Mediators of acute and chronic periradicular lesions

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Multiple mechanisms are involved in the pathologic changes associated with formation of acute and chronic periradicular lesions. Mechanical injury to the periradicular tissues can cause activation of several pathways of inflammation and release of nonspecific mediators. Continuous irritation of periradicular tissues can cause activation of several pathways of inflammation and release of nonspecific mediators. Continuous egress of antigens from a pathologically involved root canal can also result in one or a combination of the various types of immunologic reactions. A number of these reactions participate in the destruction of periradicular tissues. Because of complex interactions between the various components of these systems, the dominance of any one pathway or substance may be difficult to establish. **(ORAL SURG ORAL MED ORAL PATHOL 1994;78:511-21)**

Mechanical, chemical, or bacterial irritation of the dental pulp usually results in inflammatory changes in this tissue. Mild-to-moderate injuries of short duration cause reversible tissue damage and recovery of dental pulp. In contrast, persistent or severe injuries to the dental pulp usually cause irreversible pulpitis and pulpal neurosis. Egress of microorganisms, their by-products, and altered host tissues from infected root canals into the periradicular tissues can initiate formation and perpetuation of periradicular lesions. Radiographically these lesions appear as radiolucent areas around the portal(s) of exit of the main canal or lateral or accessory canals. Depending on their stage of development, histologic examination of periradicular lesions reveals numerous inflammatory cells such as polymorphonuclear (PMN) leukocytes, macrophages, lymphocytes, plasma cells, mast cells, basophils, and eosinophils. The interaction between the antigens (invaders) and the host defensive (defenders) mechanisms results in release of numerous mediators that curtail progression of infection and development of severe local infection (osteomyelitis) and systemic complication such as septicemia. Numerous studies have been conducted within the past 25 years to elucidate the reactions and mediators of pathogenesis of human periradicular lesions. These investigations have shed some light on the pathologic reactions and pathways responsible for periradicular bone resorption. The purpose of this article is to present an up-to-date overview of inflammatory reactions that can take place during development and progression of acute and chronic human periradicular lesions.

An interaction between exogenous irritants and defensive host cells can result in release of a number of endogenous chemical mediators such as neuropeptides, fibrinolytic peptides, kinins, complement fragments, vasoactive amines, lysosomal enzymes, cytokines, and mediators of immunologic reactions.

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NEUROPEPTIDES

Neuropeptides are proteins generated from somatosensory and autonomic nerve fibers after tissue injury. A number of neuropeptides have been characterized, including substance P (SP), calcitonin gene-related peptide (CGRP), dopamine-@-hydrolase, neuropeptide Y originating from sympathetic nerve fibers, and vasoactive intestinal polypeptides (VIP) generated from parasympathetic nerve fibers.[1]

SP is a neuropeptide present both in the peripheral and the central nervous systems. The release of SP can cause vasodilation, increased vascular permeability, and increased blood flow during inflammation. In addition, it can cause the release of histamine from mast cells and potentiate inflammatory responses.

CGRP has been localized in small-to-medium sensory nerve fibers. Like SP, it is a potent vasodilator and may play a role in the regulation of blood flow in bone, periosteum, and other sites.

SP was the first neuropeptide to be detected in dental pulp,[2] and CGRP was demonstrated in dental pulp almost 10 years later.[3-5] A number of studies have shown that sectioning of the inferior alveolar nerve results in the complete disappearance of SP- and CGRP-containing granules from nerve fibers, which suggests that these substances originate from the sensory fibers of the trigeminal ganglion.[3-7]

VIP, which was originally extracted from porcine duodenum, appears to be a stimulator of bone resorption. Hohmann et al.[8] have shown that VIP stimulates bone resorption via a prostaglandin (PG)-E_2-independent pathway. Furthermore, they have also shown the presence of functional receptors for VIP on human osteosarcoma cells.[9] VIP has been reported to be present in the dental pulp.[1] Sectioning of the inferior

