release of factors (including LPS) that immediately activate the transcriptional machinery in host cells for expression of degradative enzymes; and
4. release of factors (including proteolytic enzymes) which activate or destroy host cell effector systems (coagulation and complement components, MMPs, IgA).

Sometimes certain microorganisms can infect host cells and use the host cell signaling and effector utilities while in the same time seeking protection from the immune response.

MMPs have an important part in bone resorption process because MMPs can recruit osteoblasts and make them migrate without directly taking part in the dissolution of collagenous bone matrix. Osteoclasts express high levels of gelatinase B (MMP-9), metalloelastase (MMP-12), and MT1-MMP (MMP-14). Synthetic MMP inhibitors can inhibit true MMPs. Thus, they can disable the recruitment and migration of osteoclast or its precursor and indirectly prevent bone resorption without interfering with the collagen-degrading enzymatic machinery of the cells.[4]

According to Teronen etal, collagenases (MMP-1 and MMP-8) and gelatinases (MMP-2 and MMP-9) are present in jaw cyst wall extracts and cyst fluids, but no data exists of their role in pulpal or periapical inflammation. Previously, it has been thought that MMP-8 is only produced by developing PMN cells in bone marrow, and that the MMP-8 activity depends on the release of the enzyme from degranulation of PMN cells. However, recent findings have shown that the expression of MMP-8 exists in mesenchyme, non-PMN lineage cells, including dental pulp fibroblasts, odontoblasts, inflamed epithelial cells and plasma cells. The precise function of MMP-8 synthesized by the cells in the pulpo-dentinal complex is not clear. Therefore, a hypothesis was proposed that MMP-8 could be present in the inflamed or necrotic pulp, and the enzyme level could be related to the activity of tissue destruction of the pulp and periapical tissue.[6]

In one study [6], the western blots of teeth with acute pulpitis demonstrated large amounts of MMP-8 in the pulpal tissue. Whilst most of the MMP-8 observed in the Western blot could have mainly originated from the PMN cells numerously present in the inflamed tissue, the immunohistochemical staining revealed that other cells can also express MMP-8 in the inflamed human pulpal tissue.

In the pulp proper macrophage-like cells PMN and plasma cells have showed a clear expression of MMP-8, and the most intense MMP-8-positive cell accumulations has been observed surrounding the pulpal abscess. Also some endothelial cells of the pulp vessels have been found positive in MMP-8 staining.

The western immunoblot, jointly with immunohistochemical staining with MMP-8-specific antibody, has showed that MMP-8 (collagenase-2) is present in an inflamed pulp tissue in abundant levels. Most of the MMP-8 in pulpitis eventually originated not only from PMN leucocytes, but also from the other cells, such as macrophage-like cells and plasma cells. Because of that, pulp seems not to differ from other tissues such as periodontium influenced by inflammatoric assault.

Regarding the fact that odontoblasts and pulpal fibroblasts can express MMP-8 as well as other MMPs, the cells of the pulpo-dentinal complex may take part in the matrix remodeling and degradation during the inflammatory processes. Undeniably, interleukin-1∝ (IL-1∝), tumor necrosis factor-∝

(TNF-∝) and bacterial lipopolysaccharides can stimulate gelatinase expression in pulpal fibroblasts in vitro. Recent studies also show increased expression of collagenase-1 (MMP-1) in cultured human pulpal fibroblasts after IL-1α and TNF-∝ stimulation, and at the same time, prostaglandin E2 (PGE_2) stimulates the expression of tissue inhibitor of metalloproteinases-1 (TIMP-1). [3]

Macrophages express a variety of MMPs, including collagenase-1 (MMP-1), gelatinases (MMP-2,MMP-9), stromelysins (MMP-3, MMP-7, MMP-12) and collagenase-3 (MMP-13). The role of collagenases from macrophages may be linked to the idea that macrophage-derived MMPs could have the capability to discharge growth factors from extracellular matrix to control inflammatory reaction or only breakdown of extracellular matrix at the site of inflammation.

