PATHOGENESIS OF APICAL PERIODONTITIS AND THE CAUSES OF ENDODONTIC FAILURES

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ABSTRACT: Apical periodontitis is a sequel to endodontic infection and manifests itself as the host defense response to microbial challenge emanating from the root canal system. It is viewed as a dynamic encounter between microbial factors and host defenses at the interface between infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard tissues, destruction of other periapical tissues, and eventual formation of various histopathological categories of apical periodontitis, commonly referred to as periapical lesions. The treatment of apical periodontitis, as a disease of root canal infection, consists of eradicating microbes or substantially reducing the microbial load from the root canal and preventing re-infection by orthograde root filling. The treatment has a remarkably high degree of success. Nevertheless, endodontic treatment can fail. Most failures occur when treatment procedures, mostly of a technical nature, have not reached a satisfactory standard for the control and elimination of infection. Even when the highest standards and the most careful procedures are followed, failures still occur. This is because there are root canal regions that cannot be cleaned and obturated with existing equipments, materials, and techniques, and thus, infection can persist. In very rare cases, there are also factors located within the inflamed periapical tissue that can interfere with post-treatment healing of the lesion. The data on the biological causes of endodontic failures are recent and scattered in various journals. This communication is meant to provide a comprehensive overview of the etio-pathogenesis of apical periodontitis and the causes of failed endodontic treatments that can be visualized in radiographs as asymptomatic post-treatment periapical radiolucencies.

Key words. Apical periodontitis, periapical lesions, pathogenesis, endodontic failures.

In the long-term conflict between microbes and technology, microbes will win. (adapted from Albert Einstein)

(I) Introduction

Apical periodontitis is inflammation and destruction of periradicular tissues caused by etiological agents of endodontic origin. It is generally a sequel to endodontic infection (Fig. 1). Initially, the tooth pulp becomes infected and necrotic by an autogenous oral microflora. The endodontic environment provides a selective habitat for the establishment of a mixed, predominantly anaerobic, flora. Collectively, this habitat-adapted polymicrobial community residing in the root canal has several biological and pathogenic properties, such as antigenicity, mitogenic activity, chemotaxis, enzymatic histolysis, and activation of host cells. The microbial invaders in the root canal can advance, or their products can egress, into the periapex. In response, the host mounts an array of defenses consisting of several classes of cells, intercellular messengers, antibodies, and effector molecules. The microbial factors and host defense forces encounter, clash with, and destroy much of the periapical tissue, resulting in the formation of various categories of apical periodontitis lesions. In spite of the formidable defense, the body is unable to destroy the microbes well-entrenched in the sanctuary of the necrotic root canal, which is beyond the reaches of body defenses (Fig. 2). Therefore, apical periodontitis is not self-healing. The treatment of apical periodontitis consists of eliminating infection from the root canal and preventing re-infection by a hydraulic seal of the root canal space. Nevertheless, endodontic treatment can fail for various reasons. The pathogenesis of apical periodontitis has been well-reviewed (Stashenko, 1990; Nair, 1997; Stashenko *et al.*, 1998). However, even recent textbooks of endodontology (Cohen and Burns, 2002; Bergenholtz *et al.*, 2003) do not provide an overview of the causes of endodontic failures. The data on the biological causes of endodontic treatment failures are recent, scattered throughout various journals, and need consolidation. The purpose of this communication is to provide a comprehensive review of the pathogenesis of apical periodontitis and the causes of endodontic failures.

(II) Etiology of Apical Periodontitis

(A) CHAIN OF EVIDENCE

The presence of several distinct types of bacteria in the necrotic dental pulp was demonstrated more than a century ago (Miller, 1890). The essential role of micro-organisms in the etiology of apical periodontitis nevertheless remained uncertain for many years. Half a century later, it was shown (Kakehashi *et al.*, 1965) that no apical periodontitis developed in germ-free rats when their molar-pulps were kept exposed to the oral cavity, as compared with control rats with a conventional oral microflora in which massive periapical radiolucencies occurred. The great significance of obligate anaerobes in endodontic infections was soon established (Möller, 1966). These findings were confirmed by several researchers (Bergenholtz, 1974; Kantz and Henry, 1974; Wittgow and Sabiston, 1975). It was found that the root canals of 18 out of 19 periapically affected teeth with 'intact' crowns harbored a mixture of several species of bacteria that consisted predominantly

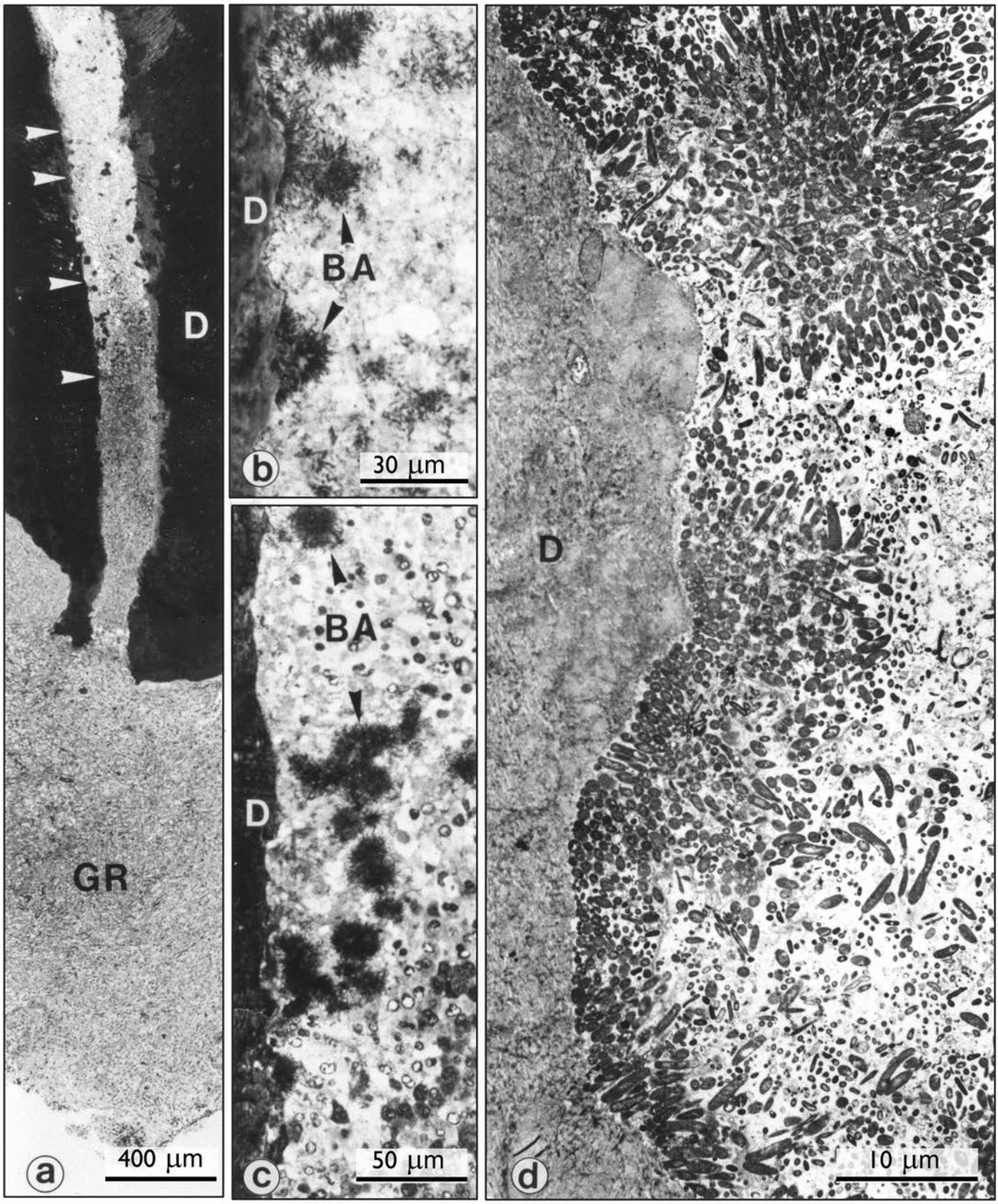

Figure 1. Endodontic microflora of a human tooth with apical periodontitis (GR). The areas between the upper two and the lower two arrowheads in **(a)** are magnified in **(b)** and **(c),** respectively. Note the dense bacterial aggregates (BA) sticking (b) to the dentinal (D) wall and also remaining suspended among neutrophilic granulocytes in the fluid phase of the root canal (c). A transmission electron microscopic view **(d)** of the pulpo-dentinal interface shows bacterial condensation on the surface of the dentinal wall, forming thick, layered biofilm. Magnifications: (a) 46x; (b) 600x; (c) 370x; and (d) 2350x. (From Nair, 1997.)

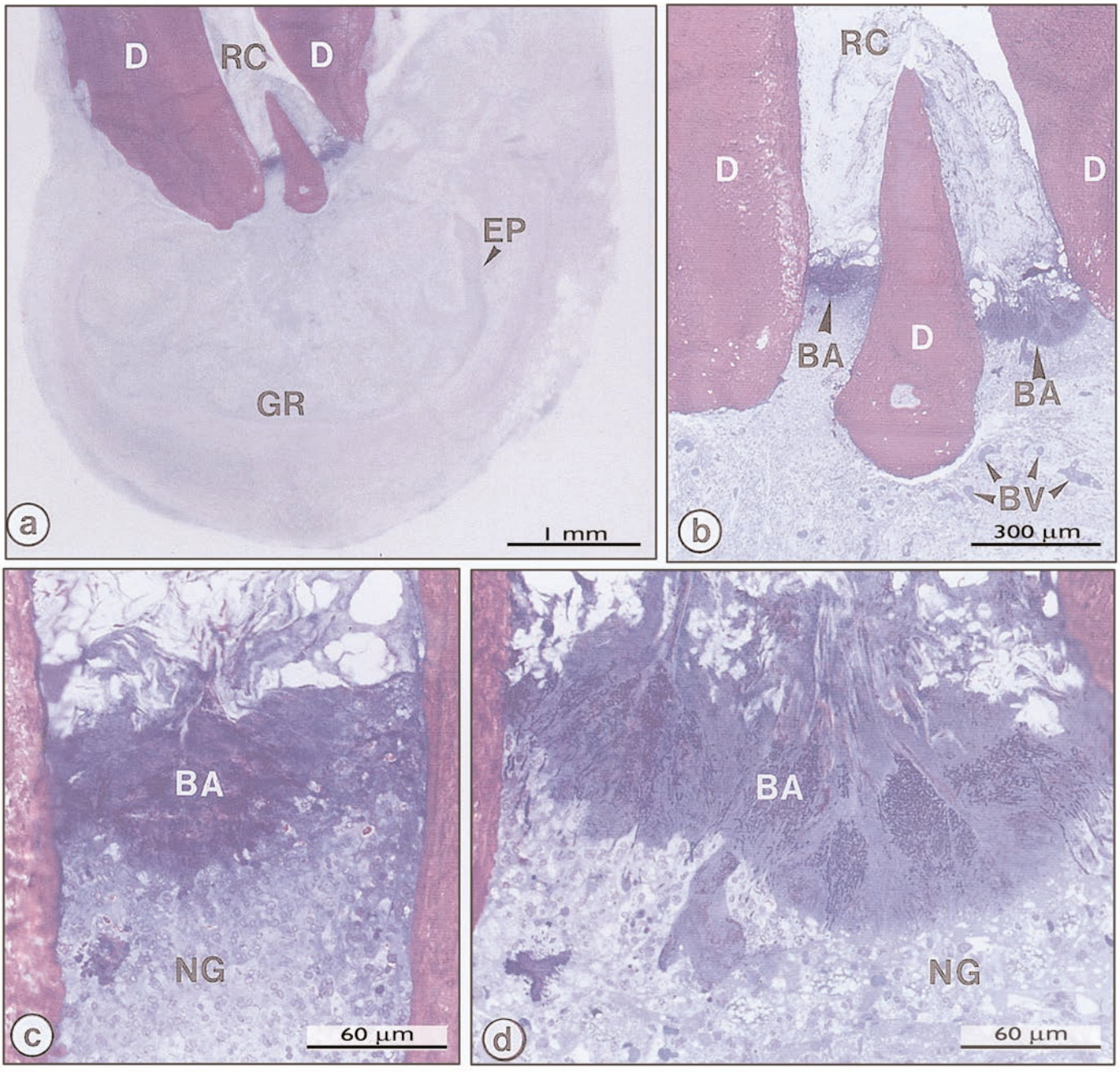

Figure 2. Well-entrenched biofilm at the apical foramen of a tooth affected with apical periodontitis (GR). The apical delta in **(a)** is magnified in (b). The canal ramifications on the left and right in **(b)** are magnified in **(c)** and **(d),** respectively. Note the strategic location of the bacterial clusters (BA) at the apical foramina. The bacterial mass appears to be held back by a wall of neutrophilic granulocytes (NG). Obviously, any surgical and/or microbial sampling procedures of the periapical tissue would contaminate the sample with the intraradicular flora. EP, epithelium. Magnifications: (a) 20x, (b) 65x, and (c,d) 350x. (From Nair, 2002.)

of strict anaerobes (Sundqvist, 1976). A series of pathobiological studies (Fabricius, 1982) subsequently determined: (a) the conditions under which the endodontic flora develops and establishes itself, and (b) the biological properties and endodontic conditions which may favor the root canal flora to become pathogenic. The ecological organization of the flora into sessile biofilms (Figs. 1, 2)—aggregates of one and co-aggregates of several species of microbes suspended in the fluid phase of infected root canals—was visualized by the application of the precise technique of correlative light and transmission electron microscopy (Nair, 1987). Today, there is a clear consensus on the essential etiological role of intraradicular micro-organisms in apical periodontitis.

(B) Portals of root canal infection

Openings in the dental hard tissue wall—resulting from caries,

clinical procedures, or trauma-induced fractures and cracks—are the most frequent portals of pulpal infection. But microbes have also been isolated from teeth with necrotic pulps and clinically intact crowns (Brown and Rudolph, 1957; Chirnside, 1957; Macdonald *et al.*, 1957; Engström and Frostell, 1961; Möller, 1966; Bergenholtz, 1974; Wittgow and Sabiston, 1975; Sundqvist, 1976; Baumgartner *et al.*, 1999). Endodontic infections of such teeth are preceded by pulpal necrosis. The teeth may appear to be clinically intact but reveal micro-cracks in hard tissues that provide portals of entry for bacteria. Bacteria from the gingival sulci or periodontal pockets have been suggested to reach the root canals of these teeth through severed periodontal blood vessels (Grossman, 1967). Pulpal infection can also occur through exposed dentinal tubules at the cervical root surface, due to gaps in the cemental coating. Microbes have also been claimed to 'seed' in the necrotic pulp *via* the blood circulation (anachoresis) (Robinson and Boling, 1941; Burke and Knighton, 1960; Gier and Mitchel, 1968; Allard *et al.*, 1979). However, bacteria could not be recovered from the root canals when the blood stream was experimentally infected, unless the root canals were over-instrumented and presumably the apical periodontal blood vessels were injured during the period of bacteremia (Delivanis and Fan, 1984). In another study (Möller *et al.*, 1981), all experimentally devitalized monkey pulps (n = 26) remained sterile for more than six months. Therefore, exposure of the dental pulp to the oral cavity is the most important route of endodontic infection.

(C) Endodontic flora of teeth with primary apical periodontitis

Contemporary knowledge of the taxonomy of infected root canal flora is based on advanced microbial culture techniques. This might soon change with the judicious application of molecular genetic techniques in endodontic microbiology (Munson *et al.*, 2002). Although a sample of the vast oral microbiota (Moore and Moore, 1994) can infect the exposed tooth pulp, culture studies have long established that only a subset of oral microflora, consisting of a limited number of species, is consistently isolated from such root canals. The root canal flora of teeth with clinically intact crowns, but having necrotic pulps and diseased periapices, is dominated (> 90%) by obligate anaerobes (Sundqvist, 1976; Byström and Sundqvist, 1981; Haapasalo, 1989; Sundqvist *et al.*, 1989), usually belonging to the genera *Fusobacterium, Porphyromonas* (formerly *Bacteroides;* Shah and Collins, 1988), *Prevotella* (formerly *Bacteroides;* Shah and Collins, 1988), *Eubacterium,* and *Peptostreptococcus.* In contrast, the microbial composition—even in the apical third of the root canal of periapically affected teeth with pulp canals exposed to the oral cavity—is not only different from but also less dominated (< 70%) by strict anaerobes (Baumgartner and Falkler, 1991). Using culture techniques (Hampp, 1957; Kantz and Henry, 1974; Dahle *et al.*, 1996), dark-field (Brown and Rudolph, 1957; Thilo *et al.*, 1986; Dahle *et al.*, 1993), and transmission electron microscopy (Nair, 1987), investigators have found spirochetes in necrotic root canals. Culture studies (for review, see Waltimo *et al.*, 2003) and the application of scanning electron microscopy (Sen *et al.*, 1995) have revealed the presence of fungi in canals of teeth with primary apical periodontitis. The presence of intraradicular viruses has so far been shown only in non-inflamed dental pulps of patients infected with immuno-deficiency virus (Glick *et al.*, 1991).

