Chapter 13

Periapical Lesions of Endodontic Origin

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Periapical lesions of endodontic origin will be discussed in this chapter in terms of function and form. An emphasis is placed on the understanding of the biological function of the lesion, with special attention to processes leading to its formation and to changes it may go through, as observed by either the clinician or the histopathologist. The content of this chapter requires some knowledge of microbiology and immunology. The reader is referred to Chapters 7 and 9 dedicated to these subjects, which provide the essential background in detail. Periapical lesions, not inflammatory in nature, will be discussed in Chapter 8.

Periapical lesions of endodontic origin are produced by an inflammatory response at the root apices of teeth with nonvital pulps. Because the pulp cavity is relatively inaccessible to the immune response, it becomes a reservoir of infection. The establishment of an inflammatory response is believed to be an attempt to prevent the spread of infection to periapical tissues. Periapical lesions of endodontic origin are generated by the immune system. The response may occur at any portal of exit from the root canal system. Since most such portals are found in the apical area, and for the sake of convenience for the reader, the terms ''periapical'' or ''apical'' will be used for inflammatory lesions of endodontic origin.

Bacterial Elimination in Periapical Lesions

The elimination of bacteria emerging from the apical foramen and the prevention of their spread to other tissues are important functions of apical periodontitis.[1,2] Even though bacteria are often present in infected root canals, most periapical lesions successfully contain them. Thus, periapical inflammatory lesions may be considered to be a successful host response to bacteria and bacterial by-products in an infected root canal system. The elimination of the invading bacteria is one of the main functions of the immune response. The key role in this process is played by the polymorphonuclear leukocytes (PMNs) that eventually phagocytize and kill the bacteria. PMNs originate from progenitor cells in the bone marrow and enter the bloodstream where they circulate for 7–10 h, ready to be called to duty. To reach the bacteria emerging from the apical foramen, PMNs must first leave the bloodstream and find their way to that site. This process is initiated by a stage termed ''margination,'' which is mediated by a locally elevated expression of attachment molecules such as intercellular attachment molecule-1 (ICAM-1) on the surface of the endothelial cells in the area.[3–5] The ''right area'' for margination is defined for the PMNs by local production of IL-1 and tumor necrosis factor (TNF) that results in an elevated expression of the attachment molecules.[3,6] Once attached, the PMNs can leave the blood vessels by passing between endothelial cells in a process termed ''diapedesis.'' PMNs follow the gradient of the chemotactic factor C5a that is generated by the activation of the complement system at the site where bacteria are present. This gradient serves as a chemotactic signal; the random motion of the PMNs becomes directional and they follow this gradient from low to high concentration. Complement may be activated directly by bacterial constituents such as bacterial endotoxin (lipopolysaccharide, LPS), using the ''alternative'' or ''properidin'' pathway. This represents the basic, innate immune response of the host. The ''classical pathway'' of complement activation is dependent on specific IgG or IgM that binds to antigens present on the bacterial

surface. This, in turn, triggers complement activation by C1q molecules of the complement cascade. Complement activation is a ''chain reaction'' type of response that generates large amounts of three types of molecules that may be considered as major life-sustaining factors. Two of them are small and readily diffusible factors, C3a and C5a, while the third is a larger molecule, C3b, that remains attached to the bacterium and serves as a signal that marks the targets for PMNs to engage and phagocytize.

Effective phagocytosis requires marking the bacteria with a dual signal, the other signal being a specific IgG molecules attached to the bacterium. PMNs have two distinct receptors, C3b receptor and Fc receptor, on their membrane that engage the relevant molecules allowing for effective phagocytosis. The latter attaches to the Fc portion of an IgG molecule that has been attached to the bacterial surface antigen via its binding sites (located at its Fab region). This mechanism is termed ''opsonization'' and is essential for the effective elimination of bacteria by the PMNs.[7] Antibodies may come from the bloodstream, but evidence for their local production will be discussed below.

It is also clear that an effective and continuous local supply of complement constituent is essential for the above-mentioned process to take place. This is achieved by an increased permeability of the capillaries in the area, induced by C3a and C5a via activation and degranulation of local mast cells. When vascular permeability is normal, large molecules are kept within the blood vessels. They serve to maintain the essential osmotic pressure that will draw water back into the venous part of the capillaries and maintain the blood and tissue volumes at equilibrium.

From the above sequence of events, it becomes apparent that even though the PMNs are responsible for phagocytizing and killing bacteria, an elaborate infrastructure of cells is required to locally provide at least two main types of molecules, without which the PMN response will be handicapped: IgG *specific* to the involved bacteria and the cytokines IL-1 and TNF.

CELLS, IMMUNOGLOBULINS AND CYTOKINES IN PERIAPICAL LESIONS

Qualitative and quantitative studies of the cellular composition of periapical granulomas have been profoundly influenced by the methodology available. Initial attempts to characterize the cells participating in these lesions were based on the classical morphology of the cells followed by the use of electron microscopy. With the introduction of immunohistological methods, the first attempts to specifically identify plasma cells in periapical lesions by their immunoglobulin content were reported.[8] In recent decades, the intensive use of monoclonal antibodies against subsets of T lymphocytes, B lymphocytes, macrophages, dendritic cells, plasma cells, and PMNs resulted in a major breakthrough in the understanding of the immunobiology of the periapical host response, in both naturally occurring human periapical lesions and those experimentally induced in the rat. The most recent approach involves molecular biology methods, such as in situ hybridization or the polymerase chain reaction.

Cells with distinct morphology, such as PMNs, mast cells, lymphocytes, plasma cells, and osteoclasts, have been easily identifiable. However, in earlier studies only cells with ''classical'' macrophage morphology could be identified as such. Monoclonal antibodies make it possible to identify macrophages and dendritic cells of diverse morphology as well as to recognize subsets of these and other cells.

EVOLUTION OF THE IMMUNOBIOLOGICAL CONCEPT OF PERIAPICAL LESIONS

The availability of new methodologies has influenced the type of studies performed and eventually affected the evolution of the immunobiological understanding of the complex nature of the periapical host response.[9–11] Initially, the commercial availability of specific antibodies directed against human IgG, IgM, IgA, and IgE allowed for the immunofluorescent or immunohistochemical detection of these molecules in periapical lesions, either in a free form or as markers of subsets of B lymphocytes and plasma cells.[12] Later, the combination of these antibodies with those directed against human complement allowed the demonstration of *activity* rather the *simple presence* of immunoglobulins in periapical lesions. Johannessen and colleagues demonstrated intracellular colocalization of IgG and C3b in macrophages in periapical inflammatory lesions, suggesting phagocytosis of bacteria via dual opsonization by both opsonins.[13]

The availability of monoclonal antibodies against various T lymphocyte subsets made it possible to explore the presence of these cells in both human periapical lesions as well as in those experimentally induced in rats. T-cells in human periapical granulomas were studied by Cymerman and colleagues.[14–16] It became apparent that both T helper ($CD4^+$) and T suppressor ($CD8^+$) lymphocytes were present in these lesions.[14] In delayed hypersensitivity in humans, a typical T helper/T suppressor relation is about 2:1.[17] It was therefore of

interest to define whether T lymphocytes in periapical lesions follow this trend. Babal and colleagues found a T helper/T suppressor ratio of <1.0 in periapical granulomas, while Barkhordar and Desouza reported a ratio of ~1.0.[15] It seemed that the predominance of T helper lymphocytes, which is typical of delayed hypersensitivity, does not exist in a chronic periapical granuloma. Nevertheless, this is not a uniform finding, as Kopp and Schwarting found a T helper/T suppressor ratio of 3:2 in periapical granulomas, which diminished to ~1.0 in periapical scars.[18].

In a rat model, Stashenko and Yu demonstrated that during the early, active, phase of lesion development, helper ($CD4^+$) T cells predominated, while at the later chronic stage, suppressor ($CD8^+$) T cells outnumbered the helper T-cell population.[19] The initial Th/Ts ratio of 1.7 lessened to <1.0 at the later stage, as compared to a Th/Ts ratio of 2.0 in peripheral blood. These findings were interpreted as the initial active function of T lymphocytes, which is later downregulated and controlled by suppressor T cells. The balance of their activities is expressed in chronic periapical lesions, such as those encountered in humans.

THE PROTECTIVE FUNCTION OF T LYMPHOCYTES IN THE PERIAPICAL GRANULOMA

A protective role of helper T lymphocyte function should eventually be expressed as a better ability of the host to prevent bacteria from spreading from the infected root canal. This may be accomplished by (1) the local production of antibodies and (2) increasing the local availability and enhancing the function of phagocytes (Figure 1). Local activation of antigen-specific T helper lymphocytes is a prerequisite for the local production of antibodies that are specific to the bacteria that periodically emerge from the root canal.[20,21] This in turn will enable effective opsonization of the bacteria, followed by phagocytosis and killing.

Local macrophage activation is accomplished mainly by interferon-γ (INF-γ) produced by the activated T helper cells (see Figure 1). Even though activation of the lymphocytes is antigen-specific, once macrophages are activated, the result is nonspecific, and their phagocytic and killing abilities, as well as cytokine production, will be greatly enhanced. IL-1 and TNF production by

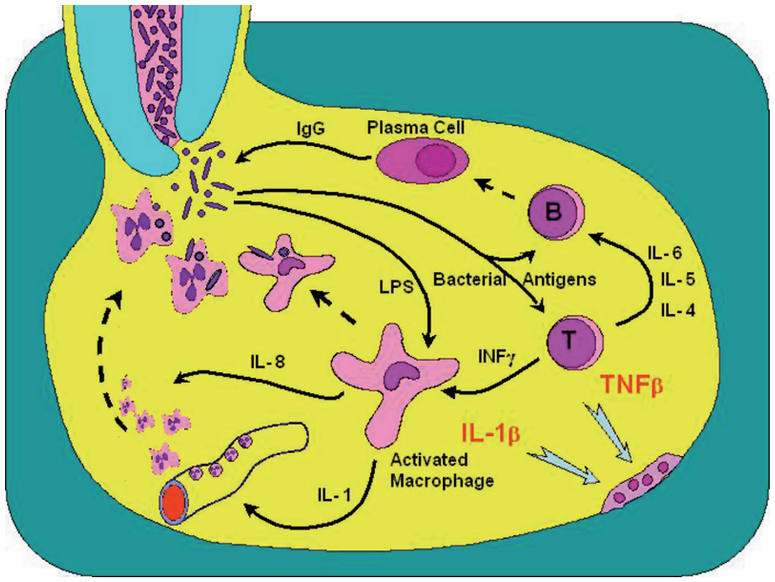

Figure 1 Protective functions of T lymphocytes and macrophages in the periapical granuloma. Specifically activated T lymphocytes produce a plethora of cytokines, some of which promote activation, proliferation, and maturation of B lymphocytes to become plasma cells producing immunoglobulins specific to the root canal bacteria. Macrophage activation is mediated by interferon-γ (IFN-γ) produced also by these cells. Activated macrophages produce IL-1 and IL-8 that are essential for effective local recruitment and activation of PMNs. Bacterial endotoxin (LPS) can bypass the T lymphocytes and directly activate macrophages. Bone resorption in apical granuloma is mediated by IL-1β (an activated macrophage product) and by TNF-β (an activated T lymphocyte product). It may be considered a negative side effect of the ongoing protective process in the apical granuloma.

activated macrophages will locally elevate ICAM-1 molecule expression by endothelial cells in the capillaries, thus enhancing the local attachment of PMNs and monocytes and enhancing their migration into the periapical area.[3,6] IL-8 produced by these macrophages will chemotactically attract the PMNs and activate them, making them more available and more competent to engage and kill the bacteria[22–24] (see Figure 1). Thus, macrophage activation has a major role in maintaining the two lines of phagocytic cell defense that are typically described in the periapical lesion: an inner area, closer to the apex, in which PMNs predominate and the area around it in which the phagocytic macrophages are observed.[25] The defensive function of helper T lymphocytes is achieved *indirectly* by allowing (1) activated specific B lymphocytes to proliferate and maturate into plasma cells that will produce antibodies and (2) the activation of nonspecific effector cells, the macrophages. In order to avoid an endless loop of mutual activation of macrophages and T lymphocytes, the process is actively controlled and downregulated by suppressor T lymphocytes.[26–28]

The essential role of the T lymphocytes in this process was well established and accepted by the late 1980s of the last century.[28,29] A turning point occurred with the first studies that utilized athymic mice and rats to study the formation of periapical inflammatory lesions to determine the critical role of T lymphocytes in the formation of these lesions.[30,31] Unexpectedly, both groups demonstrated that periapical lesions could also develop independent of T-lymphocyte activity, thus leaving the center stage to another key actor: the macrophage (as will be detailed below).

B CELLS, PLASMA CELLS, AND IMMUNOGLOBULIN PRODUCTION

The presence of *plasma cells*, the cells that produce immunoglobulins, in apical granulomas has been well documented by many investigators.[16,32–35] In addition, the presence of *B lymphocytes*, the precursors of plasma cells, has also been documented in apical granulomas.[12,36,37] Immunoglobulin-containing cells (B Ly + plasma cells) may constitute 11–50% of the mononuclear cells in apical granulomas.[12,36] Among these cells, those containing IgG are the majority (70–85%) while small fractions of them produce IgA, IgE, and IgM.[12,36] It must be kept in mind that the presence of IgG and IgG-producing cells in the periapical tissue does not necessarily mean that the immunoglobulins will be specific to the bacteria that reside in necrotic infected root canals. Baumgartner and Falkler[20] and Kettering and colleagues[21] demonstrated that antibodies specific to root canal bacteria are indeed present in the periapical tissue. Both groups independently demonstrated that specific immunoglobulins that bind to bacteria typically found in infected root canals are present in human periapical granulomas.[20,38] IgG concentrations reported in periapical exudates are four times higher than those found in the serum of the same patients.[37] This may indicate but not prove the local production of these immunoglobulins in periapical lesions. Baumgartner and Falkler[39,40] convincingly demonstrated that the local production of immunoglobulins does indeed take place in the human apical granuloma.

Immunoglobulin production by plasma cells is the end result of a complex and well-controlled process that starts with the exposure of antigen-presenting cells (APCs) to the relevant antigen. In turn, they present the antigen, in conjunction with their major histocompatibility complex class II (MHC II) surface molecule, to specific T lymphocytes. This specific signal, together with a nonspecific signal in the form of the cytokine IL-1, results in the activation of the antigen-specific set of T cells.[41] Once activated, the T cells proliferate and produce several cytokines, two of which are essential for immunoglobulin production, IL-4 and IL-5, which induce the proliferation of B lymphocytes and their maturation into plasma cells. Both serve as nonspecific signals that allow a clone of antigen-specific B cells that were exposed to the same specific antigen to respond by proliferation and maturation into specific plasma cells. These cells will eventually produce immunoglobulin that is specific to that antigen.