In the root-canal treatment, a decrease of MMP-8 in root-canal exudates will occur. The existence of MMP-8 at the second visit, even though the pulp tissue has been removed and the canals thoroughly cleaned during the first appointment, strongly indicates that MMP-8 originates from the periapical inflammation site. This could imply that the destruction of the extracellular matrix proteins shows that the inflammation is still active. Absence of MMP-8 in the root-canal exudates at the third visit would further indicate that the inflammation has gone and that the healing process has started. This is also supported by the finding that a case failing to show low MMP-8 level at this time was later diagnosed to be vertically fractured.

Thus, it can be concluded that measuring the presence and levels of MMP-8 from the root-canal exudates during the endodontic treatment could serve as a biochemical indicator for monitoring the inflammatory status of the periapical tissue. Therefore, it might be used as a diagnostic guide in deciding the treatment procedures, modalities and medication. Research in animal models shows that specific MMP inhibitors alone or in combinations have considerably decreased oedema and inflammatory tissue damage, suggesting that these inhibitors could be useful in the healing process.

MMP inhibition has also been suggested to decrease bone resorption in pathological conditions and dentinal caries progression.[6]

Il-1 is partly dependent on PGE_2 synthesis

Products of arachidonic acid metabolism have also been correlated with pulpal and periapical inflammation. Prostaglandin raises vascular permeability and stimulates bone resorption. PGE_2 is directly responsible for 10 to 15% of total bone-resorbing activity in extracts of rat periapical tissues. However, apparently about 60% of the bone-resorbing activity stimulated by IL-1 can be inhibited by indomethacin. This finding shows that IL-1 induced resorption is partly dependent on PGE_2 synthesis by cells present at the inflammatory sites.[2,12]

The participation of PGE_2 in periapical destruction in vivo is shown by indomethacin-treated rats displaying milder inflammation and less bone resorption than controls. Paradoxically, infusion of PGE_2 in vivo has actually been found to boost bone formation rather than to stimulate bone resorption, and inhibitors of PGE_2 production (such as ibuprofen) inhibit fracture repair.

Prostaglandin appears to act largely together with other mediators. This could explain their main contribution in boosting the sensitivity of osteoblasts and osteoclasts to other signals, regardless whether they actually stimulate bone resorption, as in case of pro-inflammatory cytokines, or stimulate bone formation like in case of growth factors.[2]

Regulation of pro-inflammatory cytokines: the cytokine network

The resorption and apposition of the bone can happen at the same lesion. This suggests that osteoblasts and osteoclasts are being stimulated together. This illustrates that destruction and repair work together, especially in chronic inflammation. The direct invasion of bacteria is avoided by the withdrawal of the bone tissue.[1]

Cytokines originating from CD4-positive T-cells are thought to be involved in bone resorption and formation. CD4-positive cells are categorized as helper T cells (Th), Th1 and Th2 subsets, by cytokine-producing profiles.[7]

Th1 and Th2 cells represent distinct subsets that may be discriminated by gene expression profile and function. Mediators produced by Th1 cells increase inflammation and IL-1 expression, whereas Th2 cell-derived cytokines generally decrease it .[2,8] Th1 subset synthesizes interferon (IFN-γ, IL-2 and TNF-∝, while Th2 subset synthesizes IL-4, IL-5,IL-6, IL-10 and IL-13.[2,7]

The Th2-type cytokines IL-4 and IL-10 have been reported to down-regulate the production of IL-1 and Th1-type cytokines. IL-4 and IL-10 also suppress bone resorption in vitro.[8]