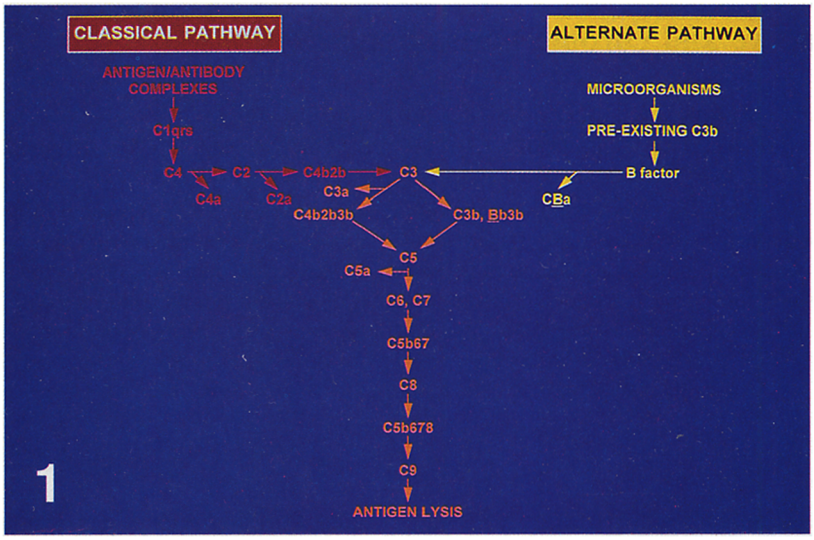

Fig. 1. Classic and alternative pathways of complement system.

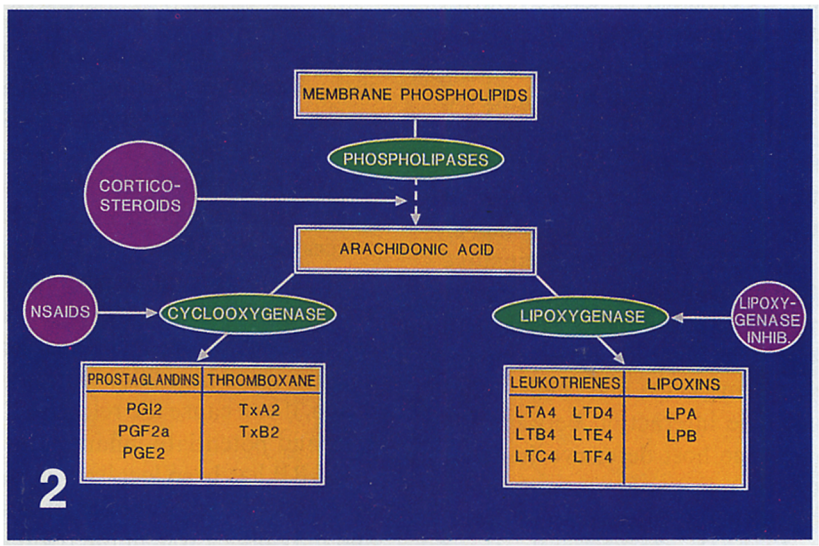

Fig. 2. Pathways of arachidonic acid metabolism.

alveolar nerve or sympathectomy did not result in the disappearance of VIP-containing granules from nerve fibers, which indicates that VIP is of parasympathetic origin.[1]

The presence of these neuropeptides has been clearly demonstrated in pulpal tissues, however, the role of neuropeptides in pathogenesis of periradicular pathosis has not been fully elucidated.

FIBRINOLYTIC PEPTIDES

Activation of the Hageman factor (blood clotting factor XII) results in the stimulation of the clotting and fibrinolytic cascades as well as kinin and complement systems. Major activators of the Hageman factor are: glass, kaolin, collagen, basement membrane, cartilage, sodium urate crystals, trypsin, kallikrein, plasmin, clotting factor XI, and bacterial lipopolysaccharides.[10]

After a tissue injury, circulating platelets immediately adhere to the subendothelial collagen and form a primary platelet plug. This initial hemostasis is followed by the coagulation cascade that can involve both an intrinsic pathway and exposure of coagulation factor XII to negatively charged collagen and an extrinsic pathway and activation of factor VII.