The invention of the polymerase chain-reaction (PCR) (Mullis and Faloona, 1987) technique and its application in microbiology (Pollard *et al.*, 1989; Spratt *et al.*, 1999) has enabled bacteria to be detected by the amplification of their DNA. These molecular methods have largely confirmed the microbial species that have been previously detected and grown by culture methods (Munson *et al.*, 2002). They have also facilitated the identification of as-yet-culture-difficult endodontic organisms, and their precise taxonomic grouping (Munson *et al.*, 2002). Although the application of molecular techniques may widen the taxonomic spectra of potential endodontic microflora, there is currently no evidence that the culture-difficult organisms reported by the highly sensitive molecular methods are viable root canal pathogens. This is because the molecular genetic methods are often applied without the enhanced precautions needed and without consideration of the limitations of the techniques (Siqueira *et al.*, 2000; Siqueira and Rôças, 2003).

(D) Pathogenicity of endodontic flora

Any microbe that infects the root canal has the potential to initiate periapical inflammation. However, the virulence and pathogenicity of individual species vary considerably and can be affected in the presence of other microbes. Although the individual species in the endodontic flora are usually of low virulence, their intraradicular survival and pathogenic properties are influenced by a combination of factors, including: (i) interactions with other micro-organisms in the root canal, to develop synergistically beneficial partners; (ii) the ability to interfere with and evade host defenses; (iii) the release of lipopolysaccharides (LPS) and other bacterial modulins; and (iv) the synthesis of enzymes that damage host tissues,

(1) Microbial interaction

There is clear evidence that microbial interaction plays a significant role in the ecological regulation and eventual development of an endodontic habitat-adapted polymicrobial flora (for reviews, see Sundqvist, 1992a,b; Sundqvist and Figdor, 2003). The importance of the mixed bacterial flora has been well-exemplified in carefully planned animal studies (Sundqvist *et al.*, 1979; Fabricius *et al.*, 1982a,b). Bacteria (*Prevotella oralis* and 11 other species) isolated from the root canals of periapically involved teeth from experimental monkeys were inoculated in various combinations or as separate species into the root canals of other monkeys (Fabricius *et al.*, 1982b). When individual bacterial species were inoculated, only mild apical periodontitis developed. But in combinations, the same bacterial species induced more severe periapical reactions. Further, *Prevotella oralis* did not establish in root canals as a mono-infection, whereas it survived and dominated the endodontic flora when introduced with the other bacterial species involved in the study. Microbial interactions that influence the ecology of the endodontic flora may be positive (synergistic) or negative associations, as a result of certain organisms influencing the respiratory and nutritional environments of the entire root canal flora (Lew *et al.*, 1971; Fabricius *et al.*, 1982a; Loesche *et al.*, 1983; Carlsson, 1990).

(2) Microbial interference

The ability of certain microbes to shirk and interfere with the host defenses has been well-elaborated (for review, see Sundqvist, 1994). Bacterial LPS can signal the endothelial cells to express leukocyte adhesion molecules that initiate extravasation of leukocytes into the area of the infection. It has been

reported that *Porphyromonas gingivalis*, an important endodontic and periodontal pathogen, and its LPS do not signal the endothelial cells to express E-selectin. *P. gingivalis* therefore has the ability to block the initial step of inflammatory response, 'hide' from the host, and multiply. The antigenicity of LPS occurs in several forms that include mitogenic stimulation of B-lymphocytes, to produce non-specific antibodies. Gram-negative organisms release membrane particles (blebs) and soluble antigens which may 'mop up' effective antibodies, to make them unavailable to act against the organism itself (Mims, 1988). *Actinomyces israelii*, a recalcitrant periapical pathogen, is easily killed by PMN *in vitro* (Figdor *et al.*, 1992). In tissues, *A. israelii* aggregate to form large cohesive colonies that cannot be killed by host phagocytes (Figdor *et al.*, 1992).

(3) LPS and other microbial modulins

LPS, also historically known as endotoxins, form an integral part of Gram-negative cell walls. They are released during disintegration of bacteria after death and also during multiplication and growth. The effects of LPS are due to their interaction with endothelial cells and macrophages. LPS not only signal the endothelial cells to express adhesion molecules but also activate macrophages to produce several molecular mediators, such as the tumor necrosis factor-α (TNF-α) and interleukins (IL) (Arden, 1979). Exogenous TNF-α administered to experimental animals can induce a lethal shock that is indistinguishable from that induced by LPS. The latter signal the presence of Gram-negative micro-organisms in the area. The impact of LPS in tissues has been aptly stated (Thomas, 1974): "...when we sense LPS, we are likely to turn on every defense at our disposal; we will bomb, defoliate, blockade, seal off and destroy all tissues in the area." The presence of LPS has been reported in samples taken from the root canal (Schein and Schilder, 1975; Dahlén and Bergenholtz, 1980) and the pulpal-dentinal wall of periapically involved teeth (Horiba *et al.*, 1990). The Gram-negative organisms of the endodontic flora multiply and also die in the apical root canal, thereby releasing LPS that egress through the apical foramen into the periapex (Yamasaki *et al.*, 1992), where they initiate and sustain apical periodontitis (Dahlén, 1980; Dahlén *et al.*, 1981).

However, LPS are not the only bacterial degradation product that can induce mammalian cells to produce cytokines. Many proteins, certain carbohydrates, and lipids of bacterial origin are now considered as belonging to a novel class of 'modulins' that induce the formation of cytokine networks and host tissue pathology (for review, see Henderson *et al.*, 1996).

(4) Enzymes

Endodontic microbes produce a variety of enzymes that are not directly toxic but may aid the spread of the organisms in host tissues. Microbial collagenase, hyaluronidase, fibrinolysins, and several proteases are examples. Microbes are also known to produce enzymes that degrade various plasma proteins involved in blood coagulation and other body defenses. The ability of some *Porphyromonas* and *Prevotella* species to break down plasma proteins—particularly IgG, IgM (Killian, 1981), and the complement factor C_3 (Sundqvist *et al.*, 1985)—is of particular significance, since these molecules are opsonins necessary for both humoral and phagocytic host defenses.

(III) Host Response

Apical periodontitis is viewed as the consequence of a dynamic encounter between root canal microbes and host defense (Nair, 1997) (Fig. 2). The latter involves cells, intercellular mediators, metabolites, effector molecules, and humoral antibodies.

(A) Cellular elements

Several classes of body cells participate in periapical defense (Fig. 3). A majority of them are from the defense systems and include polymorphonuclear leukocytes (PMN), lymphocytes, plasma cells, and monocyte/macrophages. Structural cells include fibroblasts, osteoblasts, and epithelial rests (Malassez, 1884) that play significant roles. The importance of PMN and monocyte derivatives in apical periodontitis has been shown experimentally (Stashenko *et al.*, 1995). The intensity of induced murine pulpitis and apical periodontitis can be suppressed if the animals are treated with a biological response-modifying drug, PGG glucan, that enhances the number and ability of circulating neutrophils and monocytes.

(1) PMN

The biology of PMN and their role in inflammation are well-documented (Ryan and Majno, 1977; Cotran *et al.*, 1999). The interaction of PMN with micro-organisms is of particular importance in the progression of periodontitis, at both the marginal and the periradicular sites, and has been extensively reviewed (Van Dyke and Vaikuntam, 1994). Though the PMN are essentially protective cells, they cause severe damage to the host tissues (Fig. 3a). Their cytoplasmic granules contain several enzymes that, on release, degrade the structural elements of tissue cells and extracellular matrices (Nagase *et al.*, 1992; Van Dyke and Vaikuntam, 1994). Because they are short-lived cells, PMN die in great numbers (Fig. 3a) at acute inflammatory sites (Ryan and Majno, 1977). Therefore, the accumulation and massive death of neutrophils are a major cause for tissue breakdown in acute phases of apical periodontitis.

(2) Lymphocytes

Among the three major classes of lymphocytes—T-lymphocytes, B-lymphocytes, and the natural killer (NK) cells—the T- and B-lymphocytes are of importance in apical periodontitis. They are morphologically identical (Fig. 3b) and cannot be distinguished by conventional staining or microscopic examination, but are phenotyped on the basis of surface receptors with the use of monoclonal antibodies against the latter. Cells so identified are given a 'cluster of differentiation' (CD) number.

(a) T-lymphocytes

Traditionally, the thymus-derived (T) cells have been designated after their effects or functions—for instance, the T-cells working with B-cells have been known as T-helper/inducer ($T_{h/i}$) cells, and those with direct toxic and suppressive effects on other cells have been named T-cytotoxic/suppressive ($T_{c/s}$) cells. The $T_{h/i}$ cells are $CD4^+$, and the $T_{c/s}$ cells are $CD8^+$. The $CD4^+$ cells differentiate further into two types, known as T_{h1} and T_{h2} cells. The former produce IL-2 and interferon-γ (IF-γ) and control the cell-mediated arm of the immune system. The T_{h2} cells secrete IL-4, -5, -6, and -10, that regulate the production of antibodies by the plasma cells.

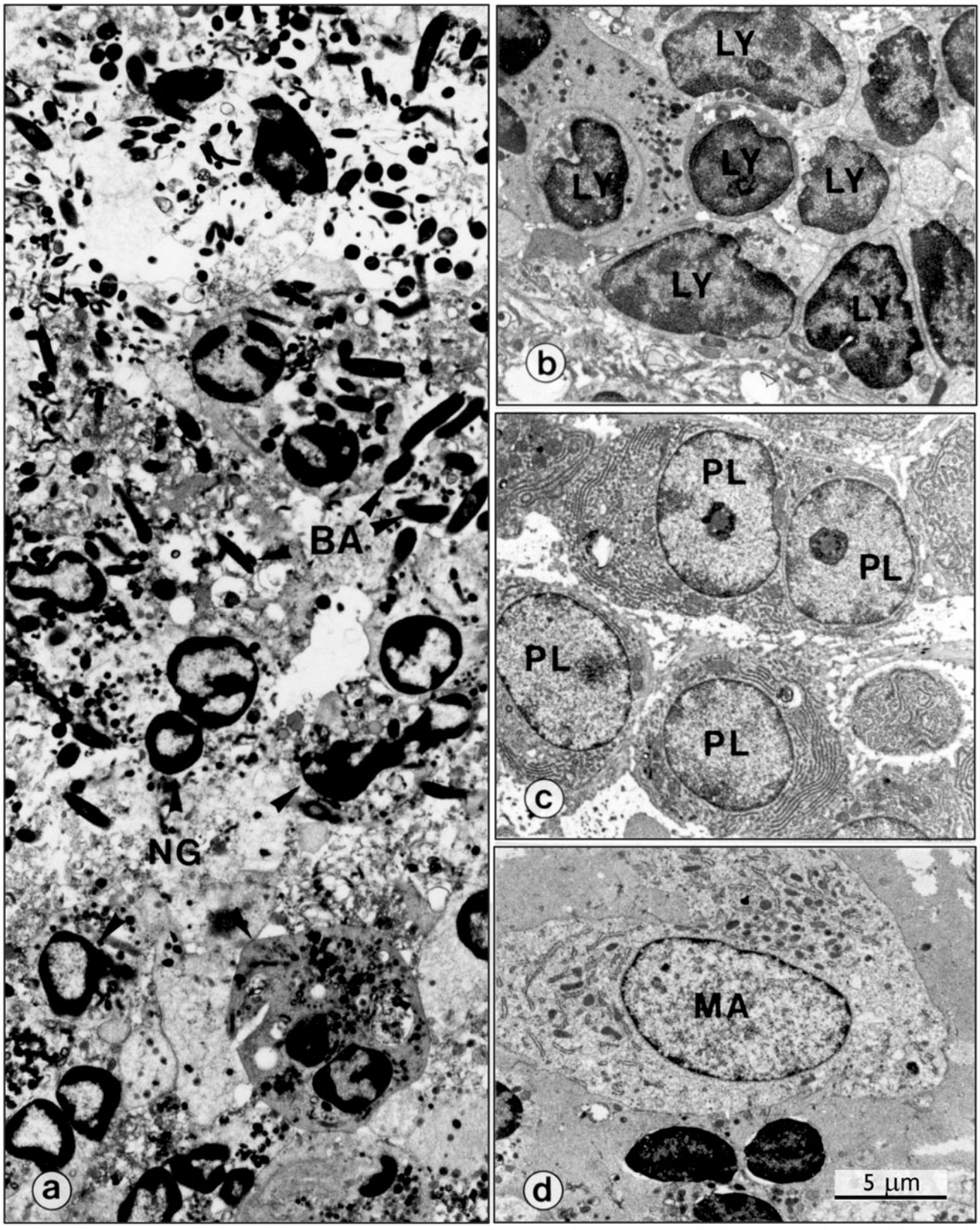

Figure 3. The primary body cells involved in the pathogenesis of apical periodontitis. Neutrophils (NG in **a**) in combat with bacteria (BA) in an exacerbating apical periodontitis. Lymphocytes (LY in **b**) are the major components of chronic apical periodontitis, but their subpopulations cannot be identified on a structural basis. Plasma cells (PL in **c**) form a significant component of chronic asymptomatic lesions. Note the highly developed rough endoplasmic reticulum of the cytoplasm and the localized condensation of heterochromatin subjacent to the nuclear membrane, which gives the typical 'cartwheel' appearance in light microscopy. Macrophages (MA in **d**) are voluminous cells with elongated or U-forming nuclei and cytoplasm with rough endoplasmic reticulum. Magnifications: (a,b,c,d) 3900x. (From Nair, 1998b.)

(b) B-lymphocytes

The lymphocytes directly responsible for antibody production are the bursa-equivalent (B) cells, named after their discovery in a chicken organ called the 'bursa of Fabricius' (Chang *et al.*, 1955). On receiving signals from antigens and the T_{h2}-cells, some of the B-cells transform into plasma cells (Fig. 3c) that manufacture and secrete antibodies.

(3) Macrophages (Metchinkoff, 1968)

Macrophages (Fig. 3d) represent the major differentiated cells of the mononuclear phagocytic system (Van Furth *et al.*, 1972; Papadimitriou and Ashman, 1989), previously known as the 'reticulo-endothelial system', that have been extensively characterized (Carr, 1980). Macrophages are activated by microorganisms, their products (LPS), chemical mediators, or foreign particles. Among the various molecular mediators that are secreted by macrophages, the cytokines IL-1, TNF-α, interferons (IFN), and growth factors are of particular importance in apical periodontitis. They also contribute serum components and metabolites, such as prostaglandins and leukotrienes, that are important in inflammation. Antigen-presenting dendritic cells are also reported in induced murine apical periodontitis (Okiji *et al.*, 1994). Whether they 'seed' in periapical lesions *via* general circulation (Jontell *et al.*, 1998) or spread locally from inflamed dental pulp is unknown.

(4) Osteoclasts

A major pathological event of apical periodontitis is the osteoclastic destruction of bone and dental hard tissues. There are extensive reviews on the origin (Nijweide and De Grooth, 1992), structure (Gay, 1992), regulation (Heersche, 1992), and 'coupling' (Puzas and Ishibe, 1992) of these cells with osteoblasts. Briefly, the pro-osteoclasts migrate through blood as monocytes to the periradicular tissues and attach themselves to the surface of bone. They remain dormant until signaled by osteoblasts to proliferate. Several daughter cells fuse to form multinucleated osteoclasts that spread over injured and exposed bone surfaces. The cytoplasmic border of the osteoclasts facing the bony surface becomes ruffled as a result of multiple infolding of the plasma membrane. Bone resorption takes place beneath this ruffled border, known as the sub-osteoclastic resorption compartment. At the periphery, the cytoplasmic 'clear zone' is a highly specialized area which regulates the biochemical activities involved in breaking down the bone. The bone destruction happens extracellularly at the osteoclast/bone interface and involves: (i) demineralization of the bone by solubilizing the mineral phase in the resorption compartment, as a result of ionic lowering of pH in the micro-environment; and (ii) enzymatic dissolution of the organic matrix. Root cementum and dentin are also resorbed in apical periodontitis by fusion macrophages designated as 'odontoclasts'. In view of their ultrastructural and histochemical similarities, they belong to the same cell population as osteoclasts (Sahara *et al.*, 1994).

(5) Epithelial cells

About 30 to 52% of all apical periodontitis lesions contain proliferating epithelium (Thoma, 1917; Freeman, 1931; Sonnabend and Oh, 1966; Seltzer *et al.*, 1969; Langeland *et al.*, 1977; Simon, 1980; Yanagisawa, 1980; Nair *et al.*, 1996). During periapical inflammation, the epithelial cell rests (Malassez, 1884) are believed to be stimulated by cytokines and growth factors to undergo division and proliferation, a process commonly described as 'inflammatory hyperplasia'. These cells participate in the pathogenesis of radicular cysts by serving as the source of epithelium. However, ciliated epithelial cells are also found in periapical lesions (Shear, 1992; Nair *et al.*, 2002), particularly in lesions affecting maxillary molars. The maxillary sinus-epithelium was suggested to be a source of those cells (Nair and Schmid-Meier, 1986; Nair *et al.*, 2002).