MHC II molecules expressing macrophages and dendritic cells were found by Kaneko and colleagues in experimentally induced periapical lesions in rats.[42,43] The presence of HLA-DR+ cells, which are the human equivalent of the MHC II-positive cells, was studied in human granulomas in a dispersed cell cytometric flow immunochemistry study.[44] Activated macrophages (HLA-DR+, $CD14^+$) and mature dendritic cells (HLA-DR+, $CD83^+$) were found in great numbers. Thus, antigen presentation is possible and likely in the confinement of the apical granuloma.

T LYMPHOCYTES IN THE PERIAPICAL GRANULOMA

T lymphocytes are the major constituents of the chronic inflammatory response at the periapical region.[16,18,19,25,34–36,45] They may constitute up to 40% of the mononuclear inflammatory cells.[35,36] During the active phase of development of an induced apical periodontitis in rats, T helper cells outnumbered T

suppressor lymphocytes with a Th/Ts ratio of 1.7.[19] When active lesion expansion subsided, by day 20, this ratio reversed to 0.9 and remained at 0.7 for the remaining 70 days of the experiment. In an established human apical granuloma, T helper ($CD4^+$) cells outnumber T suppressor cells ($CD8^+$) by a ratio of 1.5–2.0.[19] Even though large numbers of T cells are present, it is most likely that only the *activated T cells* play a role in the protective response, and these may constitute as few as 6% of the total T-cell population.[35] When activated, these cells produce a plethora of cytokines that regulate the immune response. T helper ($CD4^+$) lymphocytes may be divided according to their cytokine expression pattern and classified as either T helper 1 (Th1) cells that produce and secrete IL-2 and INF-γ or Th2 cells that produce and secrete IL-4, IL-5, IL-6, IL-10, and IL-13. Th1 type cytokines augment cytotoxic T-cell functions and stimulate proinflammatory cytokine production in other cells, such as macrophages, while Th2 type cytokines participate in B-cell stimulation to mount a humoral immune response and in downregulation of inflammatory reactions.[46,47] Among the cytokines produced by activated T cells, IL-2 is involved in T-lymphocyte proliferation while IL-4, IL-5, and IL-6 are involved in B-cell proliferation, differentiation, and maturation into plasma cells, thus leading to immunoglobulin production (see Figure 1). INF-γ is the key proinflammatory cytokine produced by activated T lymphocytes. Its major function is to activate macrophages that have several key roles, as will be discussed below. Activated macrophages, in turn, are the major source of IL-1 in the periapical lesion and, thus, may contribute to several protective processes including immunoglobulin production, at its initiation stage. TNF-β is another important cytokine produced by activated T cells, which has been implicated as a major factor in the activation of osteoclastic bone resorption in humans, as will be discussed below.[48]

MACROPHAGES IN THE PERIAPICAL GRANULOMA

Macrophages have a major role in several processes that take place in these lesions.[49] Macrophages make up to 46% of the periapical inflammatory cells found in tissue sections of human periapical granulomas.[45] When Stern and colleagues[50] dispersed periapical granulomas to cell suspensions, 30% of the resulting cells were macrophages. Macrophages were also found as the predominant inflammatory cell when Kopp and Schwarting[18] used monoclonal antibodies to identify them in human periapical lesions. Piatelli and colleagues[35] similarly reported that macrophages outnumbered T lymphocytes in human periapical granulomas. In the rat model, Kawashima and colleagues[25] recently demonstrated that macrophages were the predominant immunocompetent cell type throughout the development of the lesion. The kinetics of their presence in these experimental periapical lesions was studied by Akamine and colleagues,[32] who followed the periapical lesions for as long as 150 days. Macrophages increased in number during the first 10 days and maintained their numbers through day 60, followed by a gradual decline thereafter (Figure 2A,B).

POTENTIAL ROLE OF MACROPHAGES IN THE PERIAPICAL GRANULOMA

Macrophages have a central role in (1) innate, nonspecific immunity; (2) the onset, regulation, and outcome of antigen-specific, acquired immunity; and (3) the regulation of connective tissue destruction and repair. Macrophages are ''professional'' phagocytic cells that can internalize and kill bacteria by several mechanisms, some of which are part of innate immunity, while others require the presence of specific antibodies against the bacterium and should be considered to be part of the effector arm of specific, acquired immunity. Bacteria, new to the host, may activate the complement system

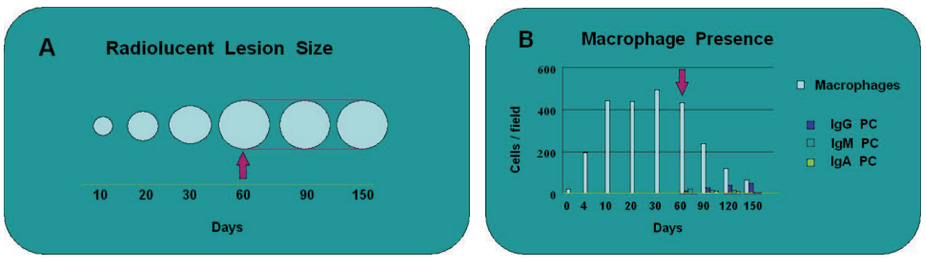

Figure 2 Macrophages and bone resorption in developing periapical lesions in rats. *A*, The periapical lesion gradually enlarges up to 60 days, and then its size remains stable. *B*, Macrophage domination in the lesion coincides with the period in which the lesion grows and diminishes afterwards, with the appearance of plasma cells. Adapted from Akamine A et al.[32]

by the alternative pathway, resulting in their opsonization by the C3b component. This, in turn, will result in their phagocytosis by the macrophages via a C3b receptor-mediated process. Other bacteria may attach to the macrophages through lectin-mediated mechanisms, leading to lectinophagocytosis that is independent of the common C3b receptor–ligand binding.[51] Once specific antibodies to a strain of bacteria are present, either by developing through the course of the current infection or as a result of a former encounter with these bacteria, a more efficient form of phagocytosis will occur, involving *dual* opsonization by IgG and C3b and engagement of both the Fc and C3b macrophage receptors.

It is the innate immunity that enables the host to survive the initial steps of infection, while the acquired, specific immunity allows the host to efficiently eliminate the invading microorganisms. Macrophages present in the periapical granuloma may contribute, by their function as phagocytes, to the effective prevention of the dissemination of bacteria from the infected root canal. Macrophages may also serve as ''APCs'' in the essential initial steps of the induction of acquired immunity. They process the antigen and present it to the antigen-specific clones of helper T lymphocytes by a process involving recognition of an MHC II molecule on macrophages by lymphocytes. Additionally, they produce the cytokine IL-1, which is an essential complementary signal for the activation of these lymphocytes. Macrophages that carry MHC II molecules, and thus may serve as APCs, have been identified in periapical granulomas in both humans and the rat model (also termed HLA-DR or Ia antigen-positive cells).[18,52]

Macrophages are considered to be a main source of the cytokines IL-1α , IL-1β, and TNF-α that contribute to the initiation and regulation of the inflammatory process. Additionally, they produce a plethora of other active molecules, including metalloproteases (collagenase and elastase) and prostaglandins that may also contribute to the destructive outcome of the periapical inflammatory process. Some of these products directly damage connective tissue constituents, while others, including the cytokines produced by the macrophages, activate other cells to exhibit either (1) destructive activities such as osteoclast activation and bone resorption or (2) constructive repair processes by activation of fibroblast proliferation and collagen production by these cells.

Though it is commonly assumed that *all* of the above-listed potential activities of the macrophage take place in the periapical granuloma, this is not entirely true. Subsets of macrophages that may exist in relatively small numbers are responsible for a specific activity. Emerging evidence indicates that some of these functions, such as active production of IL-1, involve only a small number of activated macrophages. In chronic human periapical granulomas, these cells do not exceed 2 to 3% of the macrophages present in these lesions, and these cells have been shown to express only mRNA for IL-1β.[9,53]

KINETICS OF MACROPHAGE PRESENCE IN PERIAPICAL LESIONS

The unique study by Akamine and colleagues[32] followed rat periapical lesions for as long as 150 days. An analysis of their data reveals that the active growing stage of periapical lesions in the rat, which lasted for the first 60 days, coincides with a peak of macrophage presence in this lesion. When the active growth stops and a stationary stage is reached, the presence of macrophages in the lesion gradually declines. This may be a coincidence, but nevertheless, it may indicate a significant correlation. Further support may be found in a recent study by Kawashima and colleagues that showed that macrophage infiltration in the periapical lesions was associated with bone resorption in the area.[25] In their study, macrophage infiltration preceded that of lymphocytes and gradually increased throughout the 56 days of the experiment.

IL-1 AND MACROPHAGES IN THE PERIAPICAL GRANULOMA

The presence of IL-1β in association with a subpopulation of the macrophages in periapical lesions has been reported by several investigators.[9,53,54] Artese and colleagues[53] reported that there were very few cells (2 to 3%) in established human periapical granulomas with immunoreactivity of IL-1β and TNF-α and that these cells had a macrophage morphology. Tani-Ishii and colleagues[54] used a rat model to demonstrate that IL-1α and TNF-α were associated with macrophages in the periapical lesion as soon as two days after the exposure of the pulp and persisted through the 30 days of the experiment. In contrast, TNF-β and IL-1β could not be detected in their sections.

It should be kept in mind that the presence of cytokines, in association with these cells, does not necessarily prove that they are the source of these molecules. A cytokine may potentially have been attached to or may have been taken up by the macrophages, rather than produced by them. Recently, direct proof has been provided that clearly demonstrates that the activated macrophages actually produce IL-1β in periapical lesions. In an in situ hybridization study, Hamachi and colleagues[9] demonstrated the presence of IL-1β mRNA in macrophages in human periapical lesions. This proves

not only that these cells are capable of producing cytokines in general and that cytokines are associated with them in the periapical lesion, but also that subpopulations of macrophages are actively engaged in the production of this cytokine in periapical granulomas.

BACTERIA IN PERIAPICAL LESIONS

For many years, periapical lesions were considered ''not an area in which bacteria *live*, but in which they are *destroyed*.''[55] This concept had its origin in experiments that failed to produce viable bacteria from closed apical granulomas,[56] and it gained support from the common clinical experience that once bacteria are eliminated from the root canal, most lesions will heal. With the development of adequate anaerobic cultivation methods,[57] it became apparent that this concept may be true ''in most cases'' but clearly not in all of them. Initially, the concept that viable bacteria may be present outside of the root canal in the periapical tissue was accepted for few specific bacteria.[58–61] The concept that *extraradicular infection* may survive independent of what occurs in the root canal has recently become more widely accepted.[62–68] It is widely accepted that viable bacteria may be cultured from chronic periapical abscessescellulites with a sinus tract and that some closed periapical lesions may contain viable bacteria outside of the root canal: an extraradicular infection. The latter point was initially considered to be a unique condition related only to specific bacteria, such as strains of Actinomyces and Arachnia.[58–61] However, evidence has accumulated that other bacteria may also survive outside of the root canal in certain lesions.[62–68] Even though this concept is not yet uniformly accepted,[69,70] the original concept that the periapical granuloma is *always* a sterile lesion should be reconsidered in favor of the more moderate statement that it is sterile in *most* cases. This inevitably leads to an understanding that the equilibrium between bacteria and the host, in the periapical environment, is more complicated than believed in the past.

THE EQUILIBRIUM BETWEEN BACTERIA AND THE HOST

An apical granuloma and a chronic periapical abscess with a sinus tract represent two distinct types of equilibriums established between bacteria in the root canal and the host response. The front between bacteria and the host response is established at or near the apical foramen where bacteria or bacterial products/antigens are immediately engaged by the immune system. PMNs will attempt to kill any bacteria within their reach. PMNs are relatively short-lived cells and once they migrate into the tissues they have up to a 3-day life span. Those arriving into the area adjacent to the bacteria will remain there and spontaneously die within a short period, followed by cellular disintegration. The remaining dead bacteria and remnants of the PMNs are taken up by abundant local tissue macrophages. When enzyme-containing PMNs disintegrate, they also release lytic enzymes, such as collagenase, elastase, and hyaluronidase, in the area that damage the host.[71] In histological sections taken under such conditions, only the long-living cells, such as lymphocytes, plasma cells, and macrophages, will be found. The short-lived PMNs will be found only in small numbers, either on their way from adjacent blood vessels or where they accumulate, adjacent to the apical foramen. It is important to realize that macrophages, lymphocytes, and fibroblasts, seen in the histological sections, were there a few days ago and would have been there many days after the section was taken. On the other hand, any PMN seen in the section is a new comer: it left the bloodstream a few hours beforehand and would have died, disintegrated, and disappeared within a few hours or a couple of days. They are not residents of the tissue, but rather are temporary visitors.

An apical granuloma may develop into an acute apical abscess or into a chronic periapical abscess with a sinus tract, a phenomenon termed ''phoenix abscess.''[72] A shift in the balance between bacteria and the host may be the likely cause. Such a change may be quantitative, qualitative, or both. If the amount of bacteria introduced into the lesion suddenly increases, the amount of complement-derived chemotactic factor will increase, followed by an increased number of PMNs reaching the area per given time period. More PMNs will die and more damage will be caused by the released enzymes. This rate of destruction may exceed the cleaning capacity of local macrophages and the local tissue may become solubilized by proteolytic and hydrolytic enzymes, resulting in a liquefied area surrounded by migrating PMNs in a background of chronically inflamed tissue. This results in the formation of an abscess.

If the change in the amount of bacteria is transient in nature, macrophages will clean the area, repair will take over, and the tissue will return to its previous state with connective tissue heavily infiltrated with lymphocytes, plasma cells, and macrophages.

A shift in the equilibrium could also be qualitative in nature: a shift in the bacterial population. Some bacterial strains developed a variety of phagocytosis-evading mechanisms that allow them to survive a massive host PMN attack. Some *Porphyromonas gingivalis* strains isolated from suppurating periapical lesions have the capacity to evade phagocytosis due to an antiphagocytic

capsule that they possess.[73] Other strains have a potent protease that attacks and destroys the C3 and/or C5 complement molecules, thus disrupting this essential antibacterial mechanism.[74–76] Some strains developed a unique evasion strategy and have proteases that clip off the Fc portion of IgG molecules that attach to their surface, thus creating host-derived shields made of Fab fractions that protect them from phagocytes.[77] Strains of *Actinomyces israelii* have yet another strategy: they form aggregates and biofilms that cannot be penetrated by the PMNs and are too large to be phagocytized.[59,61,78–80] A frustrated host response will continuously pour large amounts of PMNs into the battle field only to result in local tissue destruction and pus formation but without any real effect on the bacteria. A draining sinus tract is the clinical expression of the resulting chronic apical abscess.