Chemokines are believed to be among the inflammatory factors that are important in mediating the extravasation and accumulation of selective leukocyte subsets in the process of inflammation. It is suggested that the variance in chemokine receptor expressions might be the explanation to the selective recruitment in the infiltration of inflammatory cells at the local inflammation site. The chemokine receptors CXCR3 and CCR5 were expressed on Th1 cells, and CCR3 and CCR4 on Th2 cells, whereas CCR1, CCR2, and CCR5 were expressed on monocytes/ macrophages.[7]

Th1 (CD4) subset synthesizes interferon (IFN-γ, IL-2 and TNF-∝

Adaptive immune responses are also thought to play a role in local control of bone remodeling, and $CD4^+$ T lymphocytes are the source of cytokines that can induce net bone resorption in vitro. Only the intricacy of the process in human has unsettled the question of whether the illness is an outcome from inadequate immune response, or because the response from our body itself is destructive.

Instead of $CD8^+$ T-cells, it is $CD4^+$ that is associated with bone resorption in response to oral infection. At least two $CD4^+$ T-cell cytokines, IFG and IL-6, are also associated with bone loss. While the immune system is reacting to bacterial infection, the cytokines that it secretes may influence the balance of resorption and deposition that forms bone remodeling, resulting in enhanced resorption. Mice lacking the cytokine gamma interferon or interleukin-6 have also demonstrated decreased bone loss.

In one study, severe combined immunodeficient mice, which lack B and T lymphocytes, showed considerably less bone loss than did immunocompetent mice after oral infection, suggesting that lymphocytes contribute to this process. Bone loss after oral infection was decreased in mice deficient in major histocompatibility complex (MHC) class II-responsive $CD4^+$ T-cells, but no change in bone loss was observed in mice deficient in MHC class I-responsive $CD8^+$ T-cells or $NK1^+$ T-cells. Mice lacking the cytokine gamma interferon or interleukin-6 also demonstrated decreased bone loss. These results suggest that the adaptive immune response, and in particular $CD4^+$ T-cells and the pro-inflammatory cytokines that they secrete, are important effectors of bone loss consequent oral infection.[9]

Recently the involvement of interleukin (IL)-1 and tumor necrosis factor (TNF) in apical periodontitis has also been demonstrated with subsequent periapical bone resorption. One of the cytokines produced during tissue injury and by inflammatory cells that have been exposed to bacteria and their products is transforming growth factor-β_1 (TGF-β_1). It has pro-inflammatory properties, such as recruitment and activation of neutrophils, monocytes and T-cells. Furthermore, TGF-β_1 can add expansion of the inflammatory system by inducing FcγRIII (CD16) expression on blood monocytes and initiating the production of bone-resorptive cytokines, such as IL-1, TNF and IL-6. TGF-β_1 has also been recognized in many inflammatory osteolytic diseases, such as rheumatoid arthritis and periodontitis.

As the development of the lesion decreases, the amount of TGF-β_1 positive macrophages is also reduced. TGF-β_1 positive bone cells have been detected at days 30, 60, and 80 after pulp exposure, but not at day 5 to day 20.

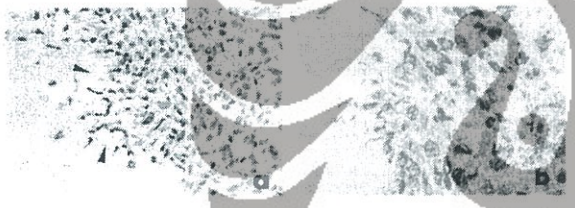
Immunohistochemical studies demonstrate that TGF-β̃positive macrophages distributed around the root apex and periapical areas show bone resorption during the active lesion phase. TGF-β_1–positive osteoblasts have been detected during the chronic stage (days 30, 60, and 80 after pulp exposure). Histologically, TGF-β_1 positive osteoblasts have a large, round nucleus with abundant cytoplasm and are found close to the area of reparative bone formation. It has been shown that the lipopolysaccharide of *Fusobacterium nucleatum*, one of the microorganisms associated with root canal infection, enhances the secretion of TGF-β_1 by monocytes. TGF-β_1 has pro-inflammatory properties, such as recruitment and activation of neutrophils, monocytes, and leukocytes.