(B) Molecular mediators

Several cytokines (Cohen *et al.*, 1974), eicosanoids, effector-molecules, and antibodies are involved in the pathogenesis and progression of apical periodontitis.

(1) Pro-inflammatory & chemotactic cytokines

They include interleukin (IL)-1, -6, and -8 and tumor necrosis factors (TNF) (Oppenheim, 1994). The systemic effects of IL-1 are identical to those observed in toxic shock. Local effects include enhancement of leukocyte adhesion to endothelial walls, stimulation of lymphocytes, potentiation of neutrophils, activation of the production of prostaglandins and proteolytic enzymes, enhancement of bone resorption, and inhibition of bone formation. IL-1β is the predominant form found in human periapical lesions and their exudates (Barkhordar *et al.*, 1992; Lim *et al.*, 1994; Matsuo *et al.*, 1994; Ataoglu *et al.*, 2002). IL-1α is primarily involved in apical periodontitis in rats (Wang and Stashenko, 1993; Tani-Ishii *et al.*, 1995). IL-6 (Hirano, 1994) is produced by both lymphoid and non-lymphoid cells under the influence of IL-1, TNF-α, and IFN-γ. It down-regulates the production and counters some of the effects of IL-1. IL-6 has been demonstrated in human periapical lesions (De Sá *et al.*, 2003) and in inflamed marginal periodontal tissues (Yamazaki *et al.*, 1994). IL-8 is a family of chemotactic cytokines (Damme, 1994) produced by monocyte/macrophages and fibroblasts under the influence of IL-1β and TNF-α. Massive infiltration of neutrophils is a characteristic of the acute phases of apical periodontitis, for which IL-8 and other chemo-attractants (such as bacterial-peptides, plasma-derived complement split-factor C_{5a}, and leukotriene B_4) are important. TNF has a direct cytotoxic effect and a general debilitating effect in chronic disease. In addition, the macrophage-derived TNF-α (Tracey, 1994) and the T-lymphocyte-derived TNF-β (Ruddle, 1994), formerly lymphotoxin, have numerous systemic and local effects similar to those of IL-1. The presence of TNF-α has been reported in human apical periodontitis lesions and root canal exudates of teeth with apical periodontitis (Artese *et al.*, 1991; Safavi and Rossomando, 1991; Ataoglu *et al.*, 2002).

(2) IFN

IFN was originally described as an antiviral agent and is now classified as a cytokine. There are three distinct IFNs, designated as -α, -β, and -γ molecules. The antiviral protein is the IFN-γ produced by virus-infected cells and normal T-lymphocytes under various stimuli, whereas the IFN-α/β proteins are produced by a variety of normal cells, particularly macrophages and B-lymphocytes.

(3) Colony-stimulating factors (CSF)

CSFs are cytokines that regulate the proliferation and differentiation of hematopoietic cells. The name originates from the early observation that certain polypeptide molecules promote the formation of granulocyte or monocyte colonies in a semi-

solid medium. Three distinct proteins of this category have been isolated, characterized, and designated as cytokines: (i) granulocyte-macrophage colony-stimulating factor (G-MCF), (ii) granulocyte colony-stimulating factor (G-CSF), and (iii) macrophage colony-stimulating factor (M-CSF). In general, CSFs stimulate the proliferation of neutrophil and osteoclast precursors in the bone marrow. They are also produced by osteoblasts (Puzas and Ishibe, 1992), thus providing one of the communication links between osteoblasts and osteoclasts in bone resorption.

(4) Growth factors

Growth factors regulate the growth and differentiation of non-hematopoietic cells. Transforming growth factors (TGFs) are produced by normal and neoplastic cells that were originally identified by their ability to induce non-neoplastic, surface-adherent colonies of fibroblasts in soft agar cultures. This process appears to be similar to the neoplastic transformation of normal to malignant cells, hence the name TGF. Based on their structural relationship to the epidermal growth factor (EGF), they are classified into TGF-α and TGF-β. The former is closely related to EGF in structure and effects but is produced primarily by malignant cells and therefore is not significant in apical periodontitis. But TGF-β is synthesized by a variety of normal cells and platelets and is involved in the activation of macrophages, proliferation of fibroblasts, synthesis of connectives tissue fibers and matrices, local angiogenesis, healing, and down-regulation of numerous functions of T-lymphocytes. Therefore, TGF-β is important to counter the adverse effects of inflammatory host responses.

(5) Eicosanoids

When cells are activated or injured by diverse stimuli, their membrane lipids are remodeled to generate compounds that serve as intra- and intercellular signals. Arachidonic acid, a 20-carbon polysaturated fatty acid present in all cell membranes, is released from membrane lipids by a variety of stimuli and is rapidly metabolized to form several C_{20} compounds, known collectively as eicosanoids (Greek: eicosi = twenty). The eicosanoids are thought of as hormones with physiological effects at very low concentrations. They mediate inflammatory response, pain, and fever, regulate blood pressure, induce blood clotting, and control several reproductive functions such as ovulation and induction of labor. Prostaglandins (PG) and leukotrienes (LT) (Samuelsson, 1983) are two major groups of eicosanoids involved in inflammation.

(a) Prostaglandins

Prostaglandins were first identified in human semen and were thought to have originated from the prostate gland—hence the name. They are formed when arachidonic acid is metabolized *via* the cyclo-oxygenase pathway (*e.g.*, PGE_2, PGD_2, PGF_{2a}, PGI_2). The PGE_2 and PGI_2 are potent activators of osteoclasts. Much of the rapid bone loss in marginal and apical periodontitis happens during episodes of acute inflammation, when the lesions are dominated by PMN, which are an important source of PGE_2. High levels of PGE_2 have been shown to be present in acute apical periodontitis lesions (McNicholas *et al.*, 1991). Apical hard-tissue resorption can be suppressed by parenteral administration of indomethacin, an inhibitor of cyclo-oxygenase (Torabinejad *et al.*, 1979).

(b) Leukotrienes

Leukotrienes (*e.g.*, LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4) are formed when arachidonic acid is oxidized *via* the lipoxygenase pathway. LTB_4 is a powerful chemotactic agent for neutrophils (Okiji *et al.*, 1991) and causes adhesion of PMN to the endothelial walls. LTB_4 (Torabinejad *et al.*, 1992) and LTC_4 (Cotti and Torabinejad, 1994) have been detected in apical periodontitis, with a high concentration of the former in symptomatic lesions (Torabinejad *et al.*, 1992).

(6) Effector molecules

One of the earliest histopathological changes that take place in both apical and marginal periodontitis is the degradation of extracellular matrices. The destruction of the matrices is caused by enzymatic effector molecules. Four major degradation pathways have been recognized: (1) osteoclastic, (2) phagocytic, (3) plasminogen-dependent, and (4) metallo-enzyme-regulated (Birkedal-Hansen, 1993). The zinc-dependent proteases that are responsible for the degradation of much of the extracellular matrix components (such as collagen, fibronectin, laminin, gelatin, and proteoglycan core proteins) belong to the superfamily of enzymes called the 'matrix metalloproteinases' (MMP). The biology of the MMP has been extensively researched and reviewed (Birkedal-Hansen *et al.*, 1992, 1993). Their presence has also been reported in apical periodontitis lesions (Teronen *et al.*, 1995; Shin *et al.*, 2002).

(7) Antibodies

These are specific weapons of the body that are produced solely by plasma cells. Different classes of immunoglobulins have been found in plasma cells (Kuntz *et al.*, 1977; Morton *et al.*, 1977; Pulver *et al.*, 1978; Jones and Lally, 1980; Stern *et al.*, 1981; Skaug *et al.*, 1982) and extracellularly (Naidorf, 1975; Kuntz *et al.*, 1977; Matsumoto, 1985; Torres *et al.*, 1994) in human apical periodontitis. The concentration of IgG in apical periodontitis was found to be nearly five times that in non-inflamed oral mucosa (Greening and Schonfeld, 1980). Immunoglobulins have also been shown in plasma cells residing in the periapical cyst wall (Toller and Holborow, 1969; Pulver *et al.*, 1978; Stern *et al.*, 1981; Smith *et al.*, 1987) and in the cyst fluid (Toller and Holborow, 1969; Selle, 1974; Skaug, 1974; Ylipaavalniemi, 1977). Their concentration in the cyst fluid was several times higher than that in blood (Selle, 1974; Skaug, 1974). The specificity of the antibodies present in apical periodontitis may be low, since LPS may act as antigens or mitogens. The resulting antibodies may be a mixture of both mono- and polyclonal varieties. The latter are non-specific to their inducer and therefore are ineffective. However, the specific monoclonal component may participate in the antimicrobial response and may even intensify the pathogenic process by forming antigen-antibody complexes (Torabinejad *et al.*, 1979). Intracanal application of an antigen against which the animal had been previously immunized resulted in the induction of a transient apical periodontitis (Torabinejad and Kriger, 1980).

(IV) Pathogenesis of Apical Periodontitis Lesions

The dynamic encounter at the periapex between the microbial and host factors outlined above results in various categories of apical periodontitis lesions (Fig. 4). The equilibrium at the periapex, in favor of or against the host defense, determines the his-

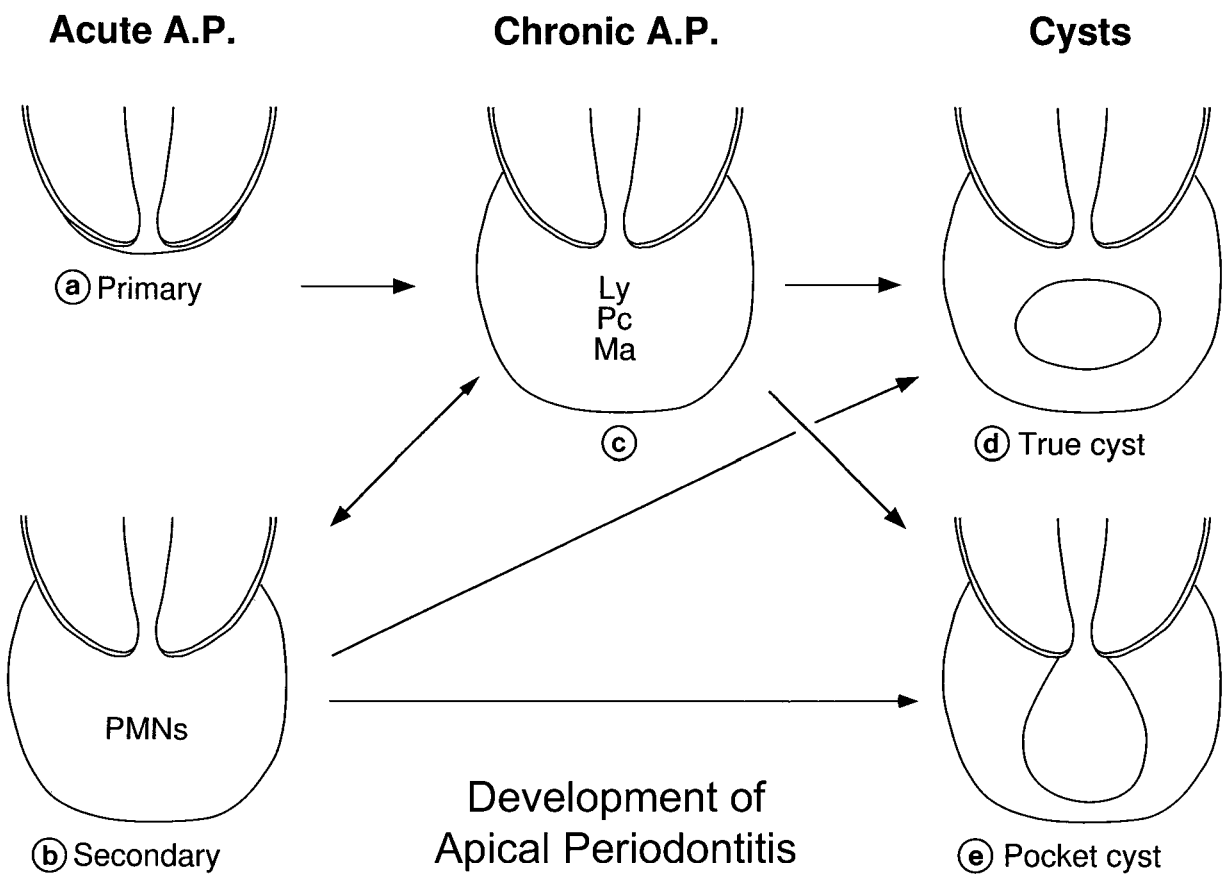

Figure 4. Pathogenesis of acute **(a,b)**, chronic **(c)**, and cystic **(d,e)** apical periodontitis (AP) lesions. The acute lesion may be primary (a) or secondary (b) and is characterized by the presence of a focus of neutrophils (PMNs). The major components of chronic lesions (c) are lymphocytes (Ly), plasma cells (Pc), and macrophages (Ma). Periapical cysts can be differentiated into true cysts (d), with completely enclosed lumina, and pocket cysts (e), with cavities open to the root canal. Arrows indicate the direction in which the lesions can change. (From Nair, 1998b.)

tological picture of the lesions.

(A) Initial apical periodontitis

This is generally caused by micro-organisms residing in or invading from the apical root canal into the periapical tissues (Fig. 5). But the inflammation can also be induced by accidental trauma, injury from instrumentation, or irritation from chemicals and endodontic materials, each of which can provoke an intense tissue-response of short duration. The process is accompanied by clinical symptoms such as pain, tooth elevation, and tenderness to pressure on the tooth. Such initial, symptomatic lesions are viewed as the acute apical periodontitis (acute periradicular periodontitis [AAE, 2003] [Fig. 4a]). It must be pointed out that, except for the outcome, there is no clear distinction between aseptic and infectious inflammations. The tissue response is generally limited to the apical periodontal ligament and the neighboring spongiosa. It is initiated by the typical neuro-vascular response of inflammation, resulting in hyperemia, vascular congestion, edema of the periodontal ligament, and extravasation of neutrophils. The latter are attracted to the area by chemotaxis, induced initially by tissue injury, bacterial products (LPS), and complement-factor C_{5a}. Since the integrity of bone, cementum, and dentin has not yet been disturbed, the periapical changes at this stage are radiographically undetectable. If non-infectious irritants induced the inflammation, the lesion may resolve, and the structure of the apical periodontium may be restored (Andreasen, 1985).