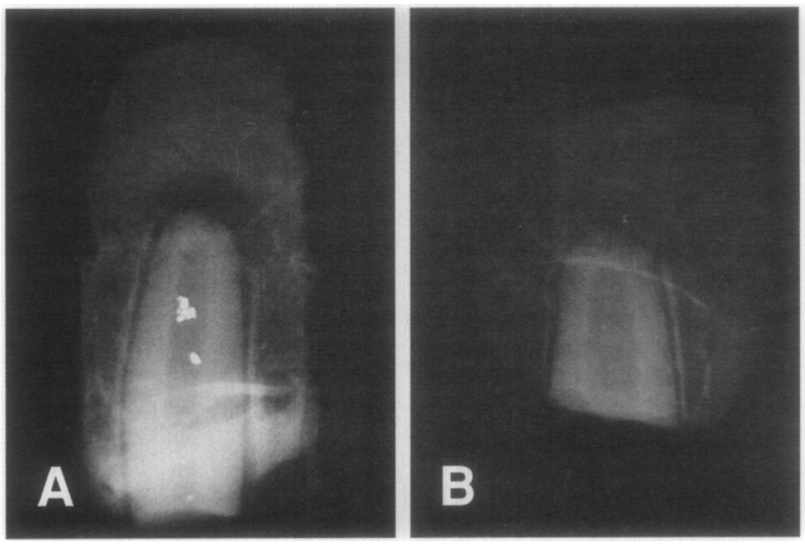

Fig. 3. **A,** Formation of lesion in periradicular tissues of feline tooth after 3 weeks of deposition of immune complexes in root canal. **B,** Deposition of saline solution in root canal of contralateral cuspid did not cause periradicular lesion.

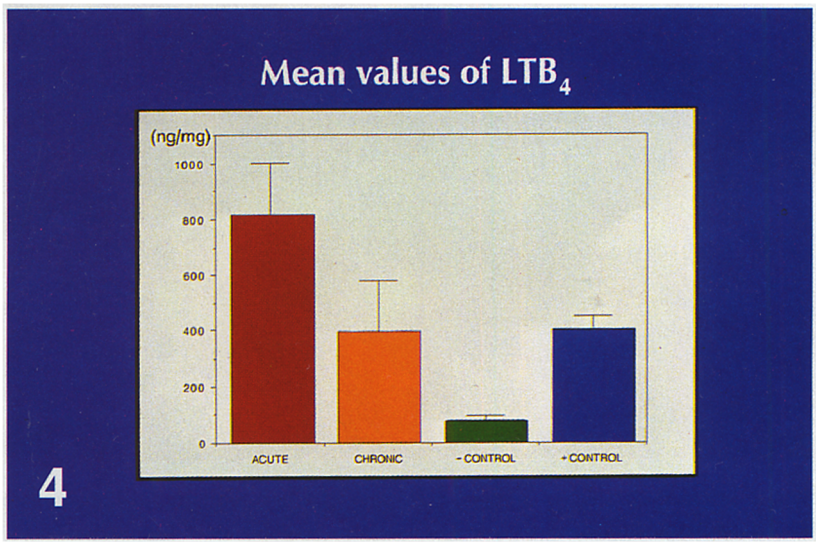

Fig. 4. High concentration of LTB4 was found in acute periradicular lesions.

As hemostasis occurs, the fibrinolytic system is activated, which causes the newly formed blood clot to dissolve. Circulating plasminogen is activated to plasmin (fibrinolysin) by the action of Factor XIIa or by a tissue factor.[10] Plasmin digests the clot and forms fibrin and fibrinogen degradation products. Release of fibrinopeptides and fibrin-degradation products result in increased vascular permeability and chemotaxis of leukocytes at the site of injury.[10]

Severance of the blood vessels in the periodontal ligament or bone during root canal instrumentation can activate intrinsic as well as extrinsic coagulation pathways. Contact of the Hageman factor with the collagen of basement membranes, with enzymes such as kallikrein or plasmin, or with endotoxins from infected root canals can activate the clotting cascade and the fibrinolytic system. Fibrinopeptides released from fibrinogen molecules and fibrin-degradation products released during the proteolysis of fibrin by plasmin can contribute to the inflammatory process.