FLARE-UPS IN PERIAPICAL LESIONS: AN IMMUNOBIOLOGICAL VIEW

A flare-up represents a shift from the equilibrium previously established between bacteria in the root canal and the host response[81] (Chapters 7 and 20). The immune response to extruded bacteria and bacterial by-products results in two main events that occur concomitantly and result in the above symptoms: a vascular and a chemotactic response. Antibodies bind to their specific bacterial antigens, thus activating the complement system via C1q and the ''classical pathway''. Large amounts of C3a and C5a are generated that drive these two main events. C5a and C3 bind to receptors on local mast cells, causing them to degranulate and release vasoactive amines, such as histamine, in the area.[12,35] The resulting vasodilatation and increased vascular permeability provide additional complement and systemic-specific plasma-derived antibodies to the ongoing response. Other large proteins also leave the blood vessels. This, in turn, results in a disturbance of the osmotic system that is responsible for the water balance between blood and adjacent tissues. Normally, fluid that leaves the blood vessels in the arterial part of the capillary bed, under the driving force of hydrostatic blood pressure, should be drawn back into the bloodstream in the venous part. This occurs due to the osmotic pressure generated by the large molecules that stay in the blood vessels. When this gradient is disrupted by the large molecules that leave the blood vessels, due to the increased permeability, water stays in the tissue, which results in edema. Since the bony crypt, in which the periapical lesion is contained, cannot expand, this process will result in increased pressure. The one direction in which this pressure may be partially relieved is moving the tooth occlusally, as far as the periodontal ligament fibers will allow. This tooth may now occlude prior to the adjacent teeth, causing an additional mechanical trauma, and the patient experiences more pain.

The other process caused by local complement activation will be a massive chemotactic signal that will attract large amounts of PMNs into the area that may result in abscess formation by the mechanisms discussed above.

PAIN IN APICAL PERIODONTITIS

Pain associated with symptomatic apical periodontitis is sometimes of moderate or severe nature, whereas most periapical lesions are not associated with pain at all.[82,83] It may be a spontaneous pain or a painful response to mechanical stimuli such as percussion, occlusal loading, or sensitivity to palpation of the periapical tissues.[84] Pain is defined as ''an unpleasant sensory and emotional experience associated with actual or potential tissue damage.''[85] Accordingly, it has peripheral, as well as central, components and is subjected to modulation at each of these levels.

The key peripheral event is the stimulation of nociceptors, neurons that are preferentially sensitive to noxious stimuli or to stimuli that would become noxious in a prolonged exposure.[85] Peripheral nociceptors may respond to mechanical, chemical, or thermal stimuli, the first two being of significance in apical periodontitis. Local sensitization of nociceptors may be induced by numerous mediators that are present in the inflamed periapical tissue (Figure 3). Some of these mediators, such as IL-1α, IL-1β, TNF-α, IL6, or nerve growth factor (NGF), are locally produced by cells of the immune system.[86–89] Others such as CGRP and Substance P derive from stimulated nerve fibers that may become abundant in the area by a sprouting process associated with chronic inflammation.[90] Blood-derived bradykinin may also be locally present.[91] Some local, peripheral mediators, such as bradykinin and serotonin, may directly induce action potential in the nociceptors.[91] The effect of others, such as prostaglandins, NGF, cytokines, serotonin, nitric oxide and adenosine, is indirect, as they lower the stimulatory threshold of these nerve fibers.[86] Pressure building up in the bony crypt, due to edema, is unable to release by swelling, as would be the case in soft tissues. Triggering of pressure nociceptors that have a lowered threshold will result in pain that is often described as an ''excruciating pain'' and may be relieved only by either natural or artificial drainage.[92]

The proalgesic effect of some cytokines such as IL-1β, IL6 and TNF-α may be partially explained by enhancement of local prostaglandin production.[88] Nevertheless,

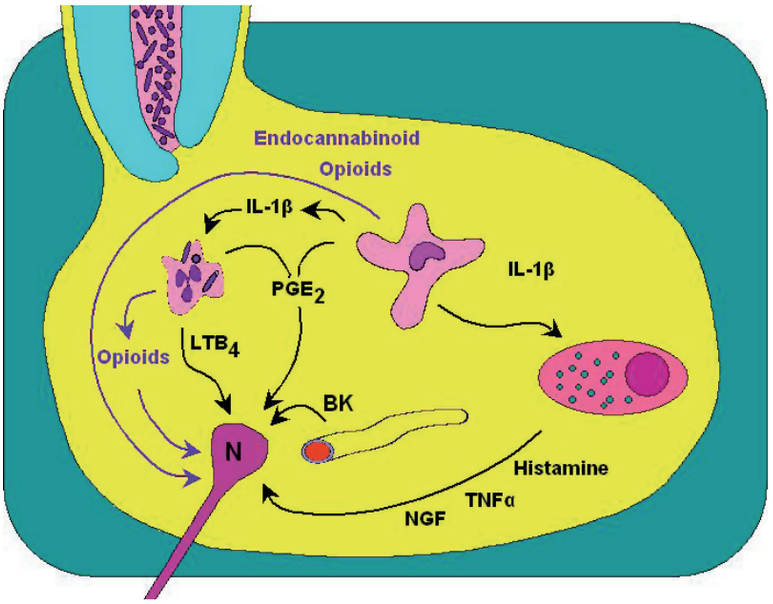

Figure 3 Local activation of nociceptors in an apical granuloma. Nociceptors (N) respond to mechanical and chemical stimuli. Bradykinin (BK) may directly induce action potential in a nociceptor. Others such as prostaglandins, nerve growth factor (NGF), and cytokines may act indirectly by lowering the stimulatory threshold of the nociceptors or by increasing their expression of BK receptors. All the mediators above have a proalgesic effect (black arrows). Opioids, endocannabinoids, and somatostatin (SIRF) generated by macrophages and PMNs have an analgesic effect (purple arrows).

some will have additional indirect effects. IL-1β enhances the production of NGF by mast cells and T lymphocytes.[93,94] NGF, in turn, binds to its receptors on the primary afferent neuron, resulting in the release of neurotransmitters.[95] It will also increase the expression of bradykinin receptors and increase the neuron's sensitivity to acidic pH that is often encountered in inflamed tissues.[96] IL-1β also enhances the local formation of another pain-inducing arachidonic acid metabolite: LTB_4.[88] In addition, bradykinin itself may initiate a cascade of cytokine release that mediates hyperalgesic state by contributing to the above-mentioned mechanisms.[97]

The presence of the above-mentioned mediators in symptomatic periapical tissues is well documented.[22,98,99,100] Nevertheless, pain is not a typical symptom of asymptomatic apical periodontitis. This may be a simple quantitative issue: it could be that only when local proalgesic mediators reach a certain threshold, pain does appear.[98,101,102] Nevertheless, it seems to be more complicated.

Local proalgesic effects of the mediators mentioned above are partially balanced by analgesic mediators derived from inflammatory cells (see Figure 3). Macrophages release somatostatin (SRIF) that has a local analgesic effect.[103] Endocannabinoids secreted by macrophages and opioids released by PMNs and macrophages most probably partially counterbalance the proalgesic mediators in the inflamed tissue.[104–108] This concept was recently elegantly supported by blocking the migration of these cells into inflammatory sites. Inhibition of this migration was achieved by specific antibodies against certain integrins and selectins that mediate margination and diapedesis. It generated a strong proalgesic effect, most probably due to the lack of the effect of the analgesic peptides in the inflammatory site.[109,110]

Central modulation of pain signals is also of major significance and is discussed in detail in Chapter 10. Pain associated with apical periodontitis is an end result of the balance between proalgesic and analgesic mediators that result in a certain level of stimulation of local peripheral nociceptors that is then transmitted centrally, where it is further modulated, resulting in a sensation of "pain."

BONE RESORPTION IN PERIAPICAL LESIONS

Bone resorption is one of the clinical hallmarks of periapical pathosis. The resorption may be viewed either as an undesirable destructive by-product of the host

response, as is the case with periodontal disease, or alternatively, as is traditionally presented, as a process by which a bone is removed from a risky area, thus allowing a ''buffer zone'' to be formed in which host response constituents engage the bacteria. In any case, it is bone resorption in the periapical area that serves as a major indicator of the presence of a periapical pathological process and the progression of its healing to the clinician. As such, it has been thoroughly studied in both humans and animal models.

POTENTIAL VERSUS ACTUAL BONE-RESORBING AGENTS

Bone resorption occurs through the activation of the bone-resorbing cells: the osteoclasts. A wide range of biologically active molecules have been demonstrated to have the capacity to activate osteoclastic bone resorption in in vitro models. These include prostaglandins,[111] bacterial endotoxin,[112] complement activation products, as well as the inflammatory cytokines IL-1α, IL-1β, TNF-α, TNF-β, IL-6, and IL-11 that, as a group, were previously referred to as ''osteoclast-activating factor.''[113] Among these, IL-1β is the most active cytokine and its bone-resorbing capacity is 13-fold of that of IL-1α and 1000-fold of that of TNF-α or TNF-β.[114] When considering the periapical bone resorption associated with infected root canals, all of these have been mentioned and most of them were demonstrated in periapical lesions.[6,12,33,48,53,98,100–102,115,116] The question was which of these *potential* bone-resorbing stimuli is actually involved in the activation of osteoclasts in these lesions?

Two studies by Wang and Stashenko[48] have provided convincing evidence that among the long list of *potential* mediators that may activate osteoclasts and cause periapical bone resorption, the main factors and those that are most important in human chronic periapical lesions are IL-1β and TNF-β. In the rat model of induced active periapical bone resorption, IL-1α and, to a lesser extent, IL-1β and TNF-β are the major bone-resorbing cytokines.[117] Both studies suggest that osteoclast activation by these cytokines may be mediated in part by de novo formation of cyclooxygenase pathway products such as prostaglandins, as the effect could be significantly blocked by nonsteroid anti-inflammatory drugs (NSAIDs).[48,117]

The formation of periapical lesions was studied in the rat model by Kakehashi, Stashenko, and others.[9,19,25,31,118,119] Following exposure and bacterial contamination of the pulp chamber and the root canal, an inflammatory response is activated in the periapical region that is associated with rapid growth of a periapical lesion, the size of which can be monitored using either radiographs or histological sections. This rapid growth persists for 15 days and is associated with ''bone-resorbing activity'' that can be detected in homogenates of the lesions and measured using an in vitro bone resorption assay.[119] Following the active resorptive phase, the lesions remain at a stable size for as long as 30 days.[120] During this stationary phase, the bone-resorbing activity declined to 10 to 30% of that in the active growing stage. This last stationary phase is considered to be equivalent to an existing chronic, periapical granuloma in humans that also contains bone-resorptive activity.[48] The cytokines defined in the above studies are found in human periapical lesions in measurable amounts. Lim and colleagues[102] found significant amounts of IL-1β in homogenates of human periapical lesions, even though none of the patients had detectable serum levels of this cytokine. Noninflamed pulp tissue, which served as a control, was also free of the cytokine. Periapical exudates were studied by Matsuo and colleagues[121] for their IL-1α and IL-1β contents. Exudates, obtained through the root canal, contained an average level of 6.57(±0.73) ng/mL of IL-1β and 3.23 (±0.66) ng/mL of IL-1α. The cytokine profile changed following root canal treatment with a tendency of IL-1α to increase and of IL-1β to decrease.[122,123]

CELLULAR SOURCES OF BONE-RESORBING CYTOKINES

Even though IL-1 and TNF may be produced by many cell types, the activated macrophage is considered to be the main source of IL-1α, IL-1β, and TNF-α.[124] On the other hand, TNF-β is commonly considered to be an activated T-lymphocyte product.[125] In view of the above, two cell types should be considered as being responsible for bone-resorbing activity in human periapical lesions: activated T cells and activated macrophages. Not all T lymphocytes or macrophages in the periapical lesion are in a state of activation. Kopp and Schwaring[18] have found that only 6% of the T lymphocytes in human periapical granulomas were activated. Artese and colleagues,[53] who also used human periapical granulomas, demonstrated that while 41% of the mononuclear inflammatory cells were macrophages, only 2 to 3% of these cells were activated and produced IL-1β and TNF-α. The activation states of these cells are closely related to each other: T helper lymphocytes may be activated in an antigen-specific manner by antigen-presenting macrophages that also produce the IL-1 required to accomplish this process. Macrophage activation, as part of the acquired, specific immune response, may be achieved by cytokines such as IFN-γ that are

produced by the activated T lymphocytes (see Figure 1). Macrophages may be activated by other routes, such as by exposure to bacterial endotoxin (LPS), as part of innate, nonspecific immunity.[126]

STUDIES IN ATHYMIC ANIMALS

Athymic rats and mice are powerful tools used to study and demonstrate the essential role of T lymphocytes in other immunobiological processes.[127,128] These animals lack T cells, and consequently T-cell function is missing, rendering a variety of immune responses inactive. Such animals were used in two studies that assumed that periapical bone resorption and the development of periapical lesions would be defective in athymic animals. The results of these studies should be viewed as a turning point in the understanding of the immunobiology of the host response and bone resorption in periapical lesions. Wallstrom and colleagues[129] demonstrated that no significant difference existed between the periapical tissue responses of conventional and athymic rats. Similar results were reported in a study by Tani-Ishii and colleagues[31] using athymic mice. They also found that periapical lesions developed in the T-cell-lacking animals at a rate that precluded the possibility that T lymphocytes are essential prerequisites in the development of these lesions. Even though T lymphocytes may, and most probably do, contribute to the process, alternative routes likely exist, enabling the formation of lesions in their absence.