When infection is involved, the neutrophils not only fight the micro-organisms (Fig. 3a) but also release leukotrienes and prostaglandins. The former (LTB_4) attract more neutrophils and macrophages into the area, and the latter activate osteoclasts. In a few days' time, the bone surrounding the periapex can be resorbed, and a radiolucent area may become detectable at the periapex (Stashenko *et al.*, 1992). This initial rapid bone resorption can be prevented by indomethacin (Torabinejad *et al.*, 1979; Torabinejad and Kriger, 1980) that inhibits cyclo-oxygenase, thus suppressing prostaglandin synthesis. Neutrophils die at the inflammatory site (Fig. 3a) and release enzymes from their cytoplasmic granules that cause destruction of the extracellular matrices and cells. The self-induced destruction of the tissues prevents the spread of infection to other parts of the body and provides space for the infiltration of specialized defense cells. During the acute phase, macrophages also appear at the periapex. Activated macrophages produce a variety of mediators, among which the pro-inflammatory (IL-1, -6, and TNF-α) and chemotactic (IL-8) cytokines are of particular importance. These cytokines intensify the local vascular response, osteoclastic bone resorption, and effector-mediated degradation of the extracellular matrices and can place the body on a 'general alert' by endocrine action, to raise the output of acute-phase

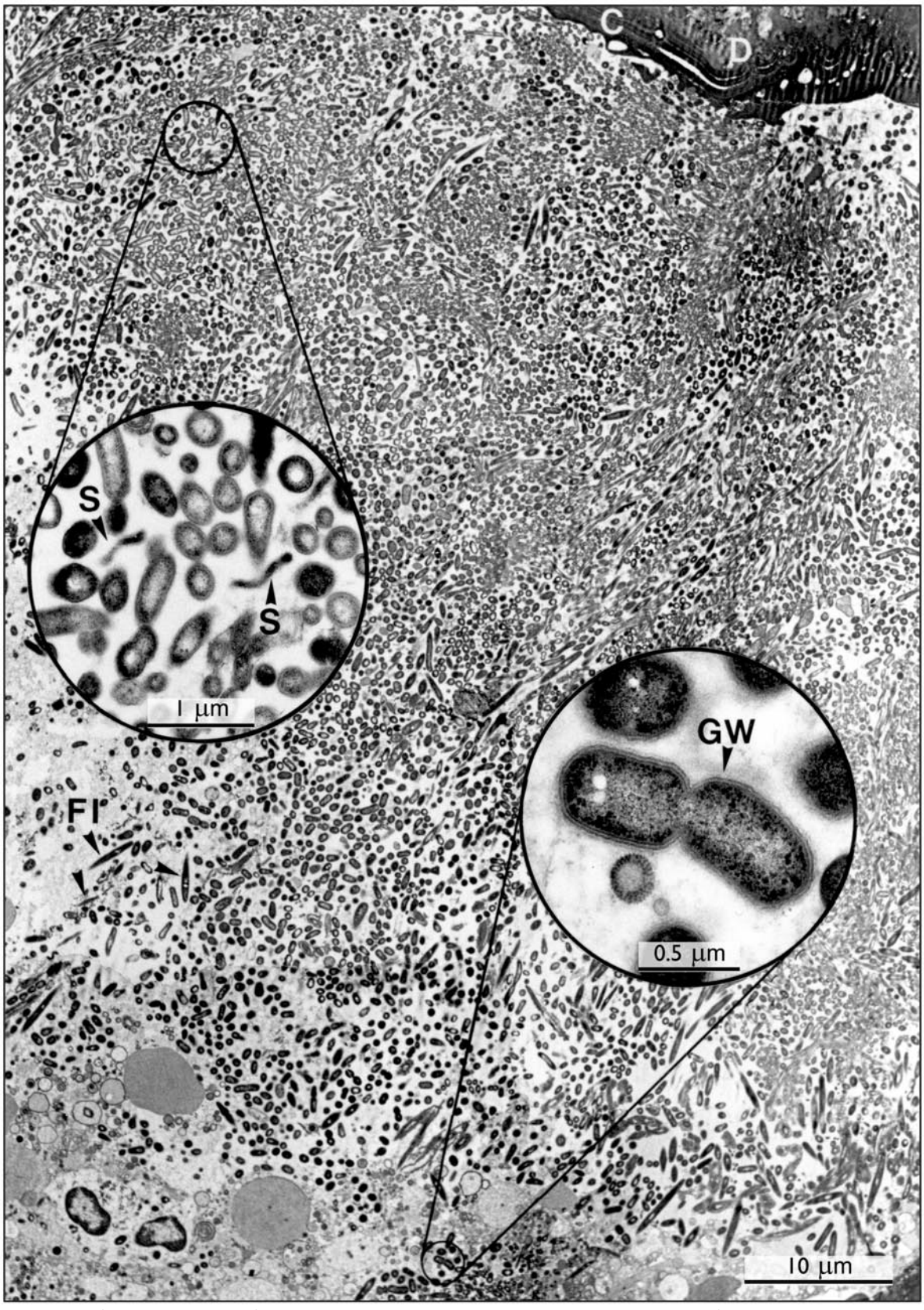

Figure 5. A microbial biofilm at the root-tip of a human tooth with secondary acute apical periodontitis of endodontic origin. The mixed bacterial flora consists of numerous dividing cocci, rods (lower inset), filaments (FI), and spirochetes (S, upper inset). Rods often reveal a Gram-negative cell wall (GW, lower inset). C, cementum; D, dentin. Magnifications: 2680x; upper inset x 19,200; lower inset x 36,400. (Adapted from Nair, 1987.)

proteins by hepatocytes (Lerner, 1994). They also act in concert with IL-6 to up-regulate the production of hematopoietic colony-stimulating factors, which rapidly mobilize the neutrophils and the pro-macrophages from bone marrow. The

acute response can be intensified, particularly in later stages, by the formation of antigen-antibody complexes (Torabinejad *et al.*, 1979; Torabinejad and Kriger, 1980). Acute primary apical periodontitis has several possible outcomes, such as: spontaneous healing, further intensification and spreading into the bone (alveolar abscess), open to the exterior (fistulation or sinus tract formation), or becoming chronic.

(B) Established chronic apical periodontitis

A prolonged presence of microbial irritants leads to a shift in the neutrophil-dominated lesion to a macrophage-, lymphocyte-, and plasma-cell-rich one, encapsulated in collagenous connective tissue. Such asymptomatic, radiolucent lesions can be visualized as a 'lull phase' following an intense phase in which PMNs die *en masse*, the 'foreign intruders' having been temporarily beaten and held in the root canal (Fig. 2). The macrophage-derived pro-inflammatory cytokines (IL-1, -6; TNF-α) are powerful lymphocyte stimulators. The quantitative data on the various types of cells residing in chronic periapical lesions may not be representative. Nevertheless, investigations based on monoclonal antibodies suggest a predominant role for T-lymphocytes and macrophages. Activated T-cells produce a variety of cytokines that down-regulate the output of pro-inflammatory cytokines (IL-1, -6, and TNF-α), leading to the suppression of osteoclastic activity and reduced bone resorption. In contrast, the T-cell-derived cytokines may concomitantly up-regulate the production of connective tissue growth factors (TGF-β), with stimulatory and proliferative effects on fibroblasts and the microvasculature. T_{h1} and T_{h2} cell populations may participate in this process (Stashenko *et al.*, 1998; Gemmell *et al.*, 2002). The option to down-regulate the destructive process explains the absence of or retarded bone resorption and the rebuilding of the collagenous connective tissue during the chronic phase of the disease. Consequently, the chronic lesions can remain 'dormant' and symptomless for long periods of time without major changes in radiographic status. But the delicate equilibrium prevailing at the periapex can be disturbed by one or more factors that may favor the micro-organisms 'stationed' within the root canal. The microbes may advance into the periapex (Fig. 5), and the lesion spontaneously becomes acute with re-occurrence of symptoms (excerbating apical periodontitis, Phoenix abscess). As a result, micro-organisms can be found extraradicularly during these acute episodes, with rapid enlargement of the radiolucent area. This radiographic feature is due to apical bone resorption occurring rapidly during the acute phases, with relative inactivity during the chronic periods. The progression of the disease, therefore, is not continuous, but happens in discrete leaps after periods of 'stability'.

Chronic apical periodontitis is commonly referred to as 'solid dental' or 'periapical granuloma'. It consists of a granulomatous tissue with infiltrate cells, fibroblasts, and a well-developed fibrous capsule. Serial sectioning shows that about 45% of all chronic periapical lesions are epithelialized (Nair *et al.*, 1996). When the epithelial cells begin to proliferate, they may do so in all directions at random, forming an irregular epithelial mass in which vascular and infiltrated connective tissue becomes enclosed. In some lesions, the epithelium may grow into the entrance of the root canal, forming a plug-like seal at the apical foramen (Malassez, 1885; Sonnabend and Oh, 1966; Nair and Schroeder, 1985). The epithelial cells generate an 'epithelial attachment' to the root surface or canal wall which, in TEM, reveals a basal lamina and hemidesmosomal structures (Nair and Schroeder, 1985). In random histological sections, the epithelium in the lesion appears as arcades and rings. The extra-epithelial tissue predominantly consists of small blood vessels, lymphocytes, plasma cells, and macrophages. Among the lymphocytes, T-cells are likely to be more numerous than B-cells (Cymerman *et al.*, 1984; Nilsen *et al.*, 1984; Torabinejad and Kettering, 1985; Kopp and Schwarting, 1989), and $CD4^+$ cells may outnumber $CD8^+$ cells (Lukic *et al.*, 1990; Piattelli *et al.*, 1991; Barkhordar *et al.*, 1992; Marton and Kiss, 1993) in certain phases of the lesions. The connective tissue capsule of the lesion consists of dense collagenous fibers that are firmly attached to the root surface, so that the lesion may be removed *in toto* with the extracted tooth.

(C) Established cystic apical periodontitis (radicular cysts)

Periapical cysts are a direct sequel to chronic apical periodontitis, but not every chronic lesion develops into a cyst. Although the reported incidence of cysts among apical periodontitis lesions varies from 6 to 55% (for review, see Nair, 1998a), investigations based on meticulous serial-sectioning and strict histopathological criteria (Sonnabend and Oh, 1966; Simon, 1980; Nair *et al.*, 1996) have showed that the actual incidence of the cysts may be well below 20%. There are two distinct categories of radicular cysts, namely, those containing cavities completely enclosed in epithelial lining (Fig. 6), and those containing epithelium-lined cavities that are open to the root canals (Fig. 7; (Simon, 1980; Nair *et al.*, 1996). The latter was originally described as 'bay cysts' (Simon, 1980) and has been newly designated as 'periapical pocket cysts' (Nair *et al.*, 1996). More than half of the cystic lesions are true apical cysts, and the remainder are apical pocket cysts (Simon, 1980; Nair *et al.*, 1996). In view of the structural difference between the two categories of cysts, the pathogenic pathways leading to their formation may differ in certain respects.

(1) Periapical true cyst

There have been many attempts to explain the pathogenesis of apical true cysts (Thoma, 1917; Rohrer, 1927; Gardner, 1962; Shear, 1963; Main, 1970; Ten Cate, 1972; Torabinejad, 1983). The genesis of true cysts has been discussed as occurring in three stages (Shear, 1992). During the first phase, the dormant epithelial cell rests (Malassez, 1884, 1885) are believed to proliferate, probably under the influence of growth factors (Thesleff, 1987; Gao *et al.*, 1996; Lin *et al.*, 1996) that are released by various cells residing in the lesion. During the second phase, an epithelium-lined cavity comes into existence. There are two long-standing theories regarding the formation of the cyst cavity. (i) The 'nutritional deficiency theory' is based on the assumption that the central cells of the epithelial strands get removed from their source of nutrition and undergo necrosis and degeneration. The products, in turn, attract neutrophilic granulocytes into the necrotic area. Such microcavities—containing degenerating epithelial cells, infiltrating leukocytes, and tissue exudate—coalesce to form the cyst cavity, lined by stratified squamous epithelium. (ii) The 'abscess theory' postulates that the proliferating epithelium surrounds an abscess formed by tissue necrosis and lysis, because of the innate nature of epithelial cells to cover exposed connective tissue surfaces. During the third phase, the cyst grows, but the exact mechanism has not yet been adequately clarified. Theories based on osmotic pressure (James, 1926; Toller, 1948, 1970) have receded to the background

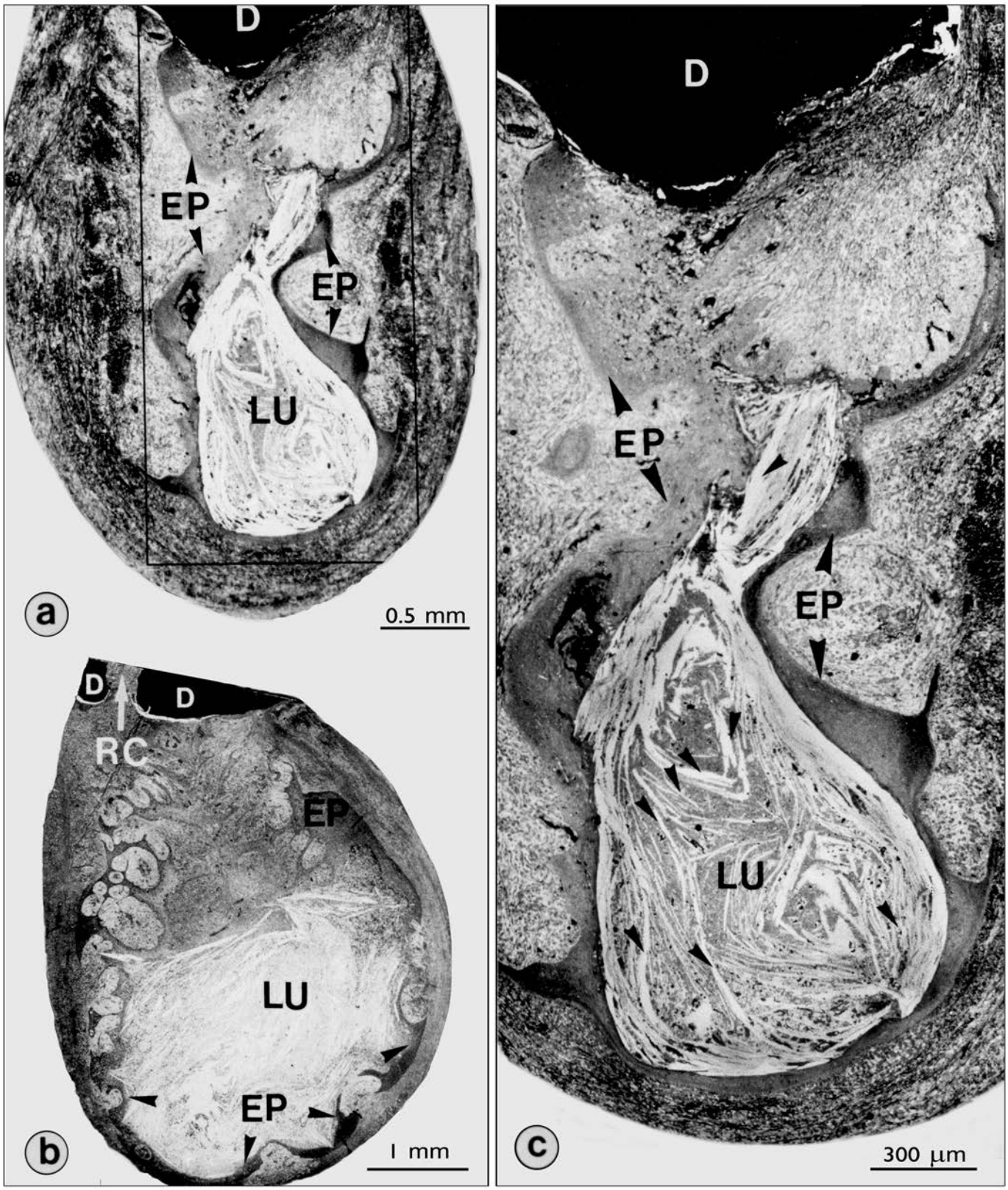

Figure 6. Structure of apical true cysts **(a,b)**. The cyst lumina (LU) are completely enclosed in stratified squamous epithelium (EP). Note the absence of any communication of the cyst lumen with the root canal (RC in b). The demarcated area in a is magnified in **c**. Arrowheads in c indicate cholesterol clefts. Magnifications; (a) 30x, (b) 17x, and **(c)** 60x. (From Nair *et al.*, 1996.)

in favor of a molecular basis for cystogenesis (Harris and Goldhaber, 1973; Harris *et al.*, 1973; Brunette *et al.*, 1979; Birek *et al.*, 1983; Teronen *et al.*, 1995). The fact that apical pocket cysts (Fig. 7), with a lumen open to the necrotic root canal, can grow

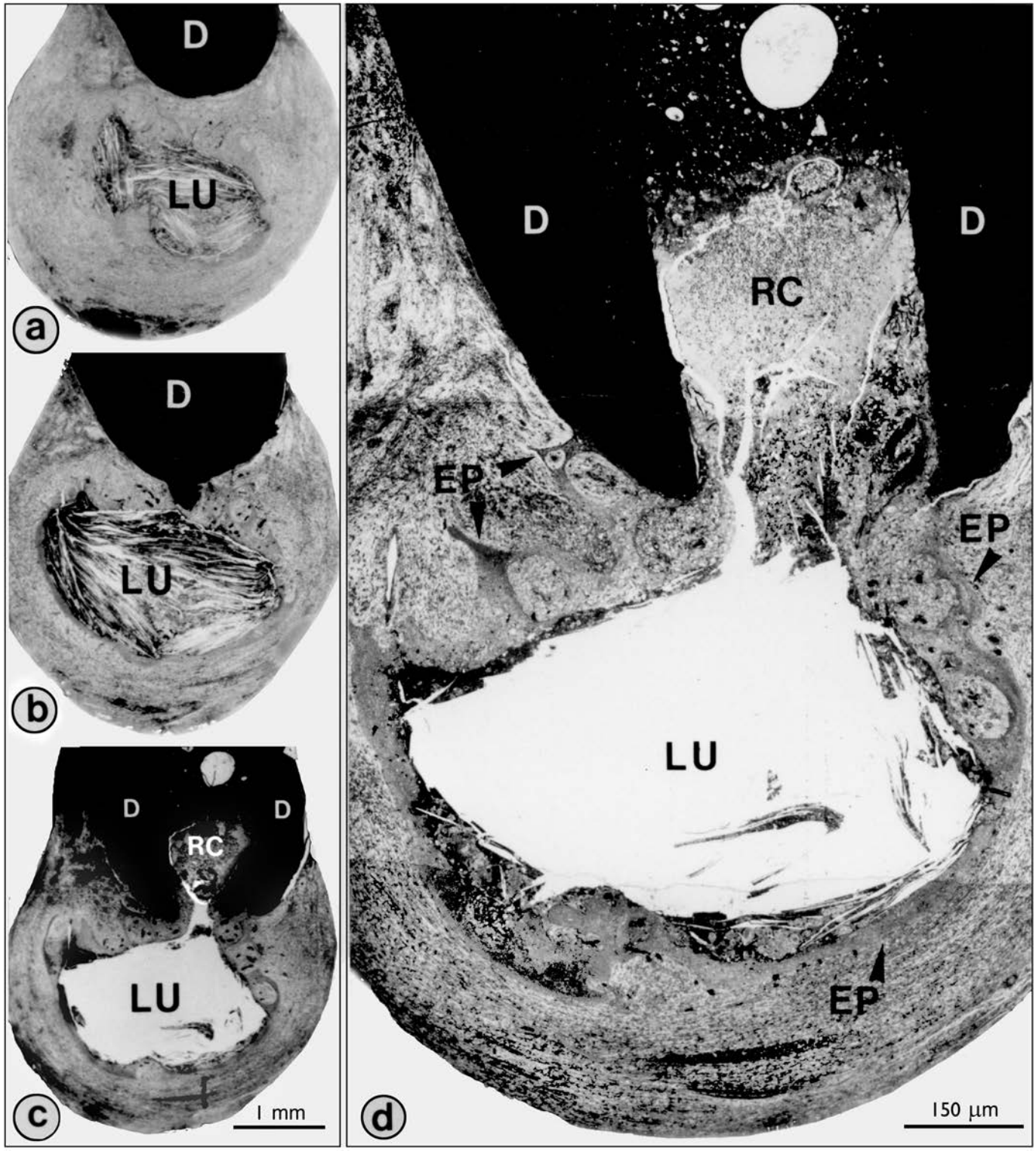

Figure 7. Structure of an apical pocket cyst. Axial sections passing peripheral to the root canal **(a,b)** give the false impression of the presence of a cyst lumen (LU) completely enclosed in epithelium. Sequential section **(c,d)** passing through the axial plane of the root canal (RC) clearly reveals the continuity of the cystic lumen (LU) with the root canal (RC). Note the pouch-like lumen (LU) of the pocket cyst with the epithelium (EP), forming a collar at the root apex. Magnifications: (a,b,c) 5x; (d) 132x. (Adapted from Nair, 1995.)

would eliminate osmotic pressure as a potential factor in the development of radicular cysts (Nair *et al.*, 1996; Nair, 1997). Although no direct evidence is yet available, the tissue dynamics and the cellular components of radicular cysts suggest possible molecular pathways for cyst expansion. The neutrophils that die in the cyst lumen provide a continuous source of prostaglandins (Formigli *et al.*, 1995), which can diffuse through the porous epithelial wall (Shear, 1992) into the surrounding tis-

sues. The cell population residing in the extra-epithelial area contains numerous T-lymphocytes (Torabinejad and Kettering, 1985), and macrophages produce a battery of cytokines, particularly the IL-1β. The prostaglandins and the inflammatory cytokines can activate osteoclasts, culminating in bone resorption. The presence of effector molecules (MMP-1 and -2) has also been reported in human periapical cysts (Teronen *et al.*, 1995).