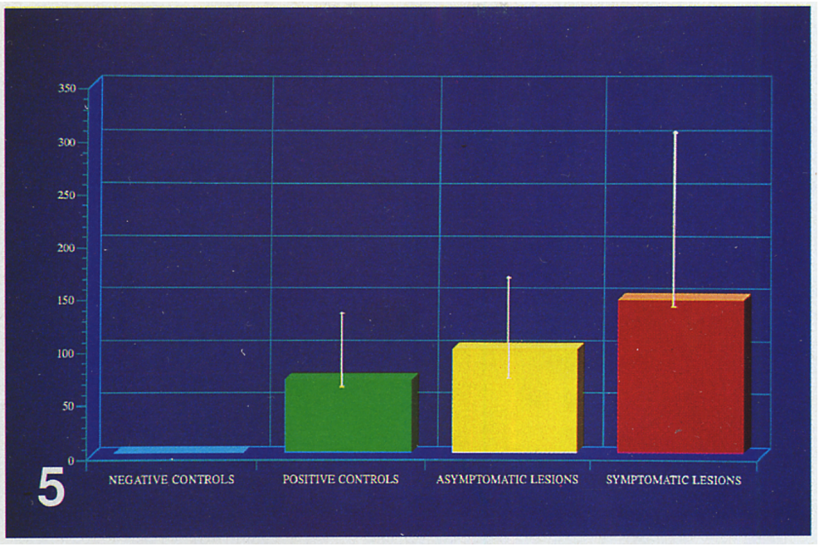

Fig. 5. Concentration of IL-1β in symptomatic and asymptomatic lesions.

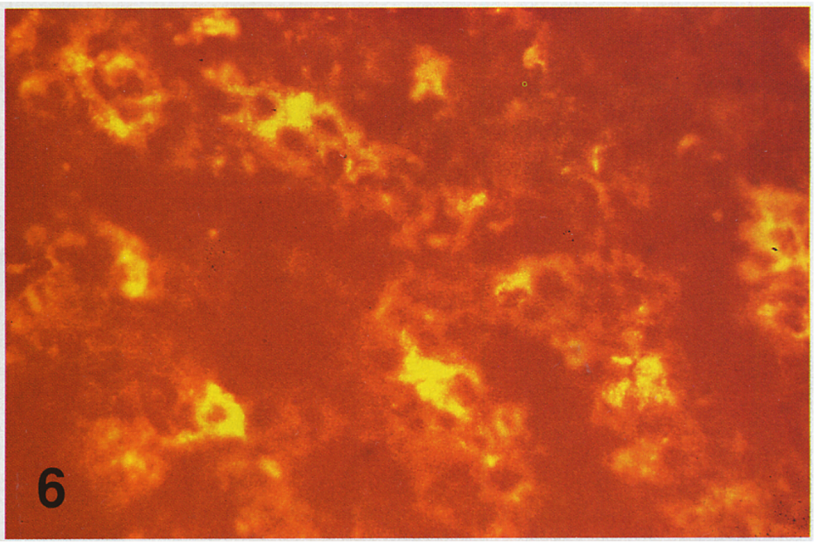

Fig. 6. Detection of immune complexes in phagocytic cells of a human periapical lesion using anticomplement immunofluorescence technique.

KININS

Release of kinins produces many signs of inflammation.[11] They can cause chemotaxis of inflammatory cells, contraction of smooth muscles, dilation of peripheral arterioles, and increased capillary permeability. They are also able to cause pain by direct action on the nerve fibers. The kinins are produced by proteolytic cleavage of kininogen by trypsin-like serine proteases, the kallikreins. The kinins are subsequently inactivated by removal of the last one or two C-terminal amino acids by the action of peptidase.[12] The kallikreins are also able to react with other systems, such as the complement and coagulation systems, to generate other trypsin-like serine proteases.[13] Elevated levels of kinins have been detected in human periradicular lesions.[14]

COMPLEMENT SYSTEM

The complement system consists of a number of distinct plasma proteins capable of interacting with each other and with other systems to produce a variety of effects.[15] Complement is able to cause cell lysis if activated on the cell membrane and also to enhance phagocytosis through interaction with complement