Activated macrophages may serve in such a route in the formation of periapical lesions. Macrophage activation may occur by a variety of pathways. Cytokines such as IFN-γ, produced by antigen-specific activated T lymphocytes, are the main immune response-related activators of the macrophage.[124,130] In their absence, bacterial endotoxin (LPS) may successfully accomplish this task.[126,131,132] This activation of the macrophage may be viewed as part of innate immunity, independent of a specific response to antigens. This may have been the mechanism by which the lesions developed in the athymic animals. The bacterial content of the infected root canals in these animals gradually developed to 46% gram-negative flora.[133] LPS, derived from these gram-negative bacteria, could activate macrophages in the periapical area, independent of T lymphocytes. These macrophages, in turn, produce their cytokines IL-1α, IL-1β and TNF-α that activate osteoclastic bone resorption. This does not preclude the participation of T cells in the process in normal animals, but rather turns the spotlight to *the main effector cell*: the macrophage. This result is in agreement with the finding that in the rat model, IL-1α is the major bone-resorbing cytokine while TNF-β, the T-cell product, could not be detected in these lesions by antibody-blocking or immunohistochemical studies.[54,117]

OSTEOCLAST ACTIVATION: UPDATED CONCEPT

The concept of osteoclast activation had a missing link for many years. It was well known that both systemic factors such as parathyroid hormone (PTH) and local factors such as IL-1β activate osteoclastic bone resorption. Osteoclasts do not have receptors for either of these agents. The presence of other cells such as osteoblasts or stromal bone marrow cells that do have the proper receptors is required.[134] An intermediate factor, produced by other bone cells and affecting the osteoclasts, had been hypothesized in an attempt to solve this enigma.[113] It was also recognized that bone cells and osteoclasts have to be *in proximity* to each other to allow the activation to take place.[134,135]

The missing link has recently been discovered.[134,136–139] (Figure 4A,B). A group of cell surface receptors and soluble ligands is responsible for up- and downregulation of osteoclastic bone resorption. When bone cells are activated by either PTH or IL-1β, they express a ligand named osteoprotegerin ligand (OPGL, RANK-Ligand) on their surface that has the following two main functions:

(1) Binding to a surface receptor RANK (receptor activator of nuclear factor κB) on mononuclear preosteoclasts, thus inducing their maturation to multinucleated mature osteoclasts
(2) Binding to RANK, also expressed on the surface of mature but inactive osteoclasts, thus activating them to become active bone–resorbing cells with a fully expressed ruffled border.[134]

This process results in increased numbers of active osteoclasts and bone resorption. Such a mechanism also explains the well-established need for cell proximity during osteoclast activation: both receptors and ligands are *surface molecules* that require proximity for binding. Inflammatory bone resorption may have yet another avenue of osteoclast activation, as activated T cells may also express OPGL on their surface, allowing them to directly activate the osteoclasts.[140–142] Downregulation of bone resorption is carried out by a soluble inhibitor, osteoprotegerin (''bone protector'', OPG), that binds to OPGL and serves as a competitive inhibitor of RANK, preventing its binding to OPGL. This soluble inhibitor was among the first to be discovered. It has been clinically tested as an osteoporosis preventing agent with promising results.[143]

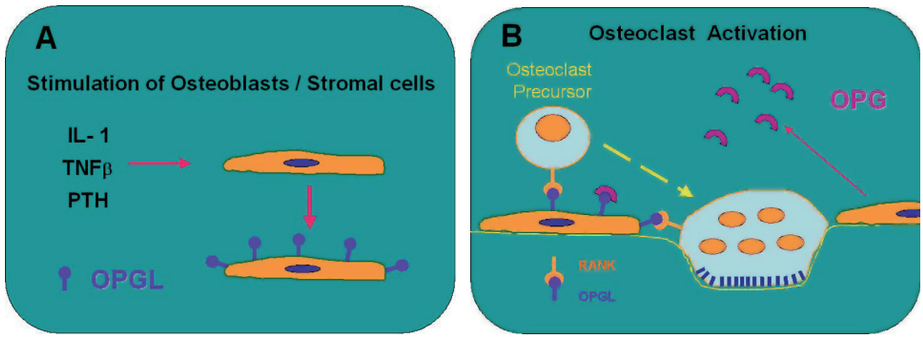

Figure 4 Osteoclast activation via osteoprotegerin ligand (OPGL) (RANK-ligand). *A*, Osteoblasts and bone marrow stromal cells express OPGL (RANK-ligand) when stimulated by either PTH (parathyroid hormone) or the cytokines IL-1 and tumor necrosis factor-β (TNF-β). *B*, Osteoclast precursors that carry the RANK-receptor on their surface are activated to develop into multinucleated osteoclasts, thus increasing the number of osteoclasts in the area. Osteoclasts that are present are activated through their RANK-receptor to become active in bone resorption, expressing a ruffled border. Osteoclast activation is downregulated by the soluble competitive inhibitor osteoprotegerin (OPG) that inhibits the binding of RANK to its ligand OPGL.

OPGL was recently demonstrated to be present in human periapical granulomas where it was found on macrophages and dendritic cells.[144,145] This, taken together with the recent demonstration of OPG production by periodontal ligament fibroblasts, may have important future implications and applications.[146]

Histopathology of Periapical Lesions of Endodontic Origin

The discussion of the histopathology of periapical lesions includes the *apical granuloma*, as well as two entities that may develop within it or from it: *apical abscess* and *apical cyst*.

The *apical granuloma* is an inflammatory lesion dominated by macrophages, lymphocytes, and plasma cells (Figure 5A,B). Abundant capillaries may be found with numerous fibroblasts and connective tissue fibers. It is often encapsulated in collagenous connective tissue. Epithelial cell proliferation is a common finding in apical granuloma. Serial sectioning reveals that epithelial proliferation occurs in 6 to 55% of these lesions.[81,147] They are believed to originate from the epithelial cell rests of Mallasez that proliferate under the influence of cytokines and growth factors generated in the periapical granuloma (Figure 6). Thus, the presence of epithelial cells in a granuloma is a common finding, and its development into a cyst may be considered to be an event that occurs with time within an existing apical granuloma.

PMNs are found in varying numbers in an apical granuloma and may reach a local dominancy within a given area of the granulomatous tissue when an abscess is formed. Thus, an apical abscess may be considered to be a transient or persistent event (in the case of a chronic apical abscess) that occurs within an existing apical granuloma. In general, the histological picture of granulomas varies considerably.[81,148] The simple, well-organized morphological description of apical periodontitis, based on a zonal pattern, hardly represents the common reality. It was originally an extrapolation from observations by Fish in an experimental model.[149] The experimental lesions were produced in guinea pigs by drilling holes in bones and packing them with wool fibers saturated with microorganisms and were not associated with infected root canals. This zonal pattern has been a conceptually useful teaching aid and, as such, has survived through the generations.[150–152] Nevertheless, one should not expect to find it in random biopsies of periapical granulomas, as it does not seem to represent the actual structural variation seen in most periapical lesions. In fact, great structural heterogeneity is the *norm* for apical periodontitis, particularly in chronic lesions.[81,148]

The hallmark of an *apical abscess* is the presence of pus: an area containing liquefied tissue. Abscesses are usually found within a pre-existing apical granuloma and represent a shift in cellular dynamics. The influx of PMNs is dramatically increased to the extent that tissue macrophages are no longer able to effectively cope with the tissue damage caused by hydrolytic enzymes released from a vast number of dying PMNs. Connective tissue constituents such as collagen and hyaluronic acid are degraded and the tissue in the center of the abscess is liquefied. On the periphery of the abscess, the tissue of

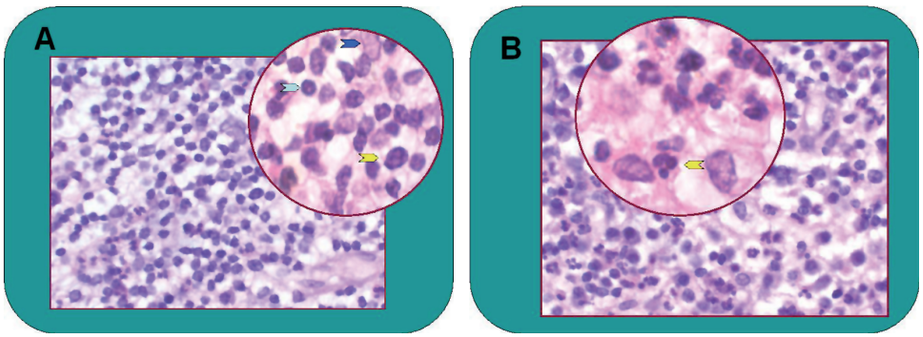

Figure 5 Apical granuloma: histopathology. *A*, The lesion is dominated by lymphocytes (green arrow) and macrophages (yellow arrow), with abundant fibroblasts (blue arrow). *B*, A chronic periapical lesion with a polymorphonuclear leukocyte (PMN) infiltrate (yellow arrow).

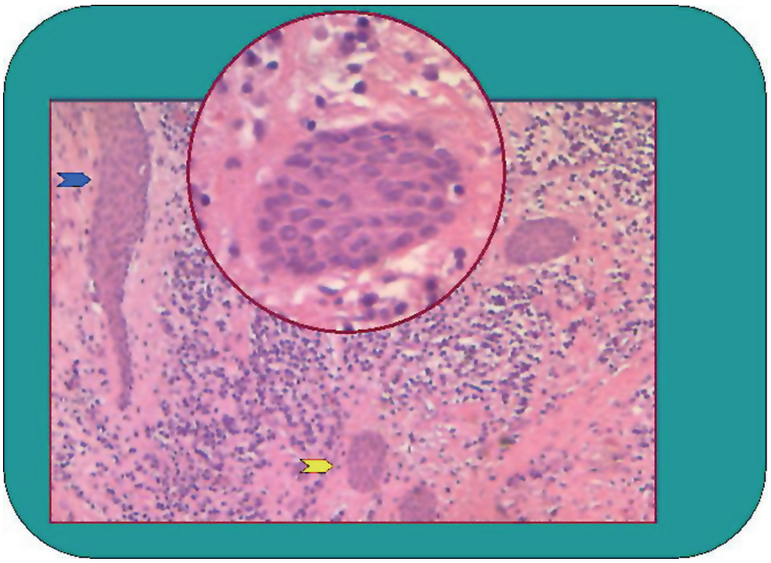

Figure 6 Epithelial strands in an apical granuloma. Epithelial strands form a network in an apical granuloma. They may be observed in a longitudinal section (blue arrow) or at cross section (yellow arrow and enlargement). This epithelium originated from the rest cells of Malassez that proliferate under the influence of cytokines and growth factors in the apical granuloma. They are the source of the epithelial lining of cysts that may develop in apical granulomas.

the apical granuloma persists and its adjacent layer is infiltrated with a large number of PMNs that are migrating from the nearest blood vessel to end their life in the pus-containing center of the abscess.

A histological continuum may be found between an apical granuloma that contains a small number of infiltrating PMNs, through a granuloma with a large number of infiltrating PMNs, and an established apical abscess. The former may be histologically termed a granuloma with acute inflammation, but only the latter warrants the term apical abscess.

An *apical cyst* is an epithelium-lined cavity that contains fluid or semisolid material and is commonly surrounded by dense connective tissue (Figure 7). Apical cysts are associated with teeth that have necrotic pulps and an infected root canal system and develop within the periapical inflammatory lesion, the granuloma. The cyst cavity is most commonly lined with stratified squamous epithelium of varying thickness that originates from the epithelial rest cells of Malassez. Nevertheless, lining with ciliary epithelium that originates from an adjacent maxillary sinus may also be

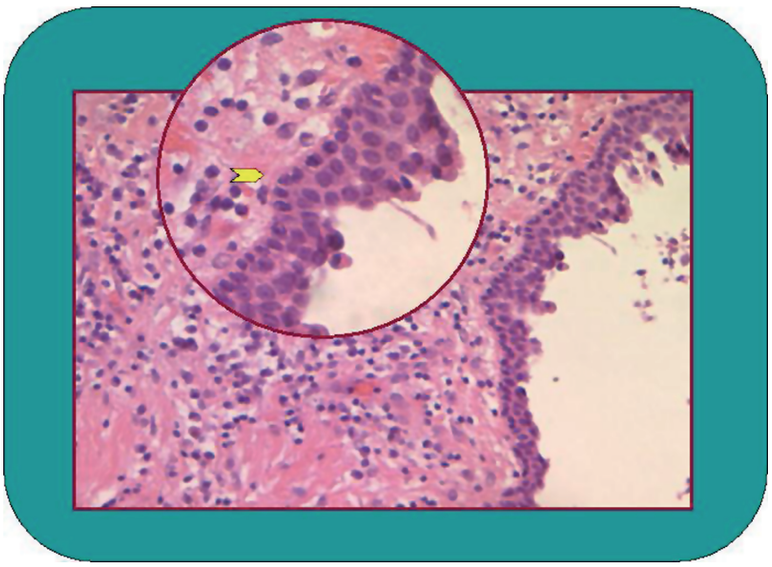

Figure 7 Apical cyst. A partial view of the wall of a space that was filled with liquid and lined with stratified cuboidal epithelium (yellow arrow and enlargement), originating from the epithelial rests of Malassez.

found.[153] The epithelial lining may be continuous, but it is often disrupted or even completely missing, most probably due to a secondary infection. Periapical cysts are divided into *pocket cysts* (bay cyst) and *true cysts*.[154–156]

A periapical pocket cyst (bay cyst) is an apical inflammatory cyst that contains a sac-like, epithelium-lined cavity that is open to and continuous with the root canal space.[154–156] True apical cysts are located within the periapical granuloma with no connection between their cavity and that of the root canal space. More than half of the apical cysts are true apical cysts while the remainder are of the pocket cyst variety.[81,154–156]

The mechanism of cyst formation in periapical inflammatory lesions has been the subject of much debate.[81,157–159] Two main theories were proposed. The ''nutritional deficiency theory'' assumes that epithelial proliferation results in an epithelial mass that is too large for nutrients to reach its core, resulting in necrosis and liquefaction of the cells in its center, thus forming the cystic cavity. The ''abscess theory'' assumes that tissue liquefaction occurred first, at the central part of an abscess, that was later lined by locally proliferating epithelium, due to the inherent nature of epithelial cells to cover the exposed connective tissue surfaces.[159] The mechanism by which cysts grow and expand is not fully understood and most probably involves inflammatory mediators that are present in the lesion.[81]

CLINICAL MANIFESTATION AND DIAGNOSTIC TERMINOLOGY

Periapical lesions of endodontic origin vary greatly in their clinical manifestation. Their classification is based on the clinical presentation at a given time point; it may shift from one diagnosis to another, with time. Such shifts may be understood and explained on the basis of the biological events discussed above.

The diagnostic terminology used here will include *normal periapical tissues*, *symptomatic apical periodontitis* (acute apical/periradicular periodontitis), *asymptomatic apical periodontitis* (chronic apical/periradicular periodontitis), *acute apical abscess* (acute periradicular abscess), *chronic apical abscess* (chronic periradicular abscess and suppurative apical/periradicular abscess), *cellulitis, condensing osteitis* (focal sclerosing osteomyelitis, periradicular osteosclerosis, sclerosing osteitis, and sclerotic bone), and *apical scar*.

Clinical manifestations that are used to make a periapical diagnosis derive from (1) subjective information derived from a patient's anamnesis and objective data derived from (2) radiographic examination, as well as (3) results of direct observation and physical examination of the patient, the subjected tooth, and surrounding tissues.

A correlation between the clinical diagnosis and that derived from histopathological examination of the tissue is limited. An apical abscess may be clinically recognized by the presence of pus; however, clinical findings and

conventional radiographs cannot predict if another lesion is a granuloma or a cyst.[160,161] Accordingly, clinical manifestations and diagnostic terminologies were discussed in this chapter separately from histopathological features of periapical lesions. A discussion of the latter will be in terms of form and function in order to allow the reader to understand the processes that lead to the clinical manifestations.

Inflammatory lesions of endodontic origin appear in response to stimuli originating from a root canal. As such, they are located around any of the root canal system portals of exit. Pathogenesis and pathology of the inflammatory lesions will be similar at any of those portals of exit; nevertheless, the clinical and radiographic manifestation may vary greatly with the location of the involved portal. The term *periradicular* rather than periapical has often been used to express this diversity.[66,102,162–165] The terms *apical* or *periapical* were used throughout this text for the sake of simplicity, as well as to express the fact that this is the most common manifestation of inflammatory lesions of endodontic origin.