(2) Periapical pocket cyst

This is probably initiated by the accumulation of neutrophils around the apical foramen in response to the bacterial presence in the apical root canal (Nair *et al.*, 1996; Nair, 1997). The micro-abscess so formed can become enclosed by the proliferating epithelium, which, on coming into contact with the root-tip, forms an epithelial collar with 'epithelial attachment' (Nair and Schroeder, 1985). The latter seals off the infected root canal with the micro-abscess from the periapical tissue milieu. When the externalized neutrophils die and disintegrate, the space they occupied becomes a microcystic sac. The presence of microbes in the apical root canal, their products, and the necrotic cells in the cyst-lumen attract more neutrophils by a chemotactic gradient. However, the pouch-like lumen—biologically outside the periapical milieu—acts as a 'death trap' for the transmigrating neutrophils. As the necrotic cells accumulate, the sac-like lumen enlarges and may form a voluminous diverticulum of the root canal space, extending into the periapical area (Nair *et al.*, 1996; Nair, 1997). Bone resorption and degradation of the matrices, occurring in association with the enlargement of the pocket cyst, are likely to follow a similar molecular pathway, as in the case of the true periapical cyst (Nair *et al.*, 1996). From the pathogenic, structural, tissue-dynamic, and host-benefit points of view, the pouch-like extension of the root canal space has much in common with a marginal periodontal pocket, justifying the name 'periapical pocket cyst' (Nair *et al.*, 1996).

(V) Causes of Endodontic Failures

Since intraradicular micro-organisms are the essential etiological agents of apical periodontitis (Kakehashi *et al.*, 1965), the aim of endodontic treatment is to eliminate infection from the root canal and prevent re-infection by obturation. When the treatment is done properly, healing of the periapical lesion usually occurs with osseous regeneration, which is characterized by gradual reduction and resolution of the radiolucency on subsequent follow-up radiographs (Strindberg, 1956; Grahnén and Hansson, 1961; Seltzer *et al.*, 1963; Storms, 1969; Molven, 1976; Kerekes and Tronstad, 1979; Molven and Halse, 1988; Sjögren *et al.*, 1990, 1997; Sundqvist *et al.*, 1998). However, for various reasons, a complete bone healing or reduction of the apical radiolucency may not occur in all root-canal-treated teeth. Cases of unresolving post-treatment periapical radiolucencies are commonly referred to as 'endodontic failures'. It is generally acknowledged that most failures occur when treatment procedures have not reached a satisfactory standard for the control and elimination of infection. Common problems that may lead to endodontic failure include inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation, and leaking temporary or permanent fillings (Sundqvist and Figdor, 1998). Even when the highest standards are met and the most careful procedures followed, failures still occur, because of the anatomical complexity of the root canal system (Hess, 1921; Perrini and Castagnola, 1998), with regions that cannot be debrided and obturated with existing instruments, materials, and techniques. In addition, there are factors beyond the root canals, within the inflamed periapical tissue, that can interfere with post-treatment healing of the lesion.

(A) Intraradicular infection

Microscopic examination of periapical tissues removed during apical surgery has long been a method for the investigation of causes of failure in root-canal-treated teeth. Early investigations of apical biopsies had several limitations, such as the use of unsuitable specimens and inappropriate methodology and criteria for analysis (Seltzer *et al.*, 1967; Andreasen and Rud, 1972b; Block *et al.*, 1976; Langeland *et al.*, 1977; Lin *et al.*, 1991). Therefore, these studies failed to yield relevant information about the reasons for apical periodontitis persisting as asymptomatic radiolucencies, even after proper conventional endodontic treatment.

In a histological analysis of apical specimens of failed cases (Seltzer *et al.*, 1967), there was not even a mention of persisting microbial infection as a potential cause of the failures. A histobacteriological study, with the use of serial-step-sectioning and special bacterial stains, found bacteria in the root canals of 14% of the 66 specimens examined (Andreasen and Rud, 1972a). Two other studies analyzed 230 and 35 endodontic surgical specimens, respectively, by routine paraffin histology (Block *et al.*, 1976; Langeland *et al.*, 1977). Although bacteria were found in 10 and 15% of the respective biopsies, intraradicular infection was detected in only a single specimen in each study. In the remaining biopsies in which bacteria were found, the data also included those specimens in which bacteria were found as 'contaminants on the surface of the tissue'. In yet another study, 'bacteria and or debris' was found in the root canals of 63% of the 86 endodontic surgical specimens (Lin *et al.*, 1991), although it is obvious that 'bacteria and debris' cannot be equated as etiological agents in endodontic treatment failures. The low reported incidence of intraradicular infections in these studies is primarily due to a methodological inadequacy, since micro-organisms easily go undetected when the investigations are based on random paraffin sections alone. This has been convincingly demonstrated (Nair, 1987; Nair *et al.*, 1990a). Consequently, persisting intraradicular infection has not been considered as a factor in endodontic failures.

To identify potential etiological agents in asymptomatic endodontic failures by microscopy, one must select the cases from teeth that have had the best possible orthograde root canal treatment, and the radiographic lesions must remain asymptomatic until surgical intervention. The specimens must be anatomically intact block-biopsies that include the apical portions of the roots and the soft tissue of the lesions. Such specimens should undergo meticulous investigation by serial or step-serial sections that are analyzed with the use of correlative light and transmission electron microscopy. A study that met these criteria and also bacterial monitoring before and during treatment revealed intraradicular micro-organisms in 6 of the 9 block biopsies (Fig. 8) (Nair *et al.*, 1990a). The findings showed conclusively that the majority of root-canal-treated teeth evincing asymptomatic apical periodontitis harbor persistent infection in the apical portion of the root canal. However, the proportion of failed cases with intraradicular infection is likely to be much higher in routine endodontic practice than the two-thirds of nine cases reported (Nair *et al.*, 1990a) for several reasons, particularly the absence of routine microbial monitoring of

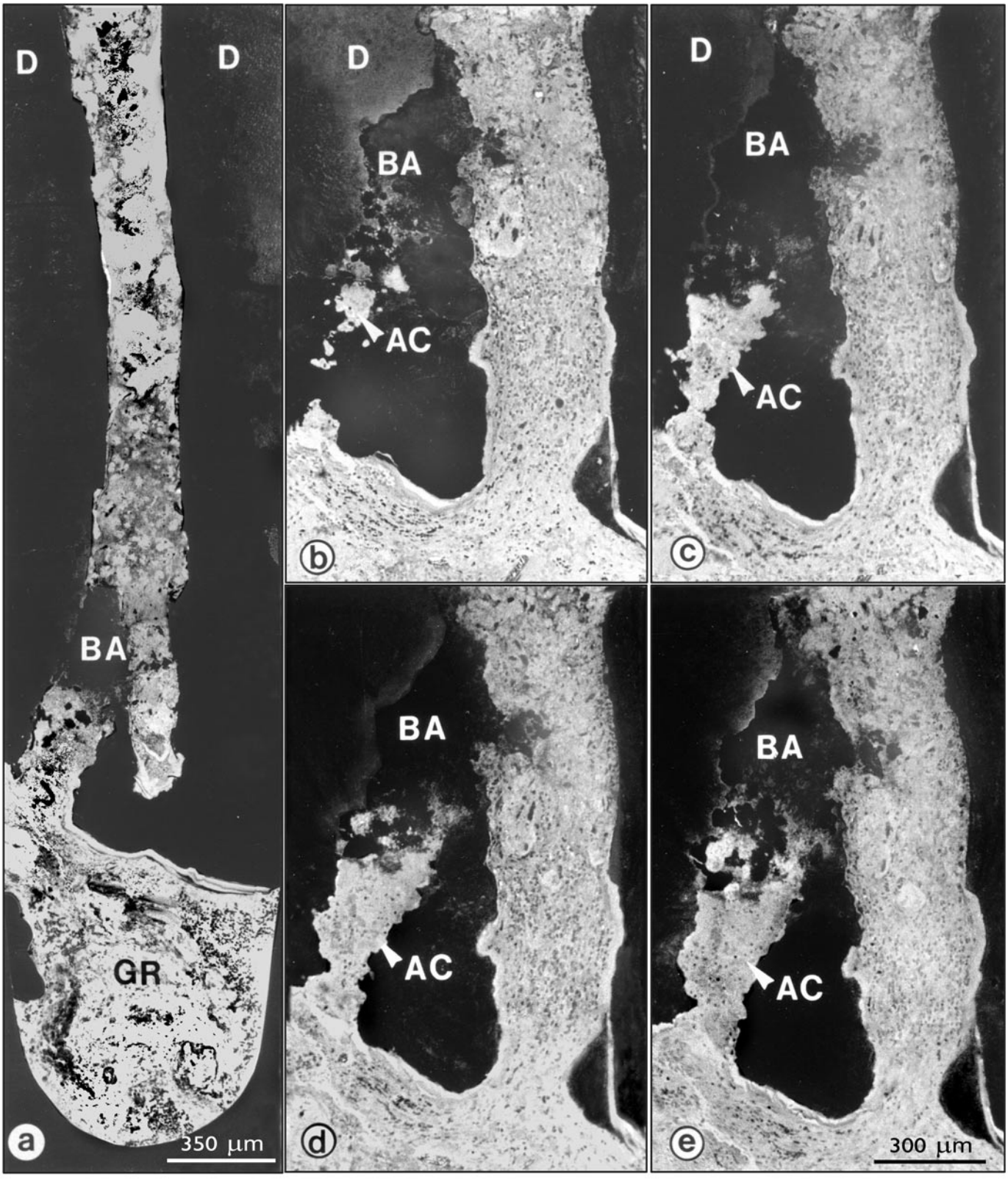

Figure 8. Axial sections through the surgically removed apical portion of the root with a therapy-resistant apical periodontitis. Note the light microscopically visible cluster of bacteria (BA in **a**) in the root canal. Serial semi-thin sections (**b** to **e**), taken at various distances from the section plane of (a), reveal the emerging and gradually widening profiles of an accessory root canal (AC) that is clogged with bacteria (BA). Magnifications: (a) 52x, (b-e) 62x. (From Nair *et al.*, 1990a.)

the root canals in 'field conditions'. At the light microscopic level, it was possible to detect bacteria in only one of the six cases (Nair *et al.*, 1990a). Micro-organisms were found as aggregates located within small canals of apical ramifications (Fig. 8)

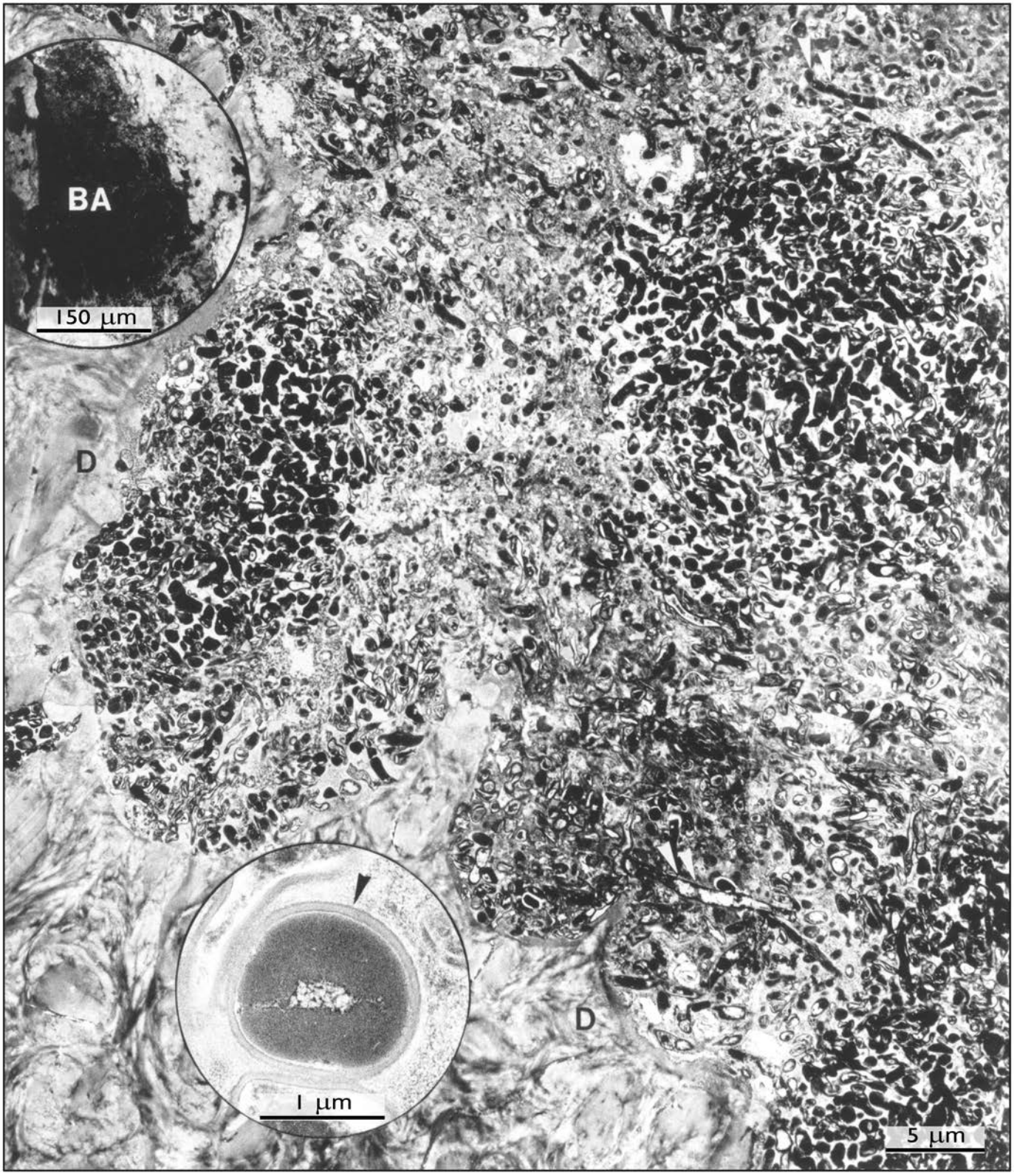

Figure 9. Transmission electron microscopic view of the bacterial mass (BA, upper inset) illustrated in (8a). Morphologically, the bacterial population appears to be composed of only Gram-positive, filamentous organisms (arrowhead in lower inset). Note the distinctive Gram-positive cell wall. The upper inset is a magnification of the bacterial cluster (BA) in (8a). Magnification: 3400x. Insets: upper, 132x; lower, 21,300x. (From Nair *et al.*, 1990a.)

of the root canal or in the space between the root fillings and the canal wall. This demonstrates the inadequacy of conventional paraffin techniques for detecting infections in apical biopsies.