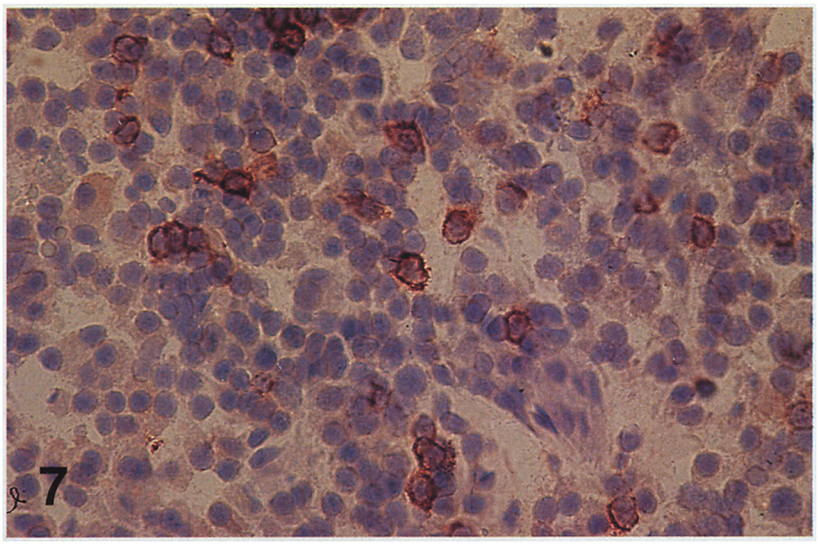

Fig. 7. Presence of numerous T lymphocytes (red cell membrane) in human periradicular lesion.

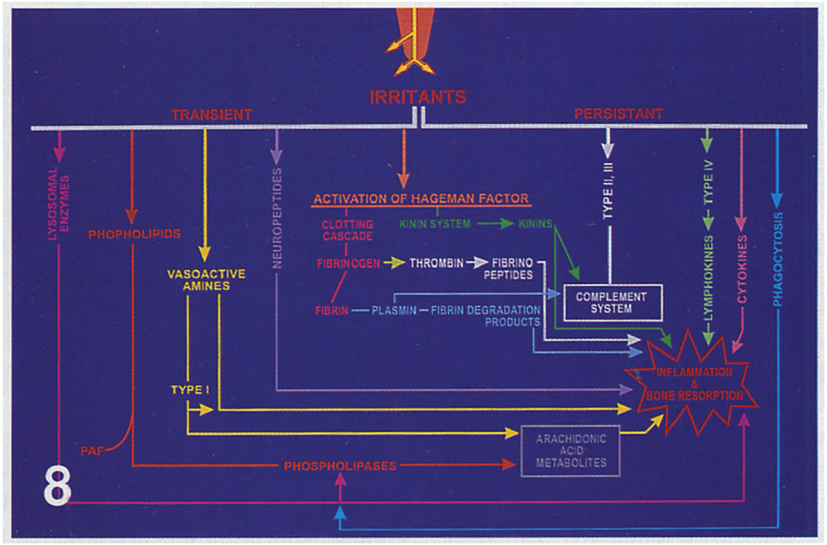

Fig. 8. Various pathways of inflammatory reactions after egress of irritants from infected root canals into periradicular tissues.

receptors on the surface of phagocytic cells. Complement can also increase vascular permeability and act as a chemotactic factor for granulocytes and macrophages. The complement system is a complex cascade that has two separate activation pathways that converge to a single protein (C3) and complete the cascade in a final and common sequence (Fig. 1). Complement can be activated through the classic pathway by antigen-antibody complexes or through the alternative pathway by directly interacting with complex carbohydrates on bacterial and fungal cell walls or with substances such as plasmin.

Several investigators have found C3 complement components in human periradicular lesions.[10] Activators of the classic and alternative pathways of the complement system include IgM, IgG, bacteria, and their by-products, lysosomal enzymes from PMN leukocytes as well as clotting factors. Most of these activators are present in periradicular lesions. Activation of the complement system in these lesions can contribute to bone resorption either by destruction of already existing bone or by inhibition of new bone formation via the production of prostaglandins (PGS).

VASOACTIVE AMINES

The two major vasoactive amines involved in inflammatory reactions are histamine and serotonin. Both exist preformed in a variety of cells, most notably in mast cells, basophils, and platelets. Release of these substances lead to increased capillary permeability and dilation and can cause smooth muscle contraction. In humans, histamine is the most important of the two substances.[15] Histamine is present in preformed granules in mast cells and is released by a number of stimuli including physical and chemical injuries, complement activation products, activated T lymphocytes, and bridging of membrane-bound IgE by allergens. These stimuli initiate a transmembrane signal in the mast cell that eventually culminates in the release of histamine and perhaps other more minor vasoactive amines.[10]

Numerous mast cells have been detected in human periradicular lesions.[16, 17] Physical or chemical injury of periradicular tissues during cleaning, shaping, or obturating the root canal system with antigenic substances can cause mast cell degranulation. The discharged vasoactive amines can initiate an inflammatory response or aggravate an existing inflammatory process in the periradicular tissues.