NORMAL PERIAPICAL TISSUES

In normal periapical tissues, the tooth is not tender to percussion or pressure and there is not any tenderness to palpation of the mucosa overlying the periapical region. There is no swelling and there are no symptoms noted by the patient.[166] Radiographically, the lamina dura is intact and the periodontal ligament space has a normal and consistent width along the entire root, which is similar to that of the adjacent teeth.[166] Recognition of ''normal'' is essential to estimate changes that may occur with disease, as well as their gradual disappearance with healing.

SYMPTOMATIC APICAL PERIODONTITIS

Symptomatic apical periodontitis occurs within a previously healthy periapical region in response to either microbiological or physical irritation.[166] The former may result from an initial emergence of bacteria or their products from an infected root canal into the apical periodontium. The latter may result from endodontic treatment when there is mechanical or chemical injury to the apical tissues. They may occur together when the physical insult carry bacteria with it, from the infected root canal into the apical periodontium.

If the insult was short in nature, such as with trauma induced by a file passing through a sterile, noninfected pulp tissue, the symptoms will usually soon subside and healing will take place. On the other hand, if the insult is continuous and persistent, such as the permanent communication between bacteria growing in the root canal and the host response in the apical periodontium, events may take one of two other routes. It may either become more and more symptomatic, a process that may develop into an acute apical abscess and facial cellulitis. Alternatively, it may take a quieter route and gradually become asymptomatic apical periodontitis, with slight or no symptoms and with typical periapical bone resorption. Factors that dictate which route will be taken are not completely clear, but they most probably involve the nature of the bacteria. Their susceptibility or ability to survive the host response may shift the balance one way or the other. Symptomatic apical periodontitis represents a shift of the balance established between the bacteria and the host, in an asymptomatic apical periodontitis lesion (see below). Such shifts may occur due to a variety of reasons, starting with naturally occurring events and ending with iatrogenically induced exacerbation.

ASYMPTOMATIC APICAL PERIODONTITIS

Asymptomatic apical periodontitis is a long-standing periapical inflammatory lesion with radiographically visible periapical bone resorption but with minimal or no clinical symptoms. Its development is uneventful and often goes unnoticed by the patient until it is discovered on a radiograph or until it develops into a symptomatic lesion. Histologically, the radiolucent area associated with asymptomatic apical periodontitis will be either a granuloma or a cyst. The radiological appearance of the lesion may take a wide range of shapes and sizes that has tempted clinicians to search for a correlation between size and morphology of the lesion and its histological nature. The appearance of the radiolucent area in conventional radiographs cannot predict its histological diagnosis.[160,161,167,168] Recent innovative technologies may be more predictive (see Chapter 15).

ACUTE APICAL ABSCESS

An acute apical abscess is characterized by rapid onset, spontaneous pain, tenderness of the tooth to pressure, pus formation, and eventual swelling of the associated tissues. At the initial stages of its formation, the process may be extremely painful, as pressure builds up in the restricted periapical space. The establishment of drainage through the root canal may, in some cases, end the agonizing process. Left to natural events, an acute apical abscess will sometimes subside. In most cases, the overlying cortical plate will eventually perforate and purulence will accumulate under the periosteum producing a painful condition. Only with the perforation of the

periosteum will the pus be able to drain and allow the major pain to subside. At this stage, a local swelling will appear and an incision for drainage should be made in the overlying tissues. In some cases, natural drainage will be established within a few days by perforation of the covering tissue. In other cases, the swelling will remain for some time before it gradually subsides.

CHRONIC APICAL ABSCESS

A sinus tract is the hallmark of the chronic apical abscess. The inflammatory process perforates one of the cortical plates and a draining sinus tract is established that allows for continuous discharge of pus forming in the periapical lesion through the oral mucosa or, in rare cases, through the skin. Typically, a stoma of a parulis can be detected that, from time to time, will discard the pus. Sometimes a sinus tract will lead to the maxillary sinus and will go unobserved. A sinus tract may also exit in the gingival sulcus and must be differentiated from periodontal disease. A chronic apical abscess is most commonly, but not always, associated with an apical radiolucency. It is asymptomatic or only slightly symptomatic, and the patient may often be unaware of its presence. This may last as long as the sinus tract is not obstructed. Even when such an obstruction occurs, it is most likely that any swelling will be of limited duration and will be limited to the local area of the sinus tract, as both the bone and the periosteum are already perforated.

CELLULITIS

Cellulitis is a symptomatic edematous inflammation associated with diffuse spreading of invasive microorganisms throughout connective tissue and facial planes. Diffuse swelling of facial or cervical tissues is its main clinical feature. Cellulitis is usually a sequel of an apical abscess that penetrated the bone, allowing the spread of pus along the paths of least resistance, between facial structures. This usually implies the facial planes between the muscles of the face or the neck (see Chapter 20). Spreading of an infection may or may not be associated with systemic symptoms such as fever and malaise. Since cellulitis is usually a sequel of an uncontrolled apical abscess, other clinical features typical of an apical abscess are also expected. Spreading of an infection into adjacent and more remote connective tissue compartments may result in serious or even life-threatening complications. Cases of Ludwig's angina,[169] orbital cellulites,[170] cavernous sinus thrombosis,[171] and even death from a brain abscess[172] originating from a spreading dental infection have been reported.

CONDENSING OSTEITIS

Condensing osteitis (focal sclerosing osteomyelitis) is a diffuse radiopaque lesion believed to represent a localized bony reaction to a low-grade inflammatory stimulus, usually seen at the apex of a tooth (or its extraction site) in which there has been a long-standing pulp pathosis. It is characterized by overproduction of bone in the periapical area, mostly around the apices of mandibular molars and premolars that had long-standing pulp pathosis. The pulp of the involved tooth may be chronically inflamed, but since such inflammation may lead to pulp necrosis, it may be expected that, at some stage, the involved pulp is nonvital. The radiopacity may or may not respond to endodontic treatment.

APICAL SCAR

Apical scar is not an inflammatory lesion, but rather an uncommon pattern of healing of an apical inflammatory lesion. It consists of a dense collagenous connective tissue in the bone at or near the apex of a root with a distinctive radiolucent presentation.[173,174] It represents a form of healing that is usually associated with a root that has been treated surgically. Perforation of both facial and lingual osseous cortices is believed to result in collagenous rather than osseous healing.[173,175] Maxillary lateral incisors are the most frequently affected teeth. Definitive clinical diagnosis is very difficult without histopathological examination. A case history may be helpful, especially when a detailed surgical report is included. Periapical inflammation that, with time, resorbed and perforated both cortices may also result in an apical scar. Since such cases are quite rare,[160,174,176] the probability that a given nonresponsive periapical lesion, with no surgical involvement, is actually an apical scar is extremely low when compared to that of a rather common posttreatment disease.

Traditional Concepts Versus a Futuristic View

The current concept and rational of endodontic treatment of periapical lesions has not changed significantly for many years. It is centered on one issue: stopping the bacterial stimulation of the host response at the apical foramen/foramina that would allow healing of the lesion. Methods used to achieve this goal include the following:

(1) Cleaning, shaping, and disinfection, aimed at thorough elimination of bacteria from the root canal system

(2) In case the above procedure fails, surgical removal of residual infected tissue in apical ramifications of the root canal system inaccessible to the above-mentioned procedure and thus may still harbor bacteria
(3) Root-end sealing of the root canal system to prevent continuous bacterial stimulus that may still exist after the completion of stages ''a'' and/or ''b'' described above.

This comprises a simple and rather mechanistic rational that has worked well for several generations. Nevertheless, by limiting itself to this concept alone, the profession may ignore, or at least make no use of the vast information and potential tools generated over the last two decades in bone biology research and in studies aimed to pharmacologically modulate destructive immune responses.

The ideas, information, and concepts that follow should not be taken as proven or approved clinical therapeutic protocols. They should rather provide a conceptual framework for understanding possibilities and new avenues of thought that will most probably become possible in the coming years. They are aimed to prepare the minds and hearts of future leaders of the profession and inspire them to look into additional therapeutic avenues. Endodontics should not limit itself only to better ways of cleaning, shaping, and obturation. Biological research may provide, in the not so far future, new concepts and methods to supplement traditional ones.

Endodontic treatment is aimed to eliminate bacteria from infected root canals that will later be sealed to prevent recontamination. With the elimination of the bacterial stimuli that evoked the periapical inflammation, the periapical lesion should resolve and repair should take place. Healing of the lesions may take many months.[177–180] It may be argued that if a given lesion eventually heals in 12, 24, or even 36 months, there is no benefit in rushing the process. Nevertheless, shortening the healing time may have clinical importance, as it may (1) allow earlier decisions in regard to the restorative treatment plan related to the treated teeth and (2) limit the period for which temporary crowns and bridges are used; temporary restorations that may leak and allow recontamination of the treated root canal.

The prolonged healing process of many periapical lesions raises the possibility that the activated cells in the lesion may maintain their state of activation long after the initial cause of their activation has been eliminated. Namely, the activation state may outlive its useful purpose and become a burden. Macrophages are known to persist in tissues for many months and if their state of activation persists, they may inhibit the fibroblasts, maintain osteoclastic activity, and inhibit osteogenesis, thus preventing both soft connective tissue and bone repair from taking place.[126,181] Indirect support for this notion may be found in a study carried out, for a totally different purpose, by Kvist and Reit.[178] Healing of periapical lesions was compared following surgical and nonsurgical retreatment. At 12 months, a significant difference was found in favor of surgical treatment that faded by 48 months to almost no difference between the groups (Figure 8). If the idea presented above is true, these results may support early surgical removal of the tissue containing activated macrophages, allowing its replacement with fresh granulation tissue that contains a fresh set of cells that will not delay repair.

If this concept is valid, it may be important and possible to monitor the state of activation of periapical macrophages by sampling the interstitial fluid of the lesion through the root canal.[121,123,182] Recently Kuo and colleagues[122] were successful in performing such measurements and were able to quantify the IL-1β concentration in apical exudates and correlate it with clinical and radiological features of the lesions. A longitudinal study that evaluates the correlation between the diminishing IL-1β content of the lesions and their gradual radiographic repair will be required to prove this point.

Assuming that such inhibitory mechanisms are involved in the prolonged and delayed repair of periapical lesions, pharmacological modulation of the process may be considered (Figure 9). Stashenko and colleagues[113] demonstrated that an IL-1 receptor antagonist may be used in animals to reduce the bone-resorbing activity and the formation of periapical lesions. Similarly, NSAIDs were successfully used for a similar purpose in both experimental and human periodontal diseases, as well as in the cat model for periapical lesions.[183,184] These two approaches were directed at either blocking the *binding* of the already produced cytokine to its target cells or *interfering with its action* on osteoclasts and osteoblasts that may involve prostaglandin production.[48,117]

Tetracyclines may be used to inhibit cytokine *secretion* by activated macrophages.[185,186] Shapira and colleagues[186] studied tetracycline inhibition of TNF and IL-1 production by LPS-activated macrophages and found its effect to be at a post-transcriptional level: both mRNA and the cytokines themselves were produced but they were not secreted into the cells' surroundings. Tetracyclines may also inhibit bone resorption by other mechanisms that are unrelated to their antimicrobial capacity. This is mediated by inhibition of connective tissue metalloproteases.[187] Accordingly, it has recently been demonstrated that systemic low-dose tetracycline inhibits the formation of periapical lesions in rats by a mechanism(s) that is unrelated to their antimicrobial effects.[188] An alternative strategy may be to try to locally ''turn off'' the activated macrophages, thus lowering the

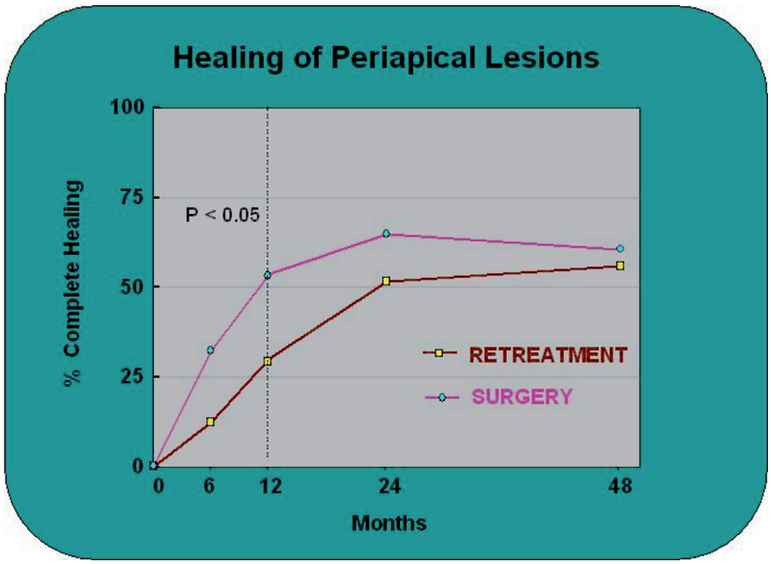

Figure 8 Healing of periapical lesions following surgical removal of the chronically inflamed tissue, compared to healing after retreatment. By 48 months, no difference was found between the groups. Nevertheless, healing was much faster when the tissue was surgically removed, with a significant difference at 12 months. Adapted from Kvist T and Reit C.[178]

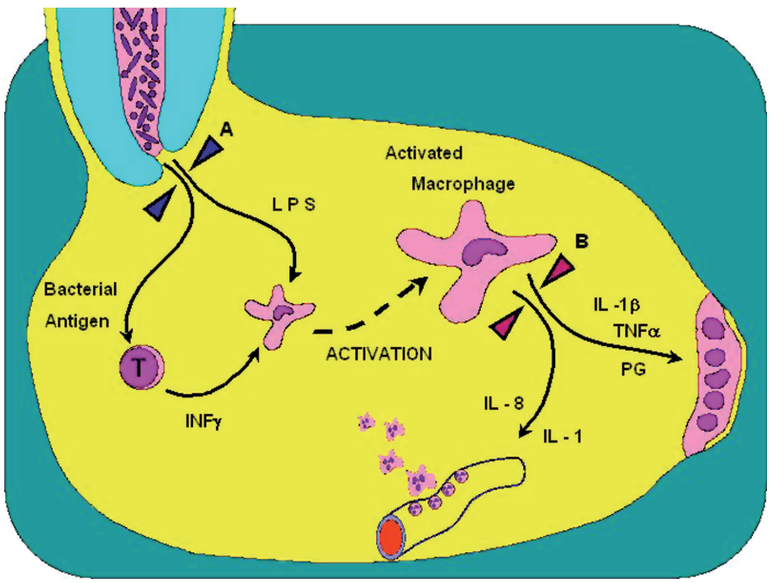

Figure 9 Potential pharmacological modulation of the healing of apical granuloma. *A*, Traditional endodontic approach: elimination of the bacteria from the root canal, followed by obturation of the root canal to prevent recontamination. *B*, Potential additional sites for pharmacological modulation. Either the local production and release of bone-resorbing cytokines or their effect on bone cells is a potential target for ''turning off'' the lesion, once its activity had outlived its useful, protective purpose.

local *production* of IL-1 in the lesion. Modulation of macrophage activation has been attempted both in vivo and in vitro using glucocorticoid steroids.[126,130,189,190] Macrophages, activated to become tumoricidal, were ''turned off'' in vivo by a process involving steroids.[189] Recently, Metzger and colleagues[126] reported that suppression of fibroblast proliferation by LPS-activated macrophages was reversed using hydrocortisone.