In a recent investigation where molecular genetic techniques were used (Siqueira and Rôças, 2004), all 22 teeth with 'no symptoms', but with unhealed post-treatment apical radio-

lucencies, revealed bacterial DNA in intraradicular samples. The selection of appropriate cases for investigation is of particular importance. It should be pointed out that five of the 22 teeth 'had temporary (coronal) restorations' that would allow for bacterial re-infection of the canals by possible coronal microleakage. Apart from the possible re-infection and/or contamination that can happen, even in teeth with permanent coronal restorations, the molecular technique does not differentiate between viable and non-viable organisms. The method has been successfully used to detect Myco*bacterium tuberculosis* DNA in pre-historic animals (Rothschild *et al.*, 2001) and ancient human remains (Salo *et al.*, 1994; Konomi *et al.*, 2002). Thus, the polymerase chain-reaction (PCR)-based methods (Mullis and Faloona, 1987) can detect minuscule amounts of viable and/or dead bacterial DNA that can then be amplified, resulting in an exponential accumulation of millions of copies of the original DNA fragments. The data derived from the molecular-genetic technique (Siqueira and Rôças, 2004), therefore, require very careful interpretation in light of the technique's many advantages and numerous limitations, so that inflated conclusions (*e.g.,* that all post-treatment apical periodontitis is caused by the presence of intraradicular infection) can be avoided.

On the basis of cell wall ultrastructure, only Gram-positive bacteria were found (Fig. 9), an observation fully in agreement with the results of purely microbiological investigations of root canals of previously root-filled teeth with persisting periapical lesions. Of the six specimens that contained intraradicular infections, four had one or more morphologically distinct types of bacteria, and two revealed yeasts (Fig. 10). The presence of endodontic yeasts in root-treated teeth with apical periodontitis was also confirmed by microbiological techniques (Waltimov *et al.*, 1997; Peciuliene *et al.*, 2001). These findings clearly associate intraradicular fungus as a potential non-bacterial, microbial cause of endodontic failures. It has been suggested that intraradicular infection can also remain within the innermost portions of infected dentinal tubules that serve as a reservoir for endodontic re-infection that might interfere with periapical healing (Shovelton, 1964; Valderhaug, 1974; Nagaoka *et al.*, 1995; Peters *et al.*, 1995; Love *et al.*, 1997; Love and Jenkinson, 2002).

(B) Endodontic flora of root-canal-treated teeth

The microbiology of root-filled canals is less well-understood than that of untreated infected necrotic dental pulps. This is probably a consequence of the search for non-microbial causes of a purely technical nature for the failure of root canal treatments. Only a few species have been found in the root canals of teeth that have undergone proper endodontic treatment but that, on follow-up, revealed persisting, asymptomatic periapical radiolucencies. The bacteria found in these cases are predominantly Gram-positive cocci, rods, and filaments. By culture techniques, species belonging to the genera *Actinomyces, Enterococcus,* and *Propionibacterium* (previously *Arachnia*) are frequently isolated from such root canals and characterized (Möller, 1966; Sundqvist and Reuterving, 1980; Happonen, 1986; Sjögren *et al.*, 1988; Fukushima *et al.*, 1990; Molander *et al.*, 1998; Sundqvist *et al.*, 1998; Hancock *et al.*, 2001; Pinheiro *et al.*, 2003). The repeated reporting of *Enterococcus faecalis* deserves to be mentioned (Möller, 1966; Fukushima *et al.*, 1990; Molander *et al.*, 1998; Sundqvist *et al.*, 1998). *E. faecalis* is rarely found in infected but untreated root canals (Sundqvist and Figdor, 1998). It is resistant to most of the intracanal medicaments, particularly to calcium hydroxide dressings (Byström *et al.*, 1985), probably due to its ability to regulate internal pH with an efficient proton pump (Evans *et al.*, 2002). *E. faecalis* can survive prolonged starvation (Figdor *et al.*, 2003). It can grow as a mono-infection in treated canals in the absence of synergistic support from other bacteria (Fabricius *et al.*, 1982b). In spite of the current focus of attention, it still remains to be shown, in controlled studies, that *E. faecalis* is the pathogen of significance in most cases of failing endodontic treatment. Microbiological (Möller, 1966; Waltimo *et al.*, 1997) and correlative electron microscopic (Nair *et al.*, 1990a) studies have shown the presence of yeasts (Fig. 10) in canals of root-filled teeth with unresolved apical periodontitis. *Candida albicans* is the most frequently isolated fungus from root-filled teeth with apical periodontitis (Molander *et al.*, 1998; Sundqvist *et al.*, 1998).

(C) Extraradicular actinomycosis

Actinomycosis is a chronic, granulomatous, infectious disease in man and animals caused by the genera *Actinomyces* and *Propionibacterium.* They are non-acid, fast, non-motile, Gram-positive organisms revealing characteristic branching filaments that end in clubs or hyphae. The intertwining filamentous colonies are often called "sulphur granules" because of their appearance as yellow specks in exudate. When crushed, the clumps of branching micro-organisms, with radiating filaments in pus, give a "starburst" appearance that prompted the name *Actinomyces*, or 'ray fungus' (Harz, 1879). The endodontic infections of actinomyces are a sequel to caries and are caused by *Actinomyces israelii* and *Propionibacterium propionicum,* commensals of the oral cavity. In tissue sections, the characteristic light microscopic feature of an actinomycotic colony is the presence of an intensely dark-staining, Gram- and PAS-positive, core with radiating peripheral filaments (Fig. 11) that give the typical "starburst" or "ray fungus" appearance. Ultrastructurally, the center of the colony consists of a very dense aggregation of branching filamentous organisms held together by an extracellular matrix (Nair and Schroeder, 1984; Figdor *et al.*, 1992). Several layers of PMN usually surround an actinomycotic colony.

Because of the ability of the actinomycotic organisms to establish extraradicularly (Fig. 11), they can perpetuate the inflammation at the periapex, even after orthograde root canal treatment. Therefore, periapical actinomycosis is important in endodontics (Sundqvist and Reuterving, 1980; Nair and Schroeder, 1984; Happonen *et al.*, 1985; Happonen, 1986; Sjögren *et al.*, 1988). *A. israelii* and *P. proprionicum* are consistently isolated and characterized from the periapical tissue of teeth which did not respond to proper conventional endodontic treatment (Happonen, 1986; Sjögren *et al.*, 1988). A strain of *A. israelii*, isolated from a case of failed endodontic treatment and grown in pure culture, was inoculated into subcutaneously implanted tissue chambers in experimental animals. Typical actinomycotic colonies were formed within the experimental host tissue. This would implicate *A. israelii* as a potential etiological factor of failed endodontic treatment. The properties that enable these bacteria to establish in the periapical tissues are not fully understood, but appear to involve their ability to build cohesive colonies that enable them to escape the host defense system (Figdor *et al.*, 1992).

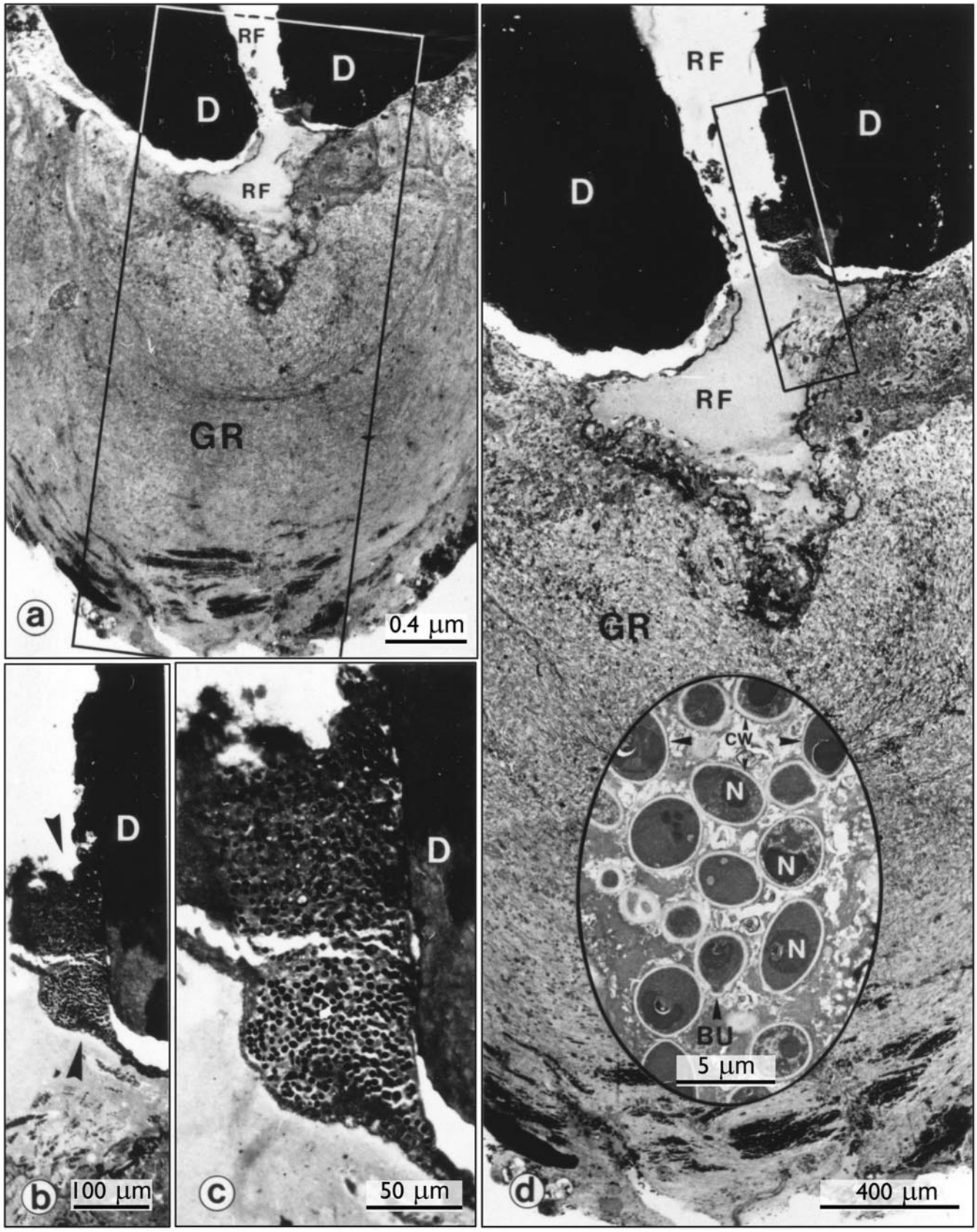

Figure 10. Fungi as a potential cause of endodontic failures. **(a)** Low-power overview of an axial section of a root-filled (RF) tooth with a persisting apical periodontitis lesion (GR). The rectangular demarcated areas in (a) and **(d)** are magnified in (d) and **(b)**, respectively. Note the two microbial clusters (arrowheads in b) further magnified in **(c)**. The oval inset in (d) is a transmission electron microscopic view of the organisms. Note the electron-lucent cell wall (CW), nuclei (N), and budding forms (BU). Magnifications: (a) 35x, (b) 130x, (c) 330x, (d) 60x, and oval inset, 3400x. (Adapted from Nair *et al.*, 1990b.)

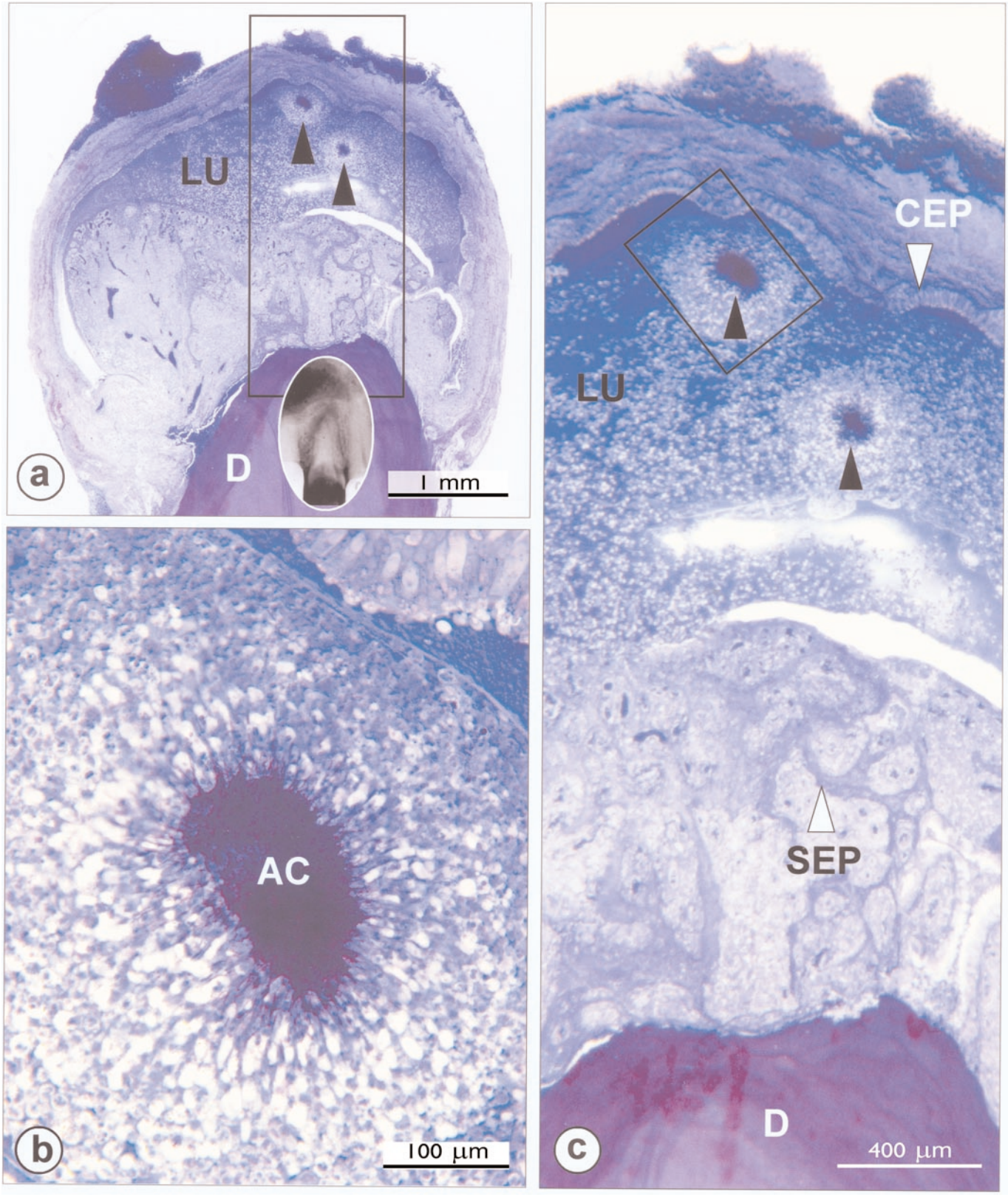

Figure 11. An *Actinomyces*-infected periapical pocket cyst affecting a human maxillary first premolar (radiographic inset). The cyst is lined with ciliated columnar (CEP) and stratified squamous (SEP) epithelia. The rectangular block in **(a)** is magnified in **(c)**. The typical 'ray-fungus' type of actinomycotic colony (AC in **b**) is a magnification of the one demarcated in (c). Note the two black-arrowheaded, distinct actinomycotic colonies (AC in c) within the lumen (LU). Magnifications: (a) 20x, (b) 60x, and (c) 210x. (From Nair *et al.*, 2002.)

(D) Other extraradicular infections

Based on classic histology (Harndt, 1926), there has been a consensus that 'solid granuloma' may not harbor infectious agents within the inflamed periapical tissue, but that micro-organisms

are consistently present in the periapical tissue of cases with clinical signs of exacerbation, abscesses, and draining sinuses.

In the late 1980s, there was a resurgence of the idea of extraradicular microbes in apical periodontitis (Tronstad *et al.*, 1987, 1990; Iwu *et al.*, 1990; Wayman *et al.*, 1992), with the controversial suggestion that extraradicular infections are the cause of many failed endodontic treatments. Several species of bacteria have been reported to be present at extraradicular locations of lesions described as "asymptomatic periapical inflammatory lesions...refractory to endodontic treatment" (Tronstad *et al.*, 1987). However, five of the eight patients had "long-standing fistulae to the vestibule..." (Tronstad *et al.*, 1987), a clear sign of abscessed apical periodontitis draining by fistulation. Obviously, the microbial samples were obtained from periapical abscesses, which always contain microbes, and not from asymptomatic periapical lesions persisting after proper endodontic treatment. Other publications also highlighted some serious problems (Iwu *et al.*, 1990; Wayman *et al.*, 1992). In one, the 16 periapical specimens studied were collected "during normal periapical curettage, apiectomy or retrograde filling" (Iwu *et al.*, 1990). Of the 58 specimens that were evaluated in another study, "29 communicated with the oral cavity through vertical root fractures or fistulas" (Wayman *et al.*, 1992). Further, the specimens were obtained during routine surgery and were "submitted by seven practitioners". An appropriate methodology is essential, and in these studies (Tronstad *et al.*, 1987; Iwu *et al.*, 1990; Wayman *et al.*, 1992), either unsuitable cases were selected for investigation or the sampling was not performed with the utmost stringency necessary for bacterial contamination to be avoided (Möller, 1966).