In addition, TGF-β_1 can stimulate mononuclear cells to synthesize IL-1, TNF, and IL-6, all of which are powerful osteolytic mediators. TGF-β_1 is also a powerful chemoattractant for osteoblasts and can increase rat calvarial and human osteoblasts. It has also been reported that TGF-β_1 can inhibit osteoclast formation or osteoclastic bone resorption and proliferation. TGF-β_1 may play dual roles in both bone resorption and deposition in induced rat periapical lesions.

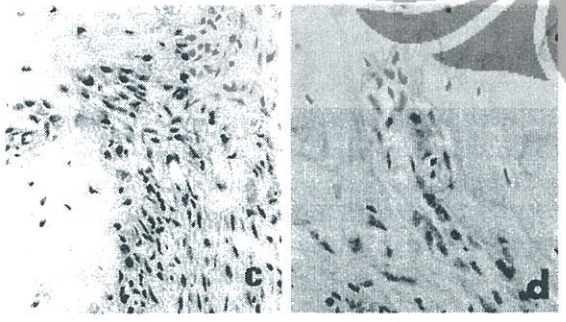
A large number of TGF-β_1 positive macrophages have been detected either around the root apex or in the surrounding area of the osteolytic sites during the active bone destruction phase.[3] The number of TGF-β_1 positive macrophages dropped as the rate of bone resorption declined. As a whole, it could be that the continuous bacterial influx from infected root canal may activate an immense production of TGF-β_1 from macrophages, resulting in the unregulated recruitment and activation of inflammatory cells, which are the accumulation of bone resorptive cytokines.

TGF-β_1 produced by osteoblasts could work as a stimulator to new bone formation and prevent unnecessary tissue breakdown in periapical lesions. Meanwhile, presence of TGF-β_1 was not detected in osteoblasts at day 5 to day 20 after pulp exposure. This could be because of suppressed production due to a high local level of TGF-β_1 released by macrophages, or an indication that osteoblasts are not activated at that period.[3]

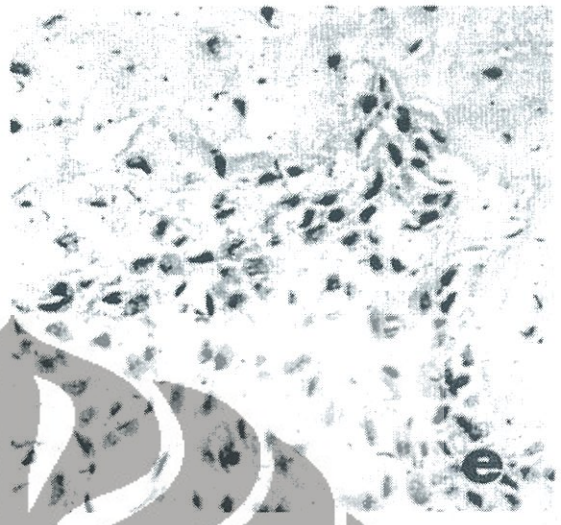
The figures below are taken from JOE vol26 no 6, june 2000.



a. many TGF-β_1 positive mononuclear cells were found around the root apex (day 10)
b. a section from picture (a) demonstrated the mononuclear, TGF-β_1 positive cells were macrophages



c. many TGF-β_1 positive osteoblasts were located along the outer surface of an osteoid layer (day 60).
d. Section of (c) showed marked osseous reversal lines around the TGF-β_1 positive osteoblasts.



e. osteoblasts in the active bone destruction phase were TGF-β_1 negative and in a fusiform shape with scant or little cytoplasm. This is at day 20.