Dexamethasone also inhibited periapical lesion formation in the rat model, most probably by a similar mechanism.[191] Such effects on macrophage activation and production of its mediators have also been reported by others and were attributed to inhibitory effects of the steroids at the gene transcription level.[130,192,193]

If and when bacteria are no longer present in the root canal, the state of activation of the macrophages may outlive its useful and beneficial purpose. Attempts to "turn off" the host response and its effects in the lesion may represent a *new biological treatment modality* that may alleviate suppression and enhance repair in these lesions (see Figure 9). Local delivery of desired pharmacological agents should be simpler in the closed environment of the periapical lesion, as compared to similar attempts in periodontal pockets. *Local sustained delivery* of drugs aimed at this goal may easily be achieved. By using biodegradable slow release devices in the form of a point that may be inserted through the root canal into periapical tissues, it may locally deliver the drug or drug combination for a predetermined period of time.

A better understanding of the immunobiology of periapical lesions may eventually result in a different endodontic practice than is encountered today. Chairside diagnostic kits that will allow definition of a periapical lesion as "active" or "healing," by sampling via the root canal prior to obturation, seem logical and possible. Similarly, pharmacological modulation of the healing process may also be near.

An alternative approach has recently emerged with the development of a device that allows enucleation of the periapical tissue, through the root canal and the apical foramen. The Apexum ™ protocol is applied just before root canal obturation. Once cleaning, shaping, and disinfection of the root canal is completed, the apical foramen is enlarged by passing a No. 35 rotary file to 1 to 2 mm beyond the apex. This passage is used to insert a specially designed nickel–titanium wire into the periapical tissue that rotates and minces the tissue (Figure 10A,B). This is followed by a biodegradable fiber rotated at a higher speed that turns the tissue into a thin suspension that is then washed out with saline using a

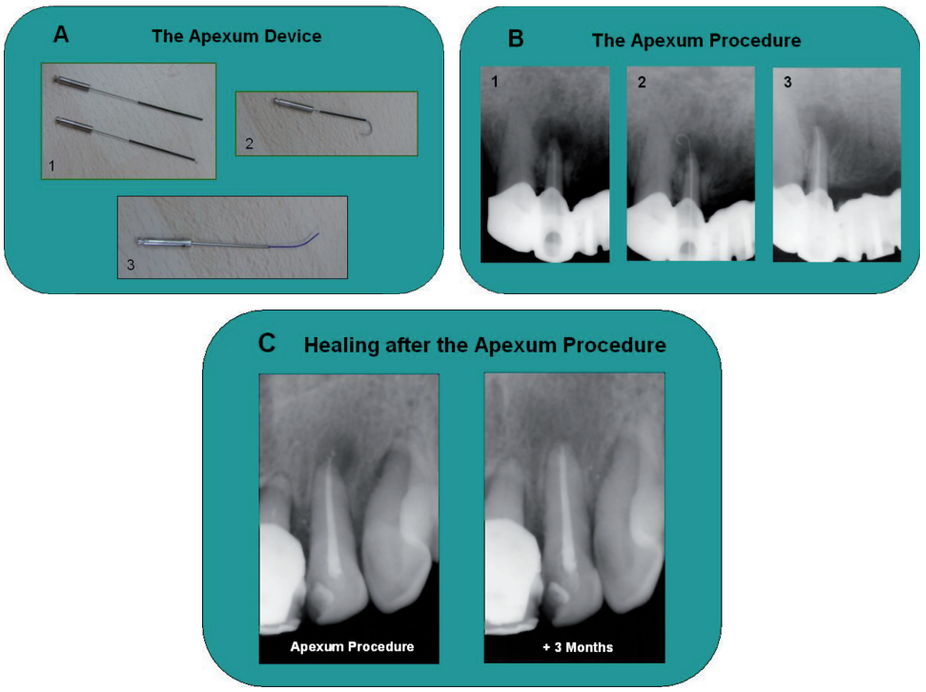

Figure 10 Apexum©: A method for minimally invasive removal of periapical tissues through the root canal. *A*, The Apexum NiTi Ablator© (1, 2). The black tube contains a nickel–titanium wire that is protruded when pushed through the apical foramen. Upon entering the periapical area, it takes a specially designed form (2) and is rotated at 300 rpm, mincing the soft tissue, while being deflected from the surrounding bone. The Apexum PGA Ablator© (3) has a fiber made of biodegradable material that is rotated in the periapical area at 5000 rpm, grinding the tissue to a thin suspension that is then washed out with saline. *B*, (1) The nickel–titanium tube is inserted to the working length. (2) The Apexum NiTi Ablator is pushed and inserted into the periapical area. (3) Once the Apexum procedure is completed, the root canal is obturated with a root canal filling. *C*, A maxillary incisor immediately after the completion of the procedure and 3 months later. Note the rapid bone healing in the periapical area.

30-G needle. A fresh blood clot forms in the periapical bony crypt. Ongoing clinical trials indicate that much faster periapical healing occurs, similar to that encountered with apical surgery (see Figure 10C).

ALVEOLAR OSTEOPOROSIS ASSOCIATED WITH BRUXISM

Ingle[194] and then Natkin and Ingle[195] have described a syndrome (''Ingle's Syndrome'') affecting adolescent females related to protrusive bruxism. By the age of 13 or 14, over one-third of the mandibular incisors had been ''ground away'' (see Figure 11A–D). Most of the lingual enamel of the maxillary incisors were also destroyed. In one case, a pulpal horn had already been exposed. In all the cases, osteoporotic bone destruction in the mental area of the mandible is apparent radiographically. Also seen radiographically, from left to right, is a graceful curve formed by the worn incisal edges of the lower anterior teeth.

When one realizes that this amount of destruction had occurred since eruption, within 6 or 7 years, one

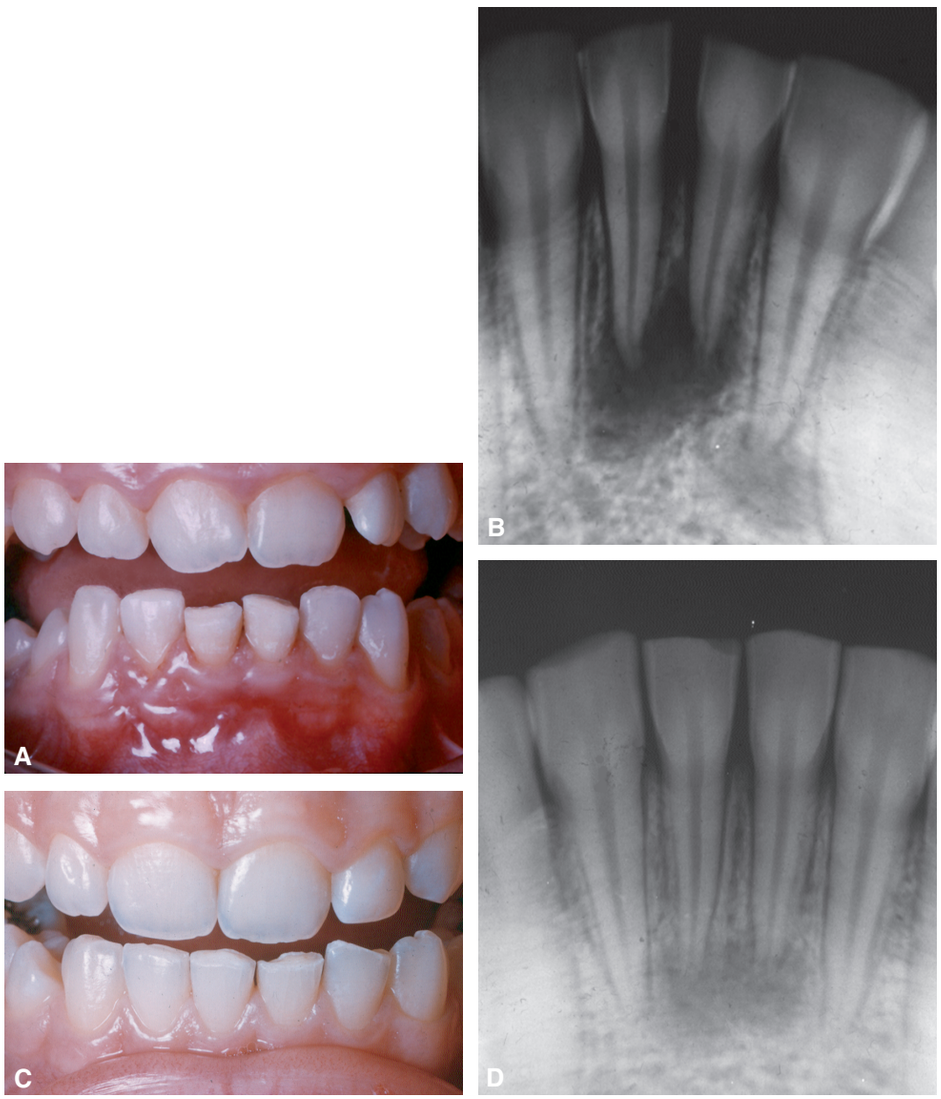

Figure 11 *A-D*, Osteoporosis and pulp death in 14-year-old identical twin sisters with the same syndrome—Adolescent Female Protrusive Bruxism. In both cases, incisal wear involving mandibular anterior teeth from compulsive protrusive bruxism. Nearly one-third of the teeth have been destroyed in 7 or 8 years. Radiographs demonstrate the osteoporotic bone loss, the periapical lesions, and the graceful curve, left to right, of the worn incisal edges common to this condition.

recognizes the intensity of the habit. The first girl seen was also a serious nail biter; ''down to the quick'' as she stated.

Not only the involved teeth were mobile, even though normal periodontal attachments were present, but also some of the pulps were necrotic. Consulting pathologists termed the bony destruction ''traumatic osteoporosis'' comparable to leg bone destruction seen in industry wherein a worker constantly presses a treadle downward, day after day.

Why do these girls grind their teeth in a protrusive motion rather than the usual lateral grinding chewing motion seen in most bruxers? The answer might be that they derive pleasure from this habit. One consulting psychiatrist suggested that this protrusive motion is the same suckling motion used by infants in nursing. Babies do not nurse by sucking (as adults do through a straw), but by moving the mandible forth and back, ''milking'' the nipple. This is a pleasurable and satisfying period of time in a baby's life. And these girls might well have some psychological need to continue the habit. Following endodontic therapy, ''night guards'' were constructed for these patients. They either threw them away after 2 or 3 months or chewed through them.

References

1. Genco CA, Cutler CW, Kapczynski D, et al. A novel mouse model to study the virulence of and host response to *Porphyromonas (Bacteroides) gingivalis*. Infect Immun 1991;59:1255–63.
2. Baumgartner JC, Falkler WA Jr, Beckerman T. Experimentally induced infection by oral anaerobic microorganisms in a mouse model. Oral Microbiol Immunol 1992;7:253–6.
3. Luscinskas FW, Cybulsky MI, Kiely JM, et al. Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. J Immunol 1991;146:1617–25.
4. Lane TA, Lamkin GE, Wancewicz EV. Protein kinase C inhibitors block the enhanced expression of intercellular adhesion molecule-1 on endothelial cells activated by interleukin-1, lipopolysaccharide and tumor necrosis factor. Biochem Biophys Res Commun 1990;172:1273–81.
5. Issekutz AC, Rowter D, Springer TA. Role of ICAM-1 and ICAM-2 and alternate CD11/CD18 ligands in neutrophil transendothelial migration. J Leukoc Biol 1999;65:117–26.
6. Kabashima H, Nagata K, Maeda K, Iijima T. Involvement of substance P, mast cells, TNF-alpha and ICAM-1 in the infiltration of inflammatory cells in human periapical granulomas. J Oral Pathol Med 2002;31:175–80.
7. Cutler CW, Kalmar JR, Arnold RR. Phagocytosis of virulent *Porphyromonas gingivalis* by human polymorphonuclear leukocytes requires specific immunoglobulin G. Infect Immun 1991;59:2097–104.
8. Naidorf IJ. Immunoglobulins in periapical granulomas: a preliminary report. J Endod 1975;1:15–18.
9. Hamachi T, Anan H, Akamine A, et al. Detection of interleukin-1 beta mRNA in rat periapical lesions. J Endod 1995;21:118–21.
10. Siqueira JF, Rocas IN, De Uzeda M, et al. Comparison of 16S rDNA-based PCR and checkerboard DNA–DNA hybridization for detection of selected endodontic pathogens. J Med Microbiol 2002;51:1090–6.
11. Sunde P, Tronstad L, Eribe R, et al. Assessment of periradicular microbiota by DNA–DNA hybridization. Endod Dent Traumatol 2000;16:191–6.
12. Pulver WH, Taubman MA, Smith DJ. Immune components in human dental periapical lesions. Arch Oral Biol 1978;23:435–43.
13. Johannessen AC, Nilsen R, Skaug N. Deposits of immunoglobulins and complement factor C3 in human dental periapical inflammatory lesions. Scand J Dent Res 1983;91:191–9.
14. Cymerman JJ, Cymerman DH, Walters J, et al. Human T lymphocyte subpopulations in chronic periapical lesions. J Endod 1984;10:9–11.
15. Barkhordar RA, Desouza YG. Human T-lymphocyte subpopulations in periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1988;65:763–6.
16. Babal P, Soler P, Brozman M, et al. In situ characterization of cells in periapical granuloma by monoclonal antibodies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1987;64:348–52.
17. Poulter LW, Seymour GJ, Duke O, et al. Immunohistological analysis of delayed-type hypersensitivity in man. Cell Immunol 1982;74:358–69.
18. Kopp W, Schwarting R. Differentiation of T lymphocyte subpopulations, macrophages, and HLA-DR-restricted cells of apical granulation tissue. J Endod 1989;15:72–75.
19. Stashenko P, Yu SM. T helper and T suppressor cell reversal during the development of induced rat periapical lesions. J Dent Res 1989;68:830–4.
20. Baumgartner JC, Falkler WA Jr. Reactivity of IgG from explant cultures of periapical lesions with implicated microorganisms. J Endod 1991;17:207–12.
21. Kettering JD, Torabinejad M, Jones SL. Specificity of antibodies present in human periapical lesions. J Endod 1991;17:213–6.