LYSOSOMAL ENZYMES

Lysosomal enzymes are potent proteolytic enzymes that are stored in small membrane-bound bodies termed lysosomes within the cytoplasm of inflammatory cells such as PMN leukocytes, macrophages, and platelets.[18] Major enzymes released from lysosomes include acid and alkaline phosphatases, lysozyme, peroxidase, cathepsins, and collagenase. Lysosomal enzymes are released by means of two principal mechanisms: (1) cytotoxic release during cell lysis; and (2) secretory release during phagocytosis.

Release of lysosomal enzymes results in increased vascular permeability and further chemotaxis of leukocytes and macrophages. In addition, lysosomal enzymes can cause cleavage of C_5 and generation of C_{5a}, a potent chemotactic component, and liberate active bradykinin from plasma kininogen.[18] Aqrabawi et al.[19] examined human periradicular lesions for the presence of lysosomal hydrolytic arylsulfatase A and B and found higher levels of these substances in lesions of endodontic origin compared with the control tissues.

ARACHIDONIC ACID METABOLITES

Arachidonic acid is a naturally occurring acid that is incorporated into phospholipids of the cell membrane. Oxidation of arachidonic acid leads to the generation of a group of biologically important products including PGs, thromboxanes, and leukotrienes (LT). Products of the arachidonic acid cascade are not preformed and stored within intracellular granules. They are synthesized from cell membrane components as a result of cell membrane injury.[20] There are several pathways by which arachidonic acid is metabolized (Fig. 2).

Prostaglandins

PGs are produced from arachidonic acid via the cyclooxygenase pathway. The PGs, particularly PGE_2 and PGI_2, have been shown to be associated with vascular permeability and pain in conjunction with the action of other chemical mediators of acute inflammation such as histamine and kinins.[10] High levels of PGs have been found in inflamed gingival and pulpal tissues.[21-24] The importance of PGs in progression of periodontal disease has been shown by the finding that an inhibitor of PGs, flurbiprofen, decreased naturally occurring periodontal disease destruction in beagle dogs.[25] Synovial tissue from patients with rheumatoid arthritis produced PGE_2 and caused bone resorption in vitro, an effect inhibited by indomethacin.[26] Tumor cells that produce large amounts of PGE_2 lead to extensive bone resorption and hypercalcemia, which are blocked by indomethacin.[27, 28] Parathyroid hormone (PTH)-related protein will also stimulate PGE_2 from human osteoblast-like cells, suggesting that PGs also may communicate bone resorptive signals locally.[29] Cytokinins such as interleukin-1 may stimulate PGs production.[30]

PGs have been implicated to play an important role in pathologic changes associated with human periradicular diseases.[31] The role of PGs in periradicular bone resorption was investigated by our[32] demonstration that the formation of periradicular lesions in cats (Fig. 3) was inhibited by systemic administration of indomethacin. Recently McNichols et al.[33] showed the presence of high levels of PGE_2 in acute periradicular abscesses.

Leukotrienes

LTs are produced from arachidonic acid via the lipooxygenase pathway (Fig. 2). The biologic activities of LTs include chemotactic effects for PMN leukocytes, eosinophils, and macrophages, increased vascular permeability, and stimulation of the release of lysosomal enzymes from PMN leukocytes and macrophages.[34] High concentrations of LT B4, a potent chemotactic agent, have been found in periradicular

lesions (Fig. 4).[35] In addition, a positive correlation was found between the concentration of this substance and the number of PMN leukocytes.

CYTOKINES

Cytokines can be derived from bone marrow mononuclear cells or from bone cells directly. They might also be incorporated into bone matrix to be released in a biologically active form during bone resorption. Cytokines may play a role not only in physiologic bone remodeling but also in inflammatory and bone remodeling diseases. The cytokines may, in fact, be the major local regulators of osteoclasts.[36]

The cytokines that have been implicated in bone resorption include interleukins (IL) 1, 3 and 6, tumor necrosis factor (TNF), and colony-stimulating factors (CSF).