Th2 subset synthesizes IL-4, IL-5, IL-6, IL-10 and IL-13[2,7]. IFN-γ-positive cells have been shown to be present in human granulomatous tissues and IL-4-producing cells in human periapical regeneration tissues. IFN- γ and IL-4 hold back bone resorption through reduction of osteoclasts. IFN- γ enhances the expression of cell adhesion molecules on vascular endothelial cells in vitro and induces differentiation of naïve CD4-positive T-cells into effector Th1 cells, while IL-4 leads to Th2 cell development.[7,8]

IFN-$\gamma^{-/-}$ mice showed significant elevations in IL-6, IL-10, IL-12, and TNF-α in lesions compared to wild-type mice, but these transformations have made no difference in IL-1α levels. Each recombinant IL-12, IL-18, and IFN-γ separately have failed to steadily transform macrophage IL-1α production in vitro. It can be concluded, at least individually, that endogenous IL-12, IL-18, and IFN-γ do not have a significant effect on the pathogenesis of infection-stimulated bone resorption in vivo, suggesting possible functional redundancy in pro-inflammatory pathways.[5]

There are several possible explanations for these findings. One of them is that, in addition to their pro-inflammatory effects, IL-12, IL-18, and IFN-γ also suppress the receptor-activator of NF-κβ ligand (RANKL)-induced osteoclast differentiation and mature osteoclast function. The effect of IFN-γ is apparent at picomolar concentrations and involves

accelerated degradation of the RANK adaptor TNF receptor-associated kinase 6. Although IL-12 does not act directly on osteoclast precursors, it can indirectly reduce RANKL-induced osteoclast differentiation alone or collaboratively with IL-18. IL-18 could also be produced by osteoblasts, enhancing granulocyte-macrophage colony-stimulating factor production by T-cells. Granulocyte-macrophage colony-stimulating factor signaling in osteoclast precursors inhibits RANKL-induced osteoclast differentiation in an IFN-γ-independent manner. It could be that the relative lack of result seen in deficiencies of IL-12, IL-18, or IFN-γ on inflammatory resorption may be a sign of the balance of two opposing processes: the loss of osteoclast inhibitory activities against the reduction in pro-inflammatory signals.[5]

Using knockout animals, the effect of a lack of the prototype Th2 anti-inflammatory cytokines IL-4, IL-6 and IL-10 has been determined. The results show that IL-10 deficiency, similarly to that of IL-4, can cause increased periapical destruction, whereas IL-6 shows only about 50% of the destruction induced by IL-10. Clearly, IL-10 has a strong inhibitory effect on periapical bone destruction, while IL-4 has no effect on bone resorption in vitro.[2]

Both IL-10-deficient and IL-6-deficient mice show increased local IL-1 production and increased infection-stimulated bone resorption in vivo. In the case of IL-10 deficiency, the increase in locally produced IL-1∝ production can be dramatic (more than 10-fold) and associated with severe bone resorption. Thus these Th2 mediators have major and non-redundant roles in suppressing bone inflammation and resorption in vivo. On the other hand, IL-4, another Th2 mediator, has shown no effect on resorption, indicating functional heterogeneity in this cytokine group. Altogether, it can be suggested that Th2 cytokines are better for immunomodulation than Th1 and Th1-cytokine-inducing cytokines in preventing inflammatory bone loss.[5,8,10]

IL-4 and IL-10 have been reported to inhibit IL-1- and TNF-∝-stimulated bone resorption in vitro. IL-1 stimulates resorption both directly and indirectly through production of cyclooxygenease-2 by osteoblasts. Both mediators inhibit bone resorption by suppressing the synthesis of cyclooxygenease-2-dependent prostaglandin E2. Both IL-4 and IL-10 also inhibit recruitment of osteoclast precursors and their differentiation to mature multinucleated osteoclasts. There was little distinction reported in the potency of resorption inhibition between IL-4 and IL-10, suggesting that the differential effects of these mediators on infection-stimulated resorption in the present study is not exerted on bone cells.[8]