Microbial contamination of periapical samples is sometimes viewed as happening from the oral cavity and other extraneous sources. Even if such 'extraneous contaminations' are avoided, contamination of periapical tissue samples with microbes from the infected root canal remains a problem. This is because micro-organisms generally live at the apical foramen (Figs. 2, 8) of teeth affected in both primary (Nair, 1987) and post-treatment apical periodontitis (Nair *et al.*, 1990a, 1999). Here, microbes can be easily dislodged during surgery and the sampling procedures. Tissue samples contaminated with intraradicular microbes may be reported as positive for the presence of an extraradicular infection. This may explain the repeated reporting—by microbial culture (Abou-Rass and Bogen, 1997; Sunde *et al.*, 2002) and molecular techniques (Gatti *et al.*, 2000; Sunde *et al.*, 2000)—of bacteria in the periapical tissue of asymptomatic post-treatment lesions, in spite of the use of strict aseptic sampling procedures.

Although there is an understandable infatuation with molecular biological techniques, they seem less suitable for solving the problem of extraradicular infection. Apart from the unavoidable contamination of the samples with intraradicular microbes, molecular genetic analysis: (1) does not differentiate between viable and non-viable organisms, (2) does not distinguish between microbes and their structural elements in phagocytes from extracellular micro-organisms in periapical tissues, and (3) exaggerates the findings by PCR amplification.

Most recently, a series of publications appeared in various journals from one research group (Sabeti *et al.*, 2003a,b,c; Sabeti and Slots, 2004) that reported the presence of certain viruses in inflamed periapical tissues and suggested an 'etiopathogenic relationship' to apical periodontitis. The data were reviewed in another short paper even before some of the original publications appeared in print (Slots *et al.*, 2003). The reported viruses are present in almost all humans in latent form from previous primary infections. It must be emphasized that the mere presence of a suspected causative agent does not imply an etiological relationship of the agent to the development and/or maintenance of the disease.

In summary, extraradicular infections do occur in: (i) acute apical periodontitis lesions (Nair, 1987); (ii) periapical actinomycosis (Sundqvist and Reuterving, 1980; Nair and Schroeder, 1984; Happonen *et al.*, 1985; Happonen, 1986; Sjögren *et al.*, 1988); (iii) association with pieces of infected root dentin that may be displaced into the periapex during root canal instrumentation (Holland *et al.*, 1980; Yusuf, 1982) or cut from the rest of the root by massive apical resorption (Valderhaug, 1974; Laux *et al.*, 2000); and (iv) infected periapical cysts (Fig. 11), particularly in periapical pocket cysts with cavities open to the root canal (Nair, 1987; Nair *et al.*, 1996,1999). Except for these special situations, the long-standing idea that solid granuloma generally do not harbor micro-organisms is still valid.

(E) Cystic apical periodontitis

There has been a long-standing difference of opinion among dental professionals as to whether periapical cysts heal after conventional root canal treatment (Nair, 1998a, 2003a). Oral surgeons hold the view that cysts do not heal and have to be removed by surgery. Many endodontists, on the other hand, are of the opinion that the majority of cysts heal after endodontic treatment. This conflict of opinion is probably an outcome of the reported high incidence of cysts among apical periodontitis and the reported high 'success rate' of root canal treatments. The recorded incidence of cysts among apical periodontitis lesions varies from 6 to 55%. Apical periodontitis cannot be differentially diagnosed into cystic and non-cystic lesions based on radiographs alone (Priebe *et al.*, 1954; Baumann and Rossman, 1956; Wais, 1958; Linenberg *et al.*, 1964; Bhaskar, 1966; Lalonde, 1970; Mortensen *et al.*, 1970). A correct histopathological diagnosis of periapical cysts is possible only through serial sectioning or step-serial sectioning of the lesions removed *in toto*. The vast discrepancy in the reported incidence of periapical cysts is probably due to differences in the interpretation of the sections. Histopathological diagnosis based on a random or limited number of serial sections usually leads to the incorrect categorization of epithelialized lesions as radicular cysts. This was clearly shown in a study, where meticulous serial sectioning was used (Nair *et al.*, 1996), in which an overall 52% of the lesions (n = 256) were found to be epithelialized, but only 15% were actually periapical cysts. In routine histopathological diagnosis, the structure of a radicular cyst in relation to the root canal of the affected tooth has not been taken into account. Since apical biopsies obtained by curettage do not include root tips of the diseased teeth, structural reference to the root canals of the affected teeth is not possible. Histopathological diagnostic laboratories and publications based on retrospective review of such histopathological reports sustain the notion that nearly half of all apical periodontitis are cysts.

An endodontic 'success rate' of 85 to 90% has been recorded for teeth with resorbing apical periodontitis (Staub, 1963; Kerekes and Tronstad, 1979; Sjögren *et al.*, 1990). However, the histological status of an apical radiolucent lesion at the time of treatment is unknown to the clinician, who is also unaware of

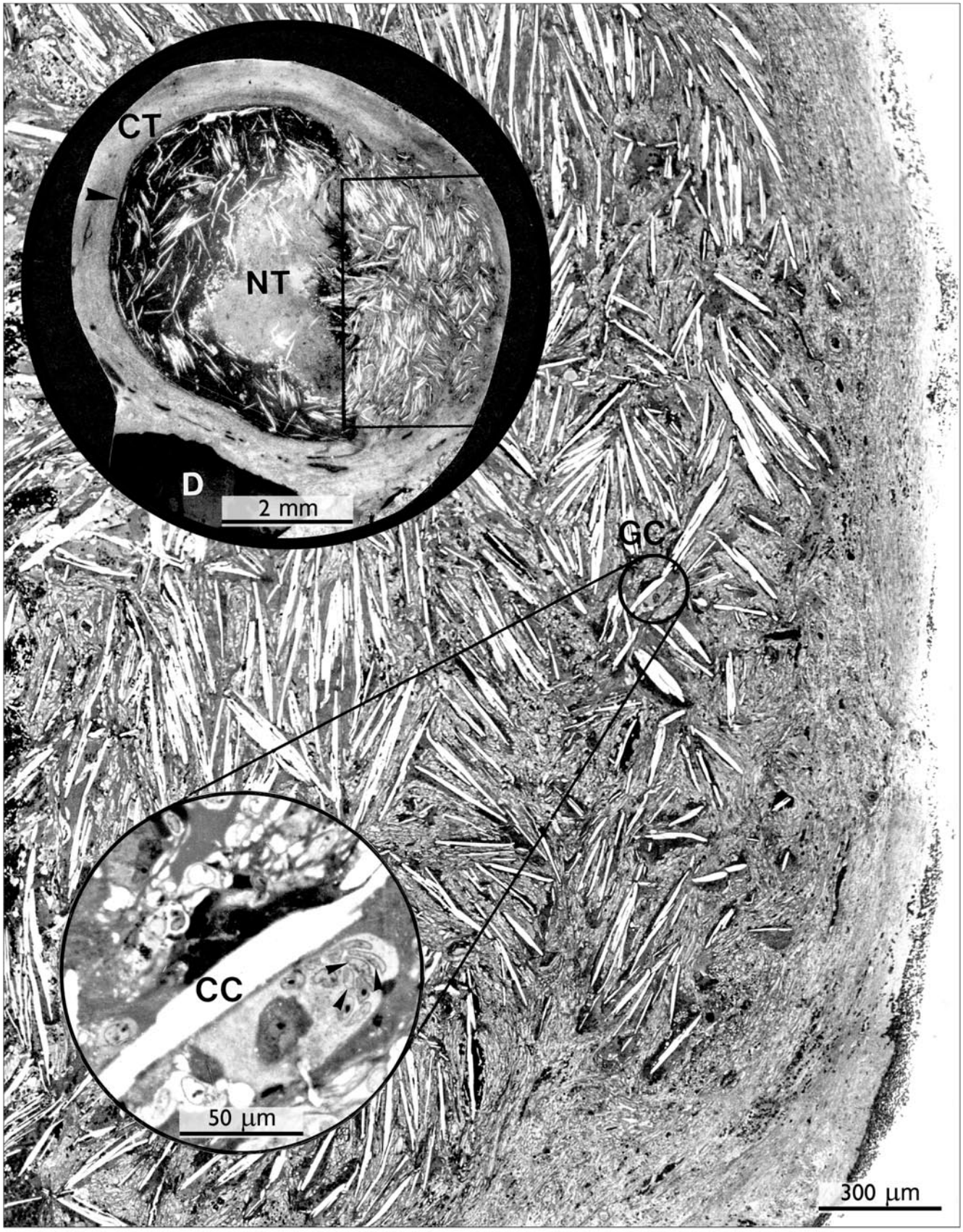

Figure 12. Cholesterol crystals and cystic condition of apical periodontitis as potential causes for endodontic failures. Overview of a histological section (upper inset) of an asymptomatic apical periodontitis that persisted after conventional root canal treatment. Note the vast number of cholesterol clefts (CC) surrounded by giant cells (GC), of which a selected one with several nuclei (arrowheads) is magnified in the lower inset. D = dentin, CT = connective tissue, NT = necrotic tissue. Magnifications: x68. Upper inset, 11s; lower inset, 412s. (From Nair, 1999.)

the differential diagnosis of the 'successful' and 'failed' cases. Nevertheless, based purely on deductive logic, a great majority of the cystic lesions should heal to account for the 'high success rate' after endodontic treatment and the reported 'high histopathological incidence' of radicular cysts. Since orthograde endodontic treatment removes much of the infectious material from the root canal and prevents re-infection by obturation, a periapical pocket cyst may heal after conventional endodontic therapy (Simon, 1980; Nair *et al.*, 1993, 1996). However, a true cyst is self-sustaining (Nair *et al.*, 1993) by virtue of its tissue dynamics and independent of the presence or absence of irritants in the root canal (Simon, 1980). Therefore, true periapical cysts, particularly those containing cholesterol crystals, are less likely to be resolved by conventional endodontic therapy (Nair, 1998a, 2003a).

(F) Foreign-body reactions

Endogenous cholesterol crystals deposited in periapical tissues (Nair *et al.*, 1993) and exogenous materials trapped in the periapical area (Nair *et al.*, 1990b; Koppang *et al.*, 1992) can perpetuate apical periodontitis after root canal treatment by initiating a foreign-body reaction at the periapex (Nair, 2003b).

(1) Cholesterol crystals

Although the presence of cholesterol crystals in apical periodontitis lesions has long been observed to be a common histopathological feature, its etiological significance in failed root canal treatments has not yet been fully appreciated (Nair, 1999). Cholesterol is a steroid lipid that is present in abundance in all "membrane-rich" animal cells (Taylor, 1988). Excess blood levels of cholesterol are suspected to play a role in atherosclerosis as a result of its deposition in the vascular walls (Yeagle, 1988, 1991). Deposition of cholesterol crystals in tissues and organs can cause ailments such as otitis media and the "pearly tumor" of the cranium (Anderson, 1996). Accumulation of cholesterol crystals occurs in apical periodontitis lesions (Shear, 1963; Bhaskar, 1966; Browne, 1971; Trott *et al.*, 1973; Nair *et al.*, 1993), with clinical significance in endodontics (Nair *et al.*, 1993; Nair, 1998a). In histopathological sections, such deposits of cholesterol appear as narrow elongated clefts, because the crystals dissolve in the fat solvents used for tissue processing and leave behind the spaces they occupied as clefts (Fig. 12). The incidence of cholesterol clefts in apical periodontitis varies from 18% to 44% of such lesions (Shear, 1963; Browne, 1971; Trott *et al.*, 1973). The crystals are believed to be formed from cholesterol released by: (i) disintegrating erythrocytes of stagnant blood vessels within the lesion (Browne, 1971); (ii) lymphocytes, plasma cells, and macrophages which die in great numbers and disintegrate in chronic periapical lesions; and (iii) the circulating plasma lipids (Shear, 1963). All these sources may contribute to the concentration and crystallization of cholesterol in the periapical area. Nevertheless, locally dying inflammatory cells may be the major source of cholesterol as a result of its release from disintegrating membranes of such cells in long-standing lesions (Seltzer, 1988; Nair *et al.*, 1993).

Cholesterol crystals are intensely sclerogenic (Abdulla *et al.*, 1967; Bayliss, 1976). They induce granulomatous lesions in dogs (Christianson, 1939), mice (Spain *et al.*, 1959; Adams *et al.*, 1963; Abdulla *et al.*, 1967; Adams and Morgan, 1967; Bayliss, 1976), and rabbits (Hirsch, 1938; Spain *et al.*, 1959; Spain and Aristizabal, 1962). In an experimental study that specifically investigated the potential association of cholesterol crystals and non-resolving apical periodontitis lesions (Nair *et al.*, 1998), pure cholesterol crystals were placed in Teflon chambers that were implanted subcutaneously into guinea pigs. The chamber contents were retrieved after 2, 4, and 32 weeks of implantation and processed for light and electron microscopy. The chambers exhibited (Fig. 13) delicate soft connective tissue that grew through perforations in the chamber wall. The crystals were densely surrounded by numerous macrophages and multinucleate giant cells that formed a well-circumscribed area of tissue reaction. The cells, however, were unable to eliminate the crystals during an observation period of eight months. The accumulation of macrophages and giant cells around cholesterol crystals suggests that the crystals induced a typical foreign-body reaction (Coleman *et al.*, 1974; Nair *et al.*, 1990b; Sjögren *et al.*, 1995).

The macrophages and giant cells that surround cholesterol crystals are not only unable to degrade the crystalline cholesterol but also are major sources of apical inflammatory and bone-resorptive mediators. Bone-resorbing activity of cholesterol-exposed macrophages due to enhanced expression of IL-1α has been shown experimentally (Sjögren *et al.*, 2002). Accumulation of cholesterol crystals in apical periodontitis lesions (Fig. 12) can adversely affect post-treatment healing of the periapical tissues, as has been shown in a long-term longitudinal follow-up of a case in which it was concluded that "the presence of vast numbers of cholesterol crystals...would be sufficient to sustain the lesion indefinitely" (Nair *et al.*, 1993). The evidence from a review of the literature clearly supports that assumption (Nair, 1999). Therefore, accumulation of cholesterol crystals in apical periodontitis lesions can prevent healing of periapical tissues after conventional root canal treatment, since orthograde root-filling re-treatment cannot remove the tissue-irritating cholesterol crystals that exist outside the root canal system.

(2) Foreign bodies

Foreign materials trapped in periapical tissue during and after endodontic treatment can perpetuate apical periodontitis persisting after root canal treatment (Nair *et al.*, 1990b; Koppang *et al.*, 1992). Endodontic clinical materials (Nair *et al.*, 1990b; Koppang *et al.*, 1992) and certain food particles (Simon *et al.*, 1982) can reach the periapex, induce a foreign-body reaction that appears radiolucent, and remain asymptomatic for several years (Nair *et al.*, 1990b).

(a) Gutta percha

The most frequently used core material for the orthograde obturation of root canals is gutta percha. The widely held view that it is biocompatible with and well-tolerated by human tissues is inconsistent with the clinical observation that extruded gutta percha is associated with delayed healing of the periapex (Strindberg, 1956; Seltzer *et al.*, 1963; Kerekes and Tronstad, 1979; Nair *et al.*, 1990b; Sjögren *et al.*, 1990). It has been experimentally shown, in guinea pigs, that large pieces of gutta percha are well-encapsulated in collagenous capsules, but that fine particles of gutta percha induce an intense, localized tissue response (Fig. 14), characterized by the presence of macrophages and giant cells (Sjögren *et al.*, 1995). The congregation of macrophages around the fine particles of gutta percha is relevant for the clinically observed impairment in the healing of apical periodontitis, when teeth are root-filled with excess.