Interleukin 1

IL-1 is produced primarily by monocytes and macrophages.[37, 38] Human monocytes produce at least two IL-1 species, IL-1α, and IL-1β.[39] IL-1β is the major form secreted by human monocytes. The chief component of osteoclast-activating factor was purified and found to be identical to IL-1β.[40] IL-1β is the most active of the cytokines in stimulating bone resorption in vitro, 15-fold more potent than IL-1α, and 1000-fold more potent than TNFs.[41] The genes for IL-1 have been cloned, and IL-1α and IL-1β are related molecules of nearly identical molecular weight (17.4 kDa) but they share only 35% sequence homology.[39]

IL-1 has been associated with increased bone resorption in vivo in several disease conditions. Because IL-1 may be produced by activated macrophages or inflammatory cells and has been identified in human dental pulp,[42] IL-1 has been implicated in the bone resorption of several chronic inflammatory diseases such as periodontal disease and periradicular lesions[43-46] (Fig. 5).

IL-3

IL-3 is a T-lymphocyte–derived substance that causes the differentiation of precursors to osteoclast-like cells.[47, 48] IL-3 has also been implicated in the bone resorption that occurs in chronic inflammatory diseases such as rheumatoid arthritis or periodontitis.[47-49]

IL-6

IL-6 is produced by a large number of cells and with a wide range of cell targets.[50] It is produced by osteoblasts but in response to other bone resorptive agents such as PTH, IL-1, and $1,25(OH)_2D_3$.[51]

IL-6 is produced during immune responses and may play a role in human resorptive diseases such as adult periodontitis[52] and rheumatoid arthritis.[53]

Tumor necrosis factor

The monocyte-macrophage–derived TNF-α and the lymphocyte-derived TNF-β (previously called lymphotoxin) have effects on bone resorption that are similar to IL-1. Their effects on osteoclasts are also indirect and are mediated through osteoblasts.[54] The effect of TNF-α to stimulate bone resorption is dependent on PG synthesis.[55]

TNFs have been detected in all samples of gingival and periradicular tissues associated with disease but were scarcely detectable in sites associated with health.[43, 56]

Colony-stimulating factors

The CSFs are a broad class of hematopoietic growth factors that support the growth and differentiation of a wide range of hematopoietic cells.

Macrophage (M)-CSF, also known as CSF-1, the effect of which is restricted to cells of the mononuclear phagocyte system,[57] may be critically important for osteoclast development. M-CSF directly stimulates proliferation of osteoclast precursors[58] perhaps in concert with IL-1 or IL-3.[59] M-CSF is produced by osteoblasts themselves and thus could serve to communicate bone-resorptive signals from osteoblasts to osteoclasts.[60-64] M-CSF production by osteoblasts is enhanced by other bone-resorbing cytokines such as IL-1 and TNF.[51, 53, 54]

Granulocyte (G) M-CSF is a T-lymphocyte–derived substance that affects a multitude of functions of mature granulocytes, monocytes, and some mesenchymal cells. GM-CSF stimulates the formation of osteoclast-like cells in long-term marrow cultures,[65] and it also stimulates the growth of osteoblast-like cells.[66] Osteoblast-like cells produce GM-CSF.[67, 68] Production of GM-CSF is stimulated by osteotropic agents such as PTH or the bacterial component lipopolysaccharide.[68] GM-CSF may act in concert with other cytokines such as IL-6[69]; it may both increase the production of other cytokines and be stimulated itself by other cytokines.[70-72] Therefore, it may cause bone resorption in the process of inflammation.

IMMUNOLOGIC REACTIONS

Immunologic reactions can be divided into antibody and cell-mediated reactions. The role of IgE and

release of vasoactive amines in pathogenesis of periradicular lesions was described earlier. In addition to IgE-mediated reactions and cell-mediated reactions, immune complex reactions can also participate in pathogenesis of acute or chronic periradicular lesions.