22. Shimauchi H, Takayama S, Narikawa-Kiji M, et al. Production of interleukin-8 and nitric oxide in human periapical lesions. J Endod 2001;27:749–52.

23. Wuyts A, Proost P, Put W, et al. Leukocyte recruitment by monocyte chemotactic proteins (MCPs) secreted by human phagocytes. J Immunol Meth 1994;174:237–47.

24. Lukic A, Vojvodic D, Majstorovic I, Colic M. Production of interleukin-8 in vitro by mononuclear cells isolated from human periapical lesions. Oral Microbiol Immunol 2006;21:296–300.

25. Kawashima N, Okiji T, Kosaka T, Suda H. Kinetics of macrophages and lymphoid cells during the development of experimentally induced periapical lesions in rat molars: a quantitative immunohistochemical study. J Endod 1996;22:311–6.

26. Stashenko P, Wang CY, Tani IN, Yu SM. Pathogenesis of induced rat periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994;78:494–502.

27. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Critic Rev Oral Biol Med 1998;9:498–521.

28. Stashenko P, Yu SM. T helper and T suppressor cell reversal during the development of induced rat periapical lesions. J Dent Res 1989;68:830–4.

29. Stashenko P, Wang CY, Tani-Ishii N, Yu SM. Pathogenesis of induced rat periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994;78:494–502.

30. Wallstrom JB, Torabinejad M, Kettering J, McMillan P. Role of T cells in the pathogenesis of periapical lesions. A preliminary report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1993;76:213–8.

31. Tani-Ishii N, Kuchiba K, Osada T, et al. Effect of T-cell deficiency on the formation of periapical lesions in mice: histological comparison between periapical lesion formation in BALB/c and BALB/c nu/nu mice. J Endod 1995;21:195–9.

32. Akamine A, Hashiguchi I, Toriya Y, Maeda K. Immunohistochemical examination on the localization of macrophages and plasma cells in induced rat periapical lesions. Endod Dent Traumatol 1994;10:121–8.

33. Babal P, Brozman M, Jakubovsky J, et al. Cellular composition of periapical granulomas and its function. Histological, immunohistochemical and electron microscopic study. Czech Med 1989;12:193–215.

34. Marton IJ, Kiss C. Characterization of inflammatory cell infiltrate in dental periapical lesions. Int Endod J 1993 26:131–6.

35. Piattelli A, Artese L, Rosini S, et al. Immune cells in periapical granuloma: morphological and immunohistochemical characterization. J Endod 1991;17:26–9.

36. Matsuo T, Ebisu S, Shimabukuro Y, et al. Quantitative analysis of immunocompetent cells in human periapical lesions: correlations with clinical findings of the involved teeth. J Endod 1992;18:497–500.

37. Matsuo T, Nakanishi T, Ebisu S. Immunoglobulins in periapical exudates of infected root canals: correlations with the clinical findings of the involved teeth. Endod Dent Traumatol 1995;11:95–9.

38. Kettering JD, Torabinejad M, Jones SL. Specificity of antibodies present in human periapical lesions. J Endod 1991;17:213–6.

39. Baumgartner JC, Falkler WA Jr. Biosynthesis of IgG in periapical lesion explant cultures. J Endod 1991;17:143–6.

40. Baumgartner JC, Falkler WA Jr. Detection of immunoglobulins from explant cultures of periapical lesions. J Endod 1991;17:105–10.

41. Mustelin T, Coggeshall KM, Isakov N, Altman A. T cell antigen receptor-mediated activation of phospholipase C requires tyrosine phosphorylation. Science 1990;247:1584–7.

42. Kaneko T, Okiji T, Kan L, et al. Ultrastructural analysis of MHC class II molecule-expressing cells in experimentally induced periapical lesions in the rat. J Endod 2001;27:337–42.

43. Kaneko T, Okiji T, Kan L, et al. An immunoelectronmicroscopic study of class II major histocompatibility complex molecule-expressing macrophages and dendritic cells in experimental rat periapical lesions. Arch Oral Biol 2001;46:713–20.

44. Lukic A, Vasilijic S, Majstorovic I, et al. Characterization of antigen-presenting cells in human apical periodontitis lesions by flow cytometry and immunocytochemistry. Int Endod J 2006;39:626–36.

45. Stern MH, Dreizen S, Mackler BF, et al. Quantitative analysis of cellular composition of human periapical granuloma. J Endod 1981;7:117–22.

46. Harris DP, Goodrich S, Mohrs K, et al. Cutting edge: the development of IL-4-producing B cells (B effector 2 cells) is controlled by IL-4, IL-4 receptor alpha, and Th2 cells. J Immunol 2005;175:7103–7.

47. Naldini A, Morena E, Filippi I, et al. Thrombin inhibits IFN-gamma production in human peripheral blood mononuclear cells by promoting a Th2 profile. J Interferon Cytokine Res 2006;26:793–9.

48. Wang CY, Stashenko P. Characterization of bone-resorbing activity in human periapical lesions. J Endod 1993;19:107–111.

49. Metzger Z. Macrophages in periapical lesions. Endod Dent Traumatol 2000;16:1–8.

50. Stern MH, Dreizen S, Mackler BF, Levy BM. Isolation and characterization of inflammatory cells from the human periapical granuloma. J Dent Res 1982;61:1408–12.

51. Ofek I, Goldhar J, Keisari Y, Sharon N. Nonopsonic phagocytosis of microorganisms. Annu Rev Microbiol 1995;49:239–76.

52. Okiji T, Kawashima N, Kosaka T, et al. Distribution of Ia antigen-expressing nonlymphoid cells in various stages of induced periapical lesions in rat molars. J Endod 1994;20:27–31.

53. Artese L, Piattelli A, Quaranta M, et al. Immunoreactivity for interleukin 1-beta and tumor necrosis factor-alpha and ultrastructural features of monocytes/macrophages in periapical granulomas. J Endod 1991;17:483–7.

54. Tani-Ishii N, Wang CY, Stashenko P. Immunolocalization of bone-resorptive cytokines in rat pulp and periapical lesions following surgical pulp exposure. Oral Microbiol Immunol 1995;10:213–9.

55. Grossman LI. Endodontic practice. 6th ed. Philadelphia, PA: Lea & Febiger; 1965.

56. Shindell E. A study of some periapical radiolucencies and their significance. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1961;14:1057–65.

57. Moller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr 1966;74:Suppl-380.

58. Happonen RP, Arstila P, Viander M, et al. Comparison of polyclonal and monoclonal antibodies to *Actinomyces* and *Arachnia* species. Scand J Dent Res 1987;95:136–43.

59. Happonen RP, Soderling E, Viander M, et al. Immunocytochemical demonstration of *Actinomyces* species and *Arachnia propionica* in periapical infections. J Oral Pathol 1985;14:405–13.

60. Sjogren U, Happonen RP, Kahnberg KE, Sundqvist G. Survival of *Arachnia propionica* in periapical tissue. Int Endod J 1988;21:277–82.

61. Figdor D, Sjogren U, Sorlin S, et al. Pathogenicity of *Actinomyces israelii* and *Arachnia propionica*: experimental infection in guinea pigs and phagocytosis and intracellular killing by human polymorphonuclear leukocytes in vitro. Oral Microbiol Immunol 1992;7:129–36.

62. Abou-Rass M, Bogen G. Microorganisms in closed periapical lesions. Int Endod J 1998;31:39–47.

63. Iwu C, MacFarlane TW, MacKenzie D, Stenhouse D. The microbiology of periapical granulomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod1990;69:502–5.

64. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol 1987;3:86–90.

65. Wayman BE, Murata SM, Almeida RJ, Fowler CB. A bacteriological and histological evaluation of 58 periapical lesions. J Endod 1992;18:152–5.

66. Siqueira JF Jr, Lopes HP. Bacteria on the apical root surfaces of untreated teeth with periradicular lesions: a scanning electron microscopy study. Int Endod J 2001;34:216–20.

67. Sunde PT, Olsen I, Lind PO, Tronstad L. Extraradicular infection: a methodological study. Endod Dent Traumatol 2000;16:84–90.

68. Noguchi N, Noiri Y, Narimatsu M, Ebisu S. Identification and localization of extraradicular biofilm-forming bacteria associated with refractory endodontic pathogens. Appl Environ Microbiol 2005;71:8738–43.

69. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004;15:348–81.

70. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249–81.

71. Ding Y, Haapasalo M, Kerosuo E, et al. Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of *Fusobacterium nucleatum, Porphyromonas gingivalis* and *Treponema denticola*. J Clin Periodontol 1997;24:237–48.

72. AAE. Glossary of endodontic terms. Chicago, IL: American Association of Endodontists; 2003.

73. Sundqvist G, Figdor D, Hanstrom L, et al. Phagocytosis and virulence of different strains of *Porphyromonas gingivalis.* Scand J Dent Res 1991;99:117–29.

74. Sundqvist GK, Carlsson J, Herrmann BF, et al. Degradation in vivo of the C3 protein of guinea-pig complement by a pathogenic strain of *Bacteroides gingivalis*. Scand J Dent Res 1984;92:14–24.

75. Cutler CW, Arnold RR, Schenkein HA. Inhibition of C3 and IgG proteolysis enhances phagocytosis of *Porphyromonas gingivalis*. J Immunol 1993;151:7016–29.

76. Sundqvist G, Carlsson J, Herrmann B, Tarnvik A. Degradation of human immunoglobulins G and M and complement factors C3 and C5 by black-pigmented *Bacteroides*. J Med Microbiol 1985;19:85–94.

77. Jansen HJ, van-der HJ, van-den KC, et al. Degradation of immunoglobulin G by periodontal bacteria. Oral Microbiol Immunol 1994;9:345–51.

78. Hirshberg A, Tsesis I, Metzger Z, Kaplan I. Periapical actinomycosis: a clinicopathologic study [see comment]. Oral Surg Oral Med Oral Pathol Oral Radiol Endod Oral Radiol Endod 2003;95:614–20.

79. Weiss EI, Shaniztki B, Dotan M, et al. Attachment of *Fusobacterium nucleatum* PK1594 to mammalian cells and its coaggregation with periopathogenic bacteria are mediated by the same galactose-binding adhesin. Oral Microbiol Immunol 2000;15:371–7.

80. Shaniztki B, Hurwitz D, Smorodinsky N, et al. Identification of a *Fusobacterium nucleatum* PK1594 galactose-binding adhesin which mediates coaggregation with periopathogenic bacteria and hemagglutination. Infect Immun 1997;65:5231–7.

81. Nair PNR. Pathobiology of primary apical periodontitis, In: Cohen S, Hargreaves KM, editors. Pathways of the pulp. 9th ed. Amsterdam: Elseiver; 2006. p. 541–79.

82. Fouad AF, Burleson J. The effect of diabetes mellitus on endodontic treatment outcome: data from an electronic patient record. J Am Dent Assoc 2003;134:43–51.

83. Mattscheck DJ, Law AS, Noblett WC. Retreatment versus initial root canal treatment: factors affecting posttreatment pain. Oral Surg Oral Med Oral Pathol Oral Radiol Endod Oral Radiol Endod 2001;92:321–4.

84. Yu CY. Role of occlusion in endodontic management: report of two cases. Aus Endod J 2004;30:110–5.

85. IASP Task Force on Taxonomy. Classification of chronic pain. In: Mersky H, Bogduk N, editors. 2nd ed. Seattle, WA: IASP Press; 1994. p. 209–14.

86. Rittner HL, Brack A, Stein C. Pro-algesic versus analgesic actions of immune cells. Curr Opin Anaesthesiol 2003;16:527–33.

87. Cunha JM, Cunha FQ, Poole S, Ferreira SH. Cytokine-mediated inflammatory hyperalgesia limited by interleukin-1 receptor antagonist. Brit J Pharmacol 2000;130:1418–24.

88. Cunha FQ, Poole S, Lorenzetti BB, Ferreira SH. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. Brit J Pharmacol 1992;107:660–4.

89. Aloe L, Simone MD, Properzi F. Nerve growth factor: a neurotrophin with activity on cells of the immune system. Micros Res Tech 1999;45:285–91.

90. Byers MR, Taylor PE, Khayat BG, Kimberly CL. Effects of injury and inflammation on pulpal and periapical nerves. J Endod 1990;16:78–84.

91. Wang H, Ehnert C, Brenner GJ, Woolf CJ. Bradykinin and peripheral sensitization. Biol Chem 2006;387:11–4.

92. Sorkin LS, Wallace MS. Acute pain mechanisms. Surg Clin North Am 1999;79:213–29.

93. Safieh-Garabedian B, Kanaan SA, Jalakhian RH, et al. Involvement of interleukin-1 beta, nerve growth factor, and prostaglandin-E2 in the hyperalgesia induced by intraplantar injections of low doses of thymulin. Brain Behav Immun 1997;11:185–200.

94. Kawamoto K, Aoki J, Tanaka A, et al. Nerve growth factor activates mast cells through the collaborative interaction with lysophosphatidylserine expressed on the membrane surface of activated platelets. J Immunol 2002;168:6412–9.

95. Price TJ, Louria MD, Candelario-Soto D, et al. Treatment of trigeminal ganglion neurons in vitro with NGF, GDNF or BDNF: effects on neuronal survival, neurochemical properties and TRPV1-mediated neuropeptide secretion. BMC Neurosci 2005;6:4.

96. Petersen M, Segond vB, Heppelmann B, Koltzenburg M. Nerve growth factor regulates the expression of bradykinin binding sites on adult sensory neurons via the neurotrophin receptor p75. Neuroscience 1998;83:161–8.

97. Ferreira SH, Lorenzetti BB, Poole S. Bradykinin initiates cytokine-mediated inflammatory hyperalgesia. Br J Pharmacol 1993;110:1227–31.

98. McNicholas S, Torabinejad M, Blankenship J, Bakland L. The concentration of prostaglandin E2 in human periradicular lesions. J Endod 1991;17:97–100.

99. Radics T, Kiss C, Tar I, Marton IJ. Interleukin-6 and granulocyte-macrophage colony-stimulating factor in apical periodontitis: correlation with clinical and histologic findings of the involved teeth. Oral Microbiol Immunol 2003;18:9–13.

100. Shimauchi H, Takayama S, Miki Y, Okada H. The change of periapical exudate prostaglandin E2 levels during root canal treatment. J Endod 1997;23:755–8.

101. Torabinejad M, Cotti E, Jung T. Concentrations of leukotriene B4 in symptomatic and asymptomatic periapical lesions. J Endod 1992;18:205–8.

102. Lim GC, Torabinejad M, Kettering J, et al. Interleukin 1-beta in symptomatic and asymptomatic human periradicular lesions. J Endod 1994;20:225–7.