However, it has also been shown that IL-10 suppresses IL-1∝ production by macrophages in a dose-dependent fashion in vitro, while IL-4 has a faint and inconsistent outcome.[8] The conclusion is that IL-10, but not IL-4, is the important endogenous suppressor of infection-stimulated bone resorption in vivo, likely acting via inhibition of IL-1∝ expression.[8]

IL-6 and IL-1 also have been reported to increase bone resorption[5,9,8], but this effect is probably inferior to their ability to stimulate osteoclast formation from precursor stem cells rather than to activate pre-existing osteoclasts.[2]

In IL-10$^{-/-}$ mice, IL-6 levels can also significantly increase in local inflammatory tissues, while the levels of IL-4 are unaffected. Although often considered to be a pro-inflammatory mediator, it is concluded that the predominant effects of IL-6 may in fact be Th2-like and anti-inflammatory. IL-6 is induced by IL-1, but can act as an response inhibitor by lowering IL-1 transcription. IL-6$^{-/-}$ also shows an increase in periapical bone destruction, but less severely than in IL-10$^{-/-}$ mice.[10] Therefore, increased expression of IL-6 may represent a compensatory mechanism that is partly useful in reducing inflammation in the absence of IL-10.[8]

The role of IL-6 is interesting because it is often considered to be pro-inflammatory.[9] IL-6 increases osteoclastogenesis, and its absence might be expected to decrease osteoclast formation as well as resorption. However, IL-6 also possesses many anti-inflammatory effects and is consistent with its category as Th2 cytokine.[2]

TRANCE or RANK-L may play a critical role in the development of osteoclasts that results in bone resorption

A new secreted glycoprotein ligand known as receptor activator of NF-κβ ligand (RANK-L) has been identified as a potential osteoclast differentiation factor. It is also known as TRANCE and ODF.[5,11] Colony stimulating factor 1 (CSF-1) together with TNF-related activation-induced cytokine (TRANCE) is vital for stromal cell mediation of osteoclastogenesis.[2,5,11]

RANK-L expression can also be increased by the bone resorbing factors, IL-11, PGE_2 and PTH.[5] Studies have shown that a T cell-derived factor, RANK-L, plays a crucial role in osteoclastogenesis.[11]

It has been shown that lesions with various degrees or radiographic bone loss are associated with marked RANK-L detection.[11] By contrast, no RANK-L mRNA signal has been detected in corresponding normal or negative control specimens. Curiously, in other experiments only minimal RANK-L expression has been found in the periodontal ligament of orthodontically extracted teeth. RANK-L may therefore play a regulatory role on osteoclasts without any impact on bone resorption.[11]

CSF (Colony Stimulating Factor) and GM-CSF (Granulocyte-Macrophage Stimulating Factor)

Differentiation of osteoclast will only occur if the appropriate microenvironment (such as the presence of CSF-1) is created by osteoblasts or stromal cells. CSF-1 is synthesised by osteogenic cells and can be modulated by PTH and bone resorbing cytokines, such as IL-1 and TNF-∝. This cytokine is also essential in osteoclastogenesis.

GM-CSF stimulates the growth of granulocytes and macrophages. It is produced by T-lymphocytes, endothelial cells, macrophages, stromal cells, fibroblasts and osteoblasts. GM-CSF acts similarly as IL-3, i.e. in high concentrations it increases the growth of early multipotential progenitor cells at early stages. But GM-CSF is not a compulsory component for osteoclast development, as is the case of CSF-1. GM-CSF has been shown to stimulate bone resorption, probably as an indirect effect related to the immune system. Macrophages activated by GM-CSF may release prostaglandins and cytokines stimulating bone resorption.[12,13]

Conclusion

Pulpal infections and periapical inflammation depend on the elements of innate and specific immunity. There are key mediators of periapical bone resorption that still need to be investigated in detail. This is required so that the destructive and protective components of this system can be identified for improved understanding and therapeutic strategies.

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