Antigen-antibody complex reactions

Immune complexes in periradicular tissues can be formed when extrinsic antigens such as bacteria or their by-products interact with either IgG or IgM antibodies. The complexes bind to platelets, which leads to the release of vasoactive amines and to increased vascular permeability and PMN leukocyte chemotaxis. The binding of immune complexes in periradicular lesions has been demonstrated in experimental animals. Simulated immune complexes placed in feline root canals led to the rapid formation of periradicular lesions, notably characterized by bone loss and the accumulation of numerous PMN leukocytes and osteoclasts.[32] Torabinejad and Kiger[73] confirmed this finding when they immunized cats with subcutaneous injections of keyhole limpet hemocyanin until the presence of circulating antibody to this antigen was detected. Challenge doses of the same antigen were then administered via the root canals. Radiographic and histologic observations suggested the development of periradicular lesions consistent with characteristics of an Arthus-type reaction.

Immune complexes in periradicular tissues in human beings have been studied as well. Torabinejad and Kettering[74] used the anticomplement immunofluorescence technique to localize immune complexes in human periradicular specimens (Fig. 6). In two separate investigations, Torabinejad et al.[75, 76] quantitated the serum concentrations of circulating immune complexes, various classes of immunoglobulins, and a C3 complement component in patients with chronic and acute periradicular lesions. The results indicated that immune complexes formed in chronic periradicular lesions are either minimal or are confined within the lesions and do not enter into the systemic circulation. In contrast, when the serum concentrations of circulating immune complexes in patients with acute abscesses were compared with those of persons without these lesions, a significant difference was found between the two groups. Complexes were present in the circulation of patients with lesions, but they were undetectable in the blood of unaffected controls.

Cell-mediated immune reactions

The presence and relative concentration of B and T lymphocytes and their subpopulations were determined in human periradicular lesions by the indirect immunoperoxidase method.[77] Many B cells, T suppressor (TS) cells, and T helper (TH) cells were detected in these lesions, but the T cells outnumbered the B cells significantly (Fig. 7). Other investigators[78-81] found approximately equal numbers of T-cell subsets in chronic lesions (TH/TS ratio < 1.0). Stashenko and Yu[80] demonstrated in developing lesions in rats that TH cells outnumber TS cells during the acute phase of lesion expansion, whereas TS cells predominate at later time periods when lesions are stabilized. On the basis of their results it appears that TH cells may participate in the development of periradicular lesions, whereas TS cells may decrease excessive immune reactivity, leading to cessation of lesion growth.

The specific role of T lymphocytes in pathogenesis of periradicular lesions has been recently studied by a number of investigators. Wallstrom and I[82] exposed the pulps of mandibular molars of athymic and conventional rats and left them open to the oral flora for 2, 4, or 8 weeks. Tissue sections were quantified by percentages of surfaces areas of bone, connective tissue, bone marrow, intrabony spaces, periradicular lesions, and numbers of osteoclasts. Statistical analysis showed no significant difference between periradicular tissue responses of the two treated groups. Waterman[83] compared periradicular lesion formation in immunosuppressed rats with that in normal rats and found no significant histologic differences between the two groups. These findings suggest that the pathogenesis of periradicular lesions is a multifactorial phenomenon and is not totally dependent on the presence of circulating lymphocytes. In addition to B and T lymphocytes, a population of large granular lymphocytes (natural killer cells) that kill neoplastics and virus-infected cells have been also detected in chronic periradicular lesions.[84] Recently, Okiji et al.[85] showed the presence of Ia antigen-expressing nonlymphoid cells in developing periradicular lesions. These cells may act as antigen-presenting cells in pathogenesis of periradicular tissues. In a histologic and histometric investigation, Yamasaki et al.[86] created pulpal exposure in the mandibular first molar of rats and studied the development of pulpal and periradicular lesions. They showed that pulpal necrosis extended gradually in coronal apical direction and periapical lesions extended initially in a mesiodistal direction.

As summarized in Fig. 8, it appears that multiple mechanisms are involved in the pathologic changes associated with formation of acute and chronic periradicular lesions. Mechanical injury to the periradicular tissues can cause activation of several pathways of inflammation and release of nonspecific mediators.

Continuous egress of antigens from a pathologically involved root canal can also result in one or a combination of the various types of immunologic reactions. Present data indicate that a number of these reactions participate in the destruction of periradicular tissues. Because of complex interactions between the various components of these systems, the dominance of any one pathway or substance may be difficult to establish. More investigations are needed to elucidate the specific role of cells or their by-products in pathogenesis of acute and chronic periradicular lesions.

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