103. Elliott DE, Blum AM, Li J, et al. Preprosomatostatin messenger RNA is expressed by inflammatory cells and induced by inflammatory mediators and cytokines. J Immunol 1998;160:3997–4003.

104. Matias I, Pochard P, Orlando P, et al. Presence and regulation of the endocannabinoid system in human dendritic cells. Eur J Biochem 2002;269:3771–8.

105. Labuz D, Berger S, Mousa SA, et al. Peripheral antinociceptive effects of exogenous and immune cell-derived endomorphins in prolonged inflammatory pain. J Neurosci 2006;26:4350–8.

106. Brack A, Labuz D, Schiltz A, et al. Tissue monocytes/macrophages in inflammation: hyperalgesia versus opioid-mediated peripheral antinociception. Anesthesiology 2004;101:204–11.

107. Rittner HL, Machelska H, Stein C. Leukocytes in the regulation of pain and analgesia. J Leukoc Biol 2005;78:1215–22.

108. Fiset ME, Gilbert C, Poubelle PE, Pouliot M. Human neutrophils as a source of nociceptin: a novel link between pain and inflammation. Biochemistry 2003;42:10498–505.

109. Machelska H, Brack A, Mousa SA, et al. Selectins and integrins but not platelet-endothelial cell adhesion molecule-1 regulate opioid inhibition of inflammatory pain. Br J Pharmacol 2004;142:772–80.

110. Machelska H, Mousa SA, Brack A, et al. Opioid control of inflammatory pain regulated by intercellular adhesion molecule-1. J Neurosci 2002;22:5588–96.

111. Klein DC, Raisz LG. Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 1970;86:1436–40.

112. Hausmann E, Raisz LG, Miller WA. Endotoxin: stimulation of bone resorption in tissue culture. Science 1970;168:862–4.

113. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med 1998;9:498–521.

114. Stashenko P, Dewhirst FE, Peros WJ, et al. Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. J Immunol 1987;138:1464–8.

115. Kawashima N, Stashenko P. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. Arch Oral Biol 1999;44:55–66.

116. Shimauchi H, Takayama S, Imai TT, Okada H. Balance of interleukin-1 beta and interleukin-1 receptor antagonist in human periapical lesions. J Endod 1998;24:116–9.

117. Wang CY, Stashenko P. The role of interleukin-1 alpha in the pathogenesis of periapical bone destruction in a rat model system. Oral Microbiol Immunol 1993;8:50–6.

118. Kakehashi S, Stanley HR, Fitzgerald RJ. The effect of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1965;20:340–9.

119. Wang CY, Stashenko P. Kinetics of bone-resorbing activity in developing periapical lesions. J Dent Res 1991;70:1362–6.

120. Wang CY, Stashenko P. Kinetics of bone-resorbing activity in developing periapical lesions. J Dent Res 1991;70:1362–6.

121. Matsuo T, Ebisu S, Nakanishi T, et al. Interleukin-1 alpha and interleukin-1 beta periapical exudates of infected root canals: correlations with the clinical findings of the involved teeth. J Endod 1994;20:432–5.

122. Kuo ML, Lamster IB, Hasselgren G. Host mediators in endodontic exudates. II. Changes in concentration with sequential sampling. J Endod 1998;24:636–40.

123. Kuo ML, Lamster IB, Hasselgren G. Host mediators in endodontic exudates. I. Indicators of inflammation and humoral immunity. J Endod 1998;24:598–603.

124. Dinarello CA. Interleukin-1. Ann NY Acad Sci 1988;546:122–132.

125. Pennica D, Nedwin GE, Hayflick JS, et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 1984;312:724–9.

126. Metzger Z, Berg D, Dotan M. Fibroblast growth in vitro suppressed by LPS-activated macrophages. Reversal of suppression by hydrocortisone. J Endod 1997;23:517–21.

127. Vos JG, Kreeftenberg JG, Kruijt BC, et al. The athymic nude rat. II. Immunological characteristics. Clin Immunol Immunopathol 1980;15:229–37.

128. Pritchard H, Riddaway J, Micklem HS. Immune responses in congenitally thymus-less mice. II. Quantitative studies of serum immunoglobulins, the antibody response to sheep erythrocytes, and the effect of thymus allografting. Clin Exp Immunol 1973;13:125–38.

129. Wallstrom JB, Torabinejad M, Kettering J, McMillan P. Role of T cells in the pathogenesis of periapical lesions. A preliminary report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1993;76:213–8.

130. Politis AD, Sivo J, Driggers PH, et al. Modulation of interferon consensus sequence binding protein mRNA in murine peritoneal macrophages. Induction by IFN-gamma and down-regulation by IFN-alpha, dexamethasone, and protein kinase inhibitors. J Immunol 1992;148:801–7.

131. Metzger Z, Hoffeld JT, Oppenheim JJ. Suppression of fibroblast proliferation by activated macrophages: involvement of H2O2 and a non-prostaglandin E product of the cyclooxygenase pathway. Cell Immunol 1986;100:501–14.

132. Metzger Z, Hoffeld JT, Oppenheim JJ. Macrophage-mediated suppression. I. Evidence for participation of both hydrogen peroxide and prostaglandins in suppression of murine lymphocyte proliferation. J Immunol 1980;124:983–8.

133. Tani-Ishii N, Wang CY, Tanner A, Stashenko P. Changes in root canal microbiota during the development of rat periapical lesions. Oral Microbiol Immunol 1994;9:129–35.

134. Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in bone cell biology. J Mol Med 2001;79:243–53.

135. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165–76.

136. Hofbauer LC, Kuhne CA, Viereck V. The OPG/RANKL/RANK system in metabolic bone diseases. J Musc Neur Interact 2004;4:268–75.

137. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165–76.

138. Suda T, Takahashi N, Udagawa N, et al. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 1999;20:345–57.

139. Teitelbaum SL. Bone resorption by osteoclasts. Science 2000;289:1504–8.

140. Kong YY, Feige U, Sarosi I, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402:304–9.

141. Taubman MA, Kawai T. Involvement of T-lymphocytes in periodontal disease and in direct and indirect

induction of bone resorption. Crit Rev Oral Biol Med 2001;12:125–35.

142. Kawai T, Matsuyama T, Hosokawa Y, et al. B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. Am J Pathol 2006;169:987–98.

143. Bekker PJ, Holloway D, Nakanishi A, et al. The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 2001;16:348–60.

144. Vernal R, Dezerega A, Dutzan N, et al. RANKL in human periapical granuloma: possible involvement in periapical bone destruction. Oral Dis 2006;12:283–9.

145. Sabeti M, Simon J, Kermani V, et al. Detection of receptor activator of NF-kappa beta ligand in apical periodontitis. J Endod 2005;31:17–8.

146. Wada N, Maeda H, Tanabe K, et al. Periodontal ligament cells secrete the factor that inhibits osteoclastic differentiation and function: the factor is osteoprotegerin/osteoclastogenesis inhibitory factor. J Periodont Res 2001;36:56–63.

147. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249–81.

148. Nair PN. Apical periodontitis: a dynamic encounter between root canal infection and host response. Periodontology 2000 1997;13:121–48.

149. Fish EW. Bone infection. J Am Dent Assoc 1939;26:691.

150. Happonen RP, Bergengoltz G. Apical periodontitis, In: Bergenholtz G, Horsted-Bindslev, Reit C, editors. Textbook of endodontology. 1st ed. Oxford: Blackwell; 2003. p. 130–44.

151. Kiss C. Cell to cell interactions. Endod Top 2004;8:88–103.

152. Marton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. [Review] [169 refs]. Oral Microbiol Immunol 2000;15:139–50.

153. Nair PN, Pajarola G, Luder HU. Ciliated epithelium-lined radicular cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:485–93.

154. Nair PN, Sjogren U, Schumacher E, Sundqvist G. Radicular cyst affecting a root-filled human tooth: a long-term post-treatment follow-up. Int Endod J 1993;26:225–33.

155. Nair PN, Pajarola G, Schroeder HE. Types and incidence of human periapical lesions obtained with extracted teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;81:93–102.

156. Simon JH. Incidence of periapical cysts in relation to the root canal. J Endod 1980;6:845–8.

157. Ten Cate AR. The epithelial cell rests of Malassez and the genesis of the dental cyst. Oral Surg Oral Med Oral Pathol Oral Radiol Endod1972;34:956–64.

158. Shafer W, Hine M, Levy B. A textbook of oral pathology. 3rd ed. Philadelphia, PA: W.B. Saunders; 1974.

159. Summers L. The incidence of epithelium in periapical granulomas and the mechanism of cavitation in apical dental cysts in man. Arch Oral Biol 1974;19:1177–80.

160. Bhaskar SN. Periapical lesions—types, incidence, and clinical features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod1966;21:657–671.

161. Natkin E, Oswald RJ, Carnes LI. The relationship of lesion size to diagnosis, incidence, and treatment of periapical cysts and granulomas. Oral Surg Oral Med Oral Pathol 1984;57:82–94.

162. Cotti E, Torabinejad M. Detection of leukotriene C4 in human periradicular lesions. Int Endod J 1994;27:82–6.

163. Cummings GR, Torabinejad M. Effect of systemic doxycycline on alveolar bone loss after periradicular surgery. J Endod 2000;26:325–7.

164. Kaufman B, Spangberg L, Barry J, Fouad AF. *Enterococcus* spp. in endodontically treated teeth with and without periradicular lesions. J Endod 2005;31:851–6.

165. Torabinejad M. Mediators of acute and chronic periradicular lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod1994;78:511–21.

166. Abbott PV. Classification, diagnosis and clinical manifestation of apical periodontitis. Endod Top 2004;8:36–54.

167. Ricucci D, Mannocci F, Ford TR. A study of periapical lesions correlating the presence of a radiopaque lamina with histological findings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod Oral Radiol Endod 2006;101:389–94.

168. Gundappa M, Ng SY, Whaites EJ. Comparison of ultrasound, digital and conventional radiography in differentiating periapical lesions. Dento-Maxillo-Facial Radiol 2006;35:326–33.

169. Hought RT, Fitzgerald BE, Latta JE, Zallen RD. Ludwig's angina: report of two cases and review of the literature from 1945 to January 1979. J Oral Surg 1980;38:849–55.

170. Bullock JD, Fleishman JA. The spread of odontogenic infections to the orbit: diagnosis and management. J Oral Maxill Surg 1985;43:749–55.

171. Fielding AF, Cross S, Matise JL, Mohnac AM. Cavernous sinus thrombosis: report of case. J Am Dent Assoc 1983;106:342–5.

172. Henig EF, Derschowitz T, Shalit M, et al. Brain abscess following dental infection. Oral Surg Oral Med Oral Pathol Oral Radiol Endod1978;45:955–8.

173. Molven O, Halse A, Grung B. Incomplete healing (scar tissue) after periapical surgery—radiographic findings 8 to 12 years after treatment. J Endod 1996;22:264–8.

174. Nair PN, Sjogren U, Figdor D, Sundqvist G. Persistent periapical radiolucencies of root-filled human teeth,

failed endodontic treatments, and periapical scars. Oral Surg Oral Med Oral Pathol Oral Radiol EndodOral Radiol Endod 1999;87:617–27.

175. Pecora G, De Leonardis D, Ibrahim N, et al. The use of calcium sulphate in the surgical treatment of a 'through and through' periradicular lesion. Int Endod J 2001;34:189–97.
176. Seltzer S, Bender IB, Smith J, et al. Endodontic failures—an analysis based on clinical, roentgenographic, and histologic findings. II. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1967;23:517–30.
177. Orstavik D. Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. Int Endod J 1996;29:150–5.
178. Kvist T, Reit C. Results of endodontic retreatment: a randomized clinical study comparing surgical and nonsurgical procedures. J Endod 1999;25:814–7.
179. Friedman S. Prognosis of initial endodontic therapy. Endod Top 2002;2:59–88.
180. Fristad I, Molven O, Halse A. Nonsurgically retreated root filled teeth—radiographic findings after 20–27 years. Int Endod J 2004;37:12–8.
181. Stashenko P, Dewhirst FE, Rooney ML, et al. Interleukin-1 beta is a potent inhibitor of bone formation in vitro. J Bone Miner Res 1987;2:559–565.
182. Shimauchi H, Miki Y, Takayama S, et al. Development of a quantitative sampling method for periapical exudates from human root canals. J Endod 1996;22:612–5.
183. Williams RC, Jeffcoat MK, Howell TH, et al. Altering the progression of human alveolar bone loss with the nonsteroidal anti-inflammatory drug flurbiprofen. J Periodontol 1989;60:485–90.
184. Torabinejad M, Clagett J, Engel D. A cat model for the evaluation of mechanisms of bone resorption: induction of bone loss by simulated immune complexes and inhibition by indomethacin. Calcif Tissue Int 1979;29:207–14.
185. Shapira L, Barak V, Soskolne WA, et al. Effects of tetracyclines on the pathologic activity of endotoxin: in vitro and in vivo studies. Adv Dent Res 1998;12:119–22.
186. Shapira L, Soskolne WA, Houri Y, et al. Protection against endotoxic shock and lipopolysaccharide-induced local inflammation by tetracycline: correlation with inhibition of cytokine secretion. Infect Immun 1996;64:825–8.
187. Golub LM, Lee HM, Ryan ME, et al. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. Adv Dent Res 1998;12:12–26.
188. Metzger Z, Belkin D, Kariv N, et al. Low-dose doxycycline inhibits development of periapical lesions in rats. Int Endod J [In press].
189. Schultz RM, Chirigos MA, Stoychkov JN, Pavilidis RJ. Factors affecting macrophage cytotoxic activity with particular emphasis on corticosteroids and acute stress. J Reticuloendothel Soc 1979;26:83–91.
190. Nakamura Y, Murai T, Ogawa Y. Effect of in vitro and in vivo administration of dexamethasone on rat macrophage functions: comparison between alveolar and peritoneal macrophages. Eur Respir J 1996;9:301–6.
191. Metzger Z, Klein H, Klein A, Tagger M. Periapical lesion development in rats inhibited by dexamethasone. J Endod 2002;28:643–5.
192. Knudsen PJ, Dinarello CA, Strom TB. Glucocorticoids inhibit transcriptional and post-transcriptional expression of interleukin 1 in U937 cells. J Immunol 1987;139:4129–34.
193. Waage A, Slupphaug G, Shalaby R. Glucocorticoids inhibit the production of IL6 from monocytes, endothelial cells and fibroblasts. Eur J Immunol 1990;20:2439–43.
194. Ingle JI. Alveolar osteoporosis and pulpal death associated with compulsive bruxism. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1960;13 Nov:1371–81.
195. Natkin, E, Ingle JI. A further report on alveolar osteoporosis and pulpal death associated with compulsive bruxism. Periodont J Am Soc Periodont 1963; 1 Nov/Dec:260–3.