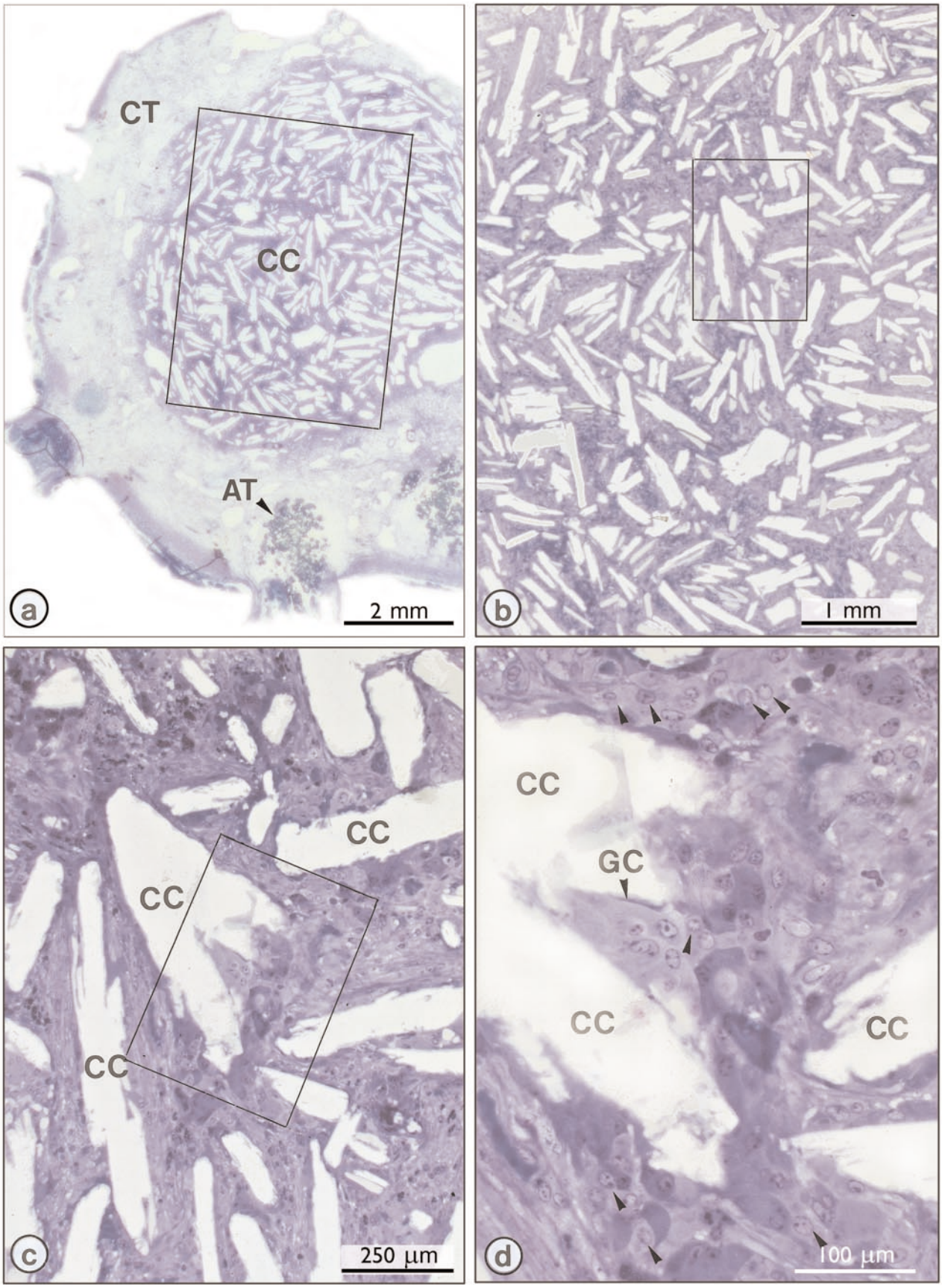

Figure 13. Photomicrograph **(a)** of guinea pig tissue reaction to aggregates of cholesterol crystals after an observation period of 32 weeks. The rectangular demarcated areas in (a), (b), and (c) are magnified in **(b)**, **(c)**, and **(d)**, respectively. Note the rhomboid clefts left by cholesterol crystals (CC) surrounded by giant cells (GC) and numerous mononuclear cells (arrowheads in d). AT = adipose tissue, CT = connective tissue. Magnifications: (a) 10x, (b) 21x, (c) 82x, and (d) 220x. (From Nair, 1999.)

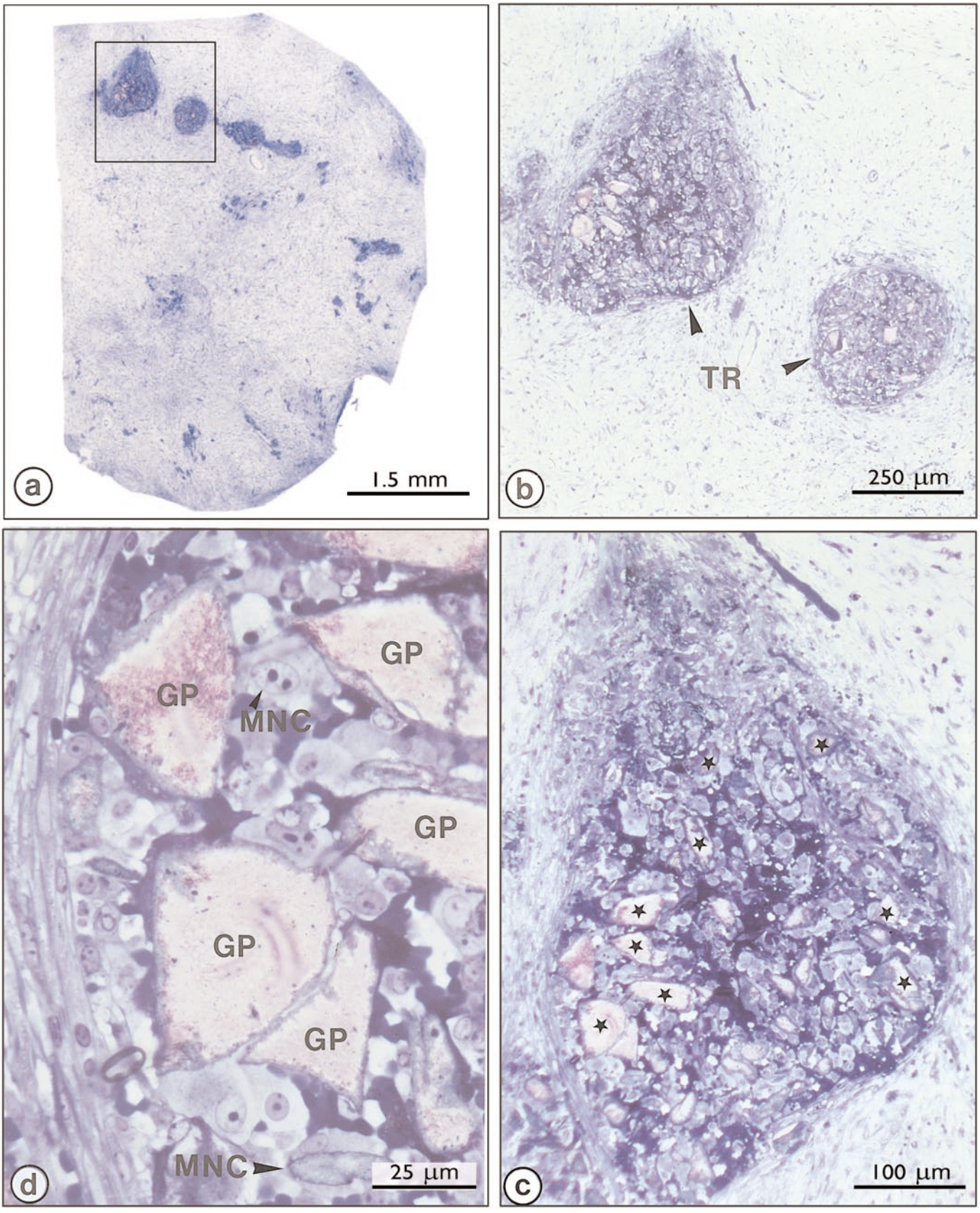

Figure 14. Disintegrated gutta-percha as potential cause of endodontic failures. As clusters of fine particles **(a)**, they induce intense circumscribed tissue reaction (TR) around the particles. The regular demarcated area in (a) is magnified in **(b)**. Note that the fine particles of gutta-percha (* in **c**, GP in **d**) are surrounded by numerous mononuclear cells (MNC). Magnifications: (a) 30x, (b) 80x, (c) 200x, and (d) 750x. (From Nair, 2002.)

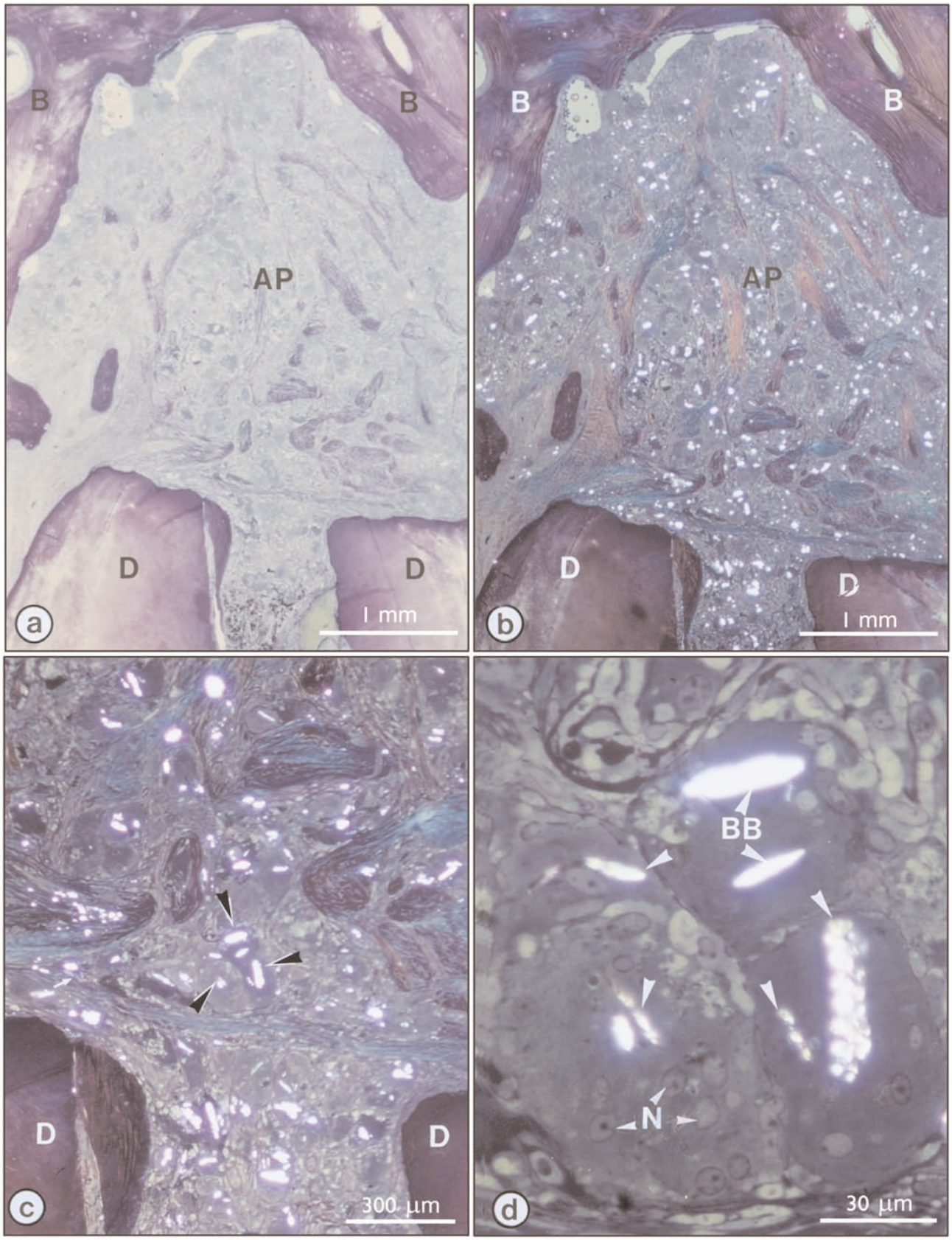

Figure 15. Talc-contaminated gutta percha as a potential cause of endodontic failure. Note the apical periodontitis (AP) characterized by foreign-body giant cell reaction to gutta-percha cones contaminated with talc **(a)**. The same field when viewed in polarized light **(b)**. Note the birefringent bodies distributed throughout the lesion (b). The apical foramen is magnified in **(c)**, and the dark-arrowheaded cells in (c) are further enlarged in **(d)**. Note the birefringence (BB) emerging from slit-like inclusion bodies in multinucleated (N) giant cells. B, bone; D, dentin. Magnifications: (a,b) 25x; (c) 66x; and (d) 300x. (From Nair, 1998b.)

Gutta percha cones contaminated with tissue-irritating materials can induce a foreign-body reaction at the periapex. In an investigation on nine asymptomatic apical periodontitis lesions that were removed as surgical block biopsies and analyzed by correlative light and electron microscopy, one biopsy (Fig. 15a) revealed the involvement of contaminated gutta percha (Nair *et al.*, 1990b). The radiolucency grew in size but remained asymtomatic for a decade of post-treatment follow-up. The lesion was characterized by the presence of vast numbers of multinucleated giant cells with birefringent inclusion bodies (Figs. 15b, 15c, 15d). In transmission electron microscopy, the birefringent bodies were highly electron-dense. An x-ray microanalysis of the inclusion bodies, by scanning transmission electron microscopy (STEM), revealed the presence of magnesium and silicon. These elements are presumably the remnants of a talc-contaminated gutta percha that protruded into the periapex and had been resorbed during the follow-up period.

(b) Plant materials

Vegetable food particles, particularly leguminous seeds (pulses), and endodontic clinical materials of plant origin can get lodged in the periapical tissue before and/or during endodontic treatment and cause treatment failures. Oral pulse granuloma is a distinct histopathological entity. The lesions are also referred to as giant cell hyalin angiopathy (Dunlap and Barker, 1977), vegetable granuloma (Harrison and Martin, 1986), and food-induced granuloma (Brown and Theaker, 1987). Pulse granuloma has been reported in lungs (Head, 1956), stomach walls, and peritoneal cavities (Sherman and Moran, 1954). Experimental lesions have been induced in animals by intratracheal, intraperitoneal, and submucous introduction of leguminous seeds (Knoblich, 1969; Talacko and Radden, 1988a). Periapical pulse granulomas are associated with tooth damaged by caries and with the antecedence of endodontic treatment (Simon *et al.*, 1982; Talacko and Radden, 1988b). Pulse granulomas are characterized by the presence of intensely iodine- and PAS-positive hyaline rings or bodies surrounded by giant cells and inflammatory cells (Mincer *et al.*, 1979; Simon *et al.*, 1982; Talacko and Radden, 1988a,b). Leguminous seeds are the most frequently involved vegetable food material in such granulomatous lesions. This indicates that certain components in pulses such as antigenic proteins and mitogenic phytohemagglutinins may be involved in the pathological tissue response (Knoblich, 1969). The pulse granulomas are clinically significant because particles of vegetable food materials can reach the periapical tissue *via* root canals of teeth exposed to the oral cavity by trauma, caries damage, or endodontic procedures (Simon *et al.*, 1982).

Apical periodontitis developing against particles of predominantly cellulose-containing materials that are used in endodontic practice (White, 1968; Koppang *et al.*, 1987, 1989; Sedgley and Messer, 1993) has been denoted as 'cellulose granuloma'. The cellulose in plant materials is a granuloma-inducing agent (Knoblich, 1969). Endodontic paper points (Fig. 16) are utilized for microbial sampling and drying of root canals. Sterile and medicated cotton wool has been used as an apical seal. Particles of these materials can dislodge or get pushed into the periapical tissue (White, 1968) to induce a foreign-body reaction at the periapex. The resultant clinical situation may be a "prolonged, extremely troublesome and disconcerted course of events" (White, 1968). The presence of cellulose fibers in periapical biopsies with a history of previous endodontic treatment has been reported (Koppang *et al.*, 1987, 1989; Sedgley and Messer, 1993). The endodontic paper points and cotton wool consist of cellulose that cannot be degraded by human body cells. They remain in tissues for long periods of time (Sedgley and Messer, 1993) and induce a foreign-body reaction around them. In polarized light, the particles are birefringent, due to the regular structural arrangement of the molecules within cellulose (Koppang *et al.*, 1989). Infected paper points can protrude through the apical foramen (Fig. 16) and allow a biofilm to grow around it. This will sustain and even intensify the apical periodontitis after root canal treatment, which eventually can result in treatment failure.

(c) Other foreign materials

These include amalgam, endodontic sealants, and calcium salts derived from periapically extruded $Ca(OH)_2$. In a histological and x-ray microanalytical investigation of 29 apical biopsies, 31% of the specimens were found to contain materials compatible with amalgam and endodontic sealer components (Koppang *et al.*, 1992).

(G) Scar-tissue healing

There is evidence that unresolved periapical radiolucencies may occasionally be due to healing of the lesion by scar tissue that may be mistaken as a radiographic sign of failed endodontic treatment (Penick, 1961; Bhaskar, 1966; Seltzer *et al.*, 1967; Nair *et al.*, 1999).

In summary, there are five biological factors that contribute to persistent periapical radiolucency after root canal treatment: (1) intraradicular infection in the apical root canal system; (2) extraradicular infection, mostly in the form of periapical actinomycosis; (3) cystic lesions; (4) foreign-body reaction to crystalline substances of endogenous origin (cholesterol crystals), extruded root canal filling or other foreign materials; and (5) scar-tissue healing of the lesion. It must be emphasized that of all these factors, microbial infection persisting in the apical portion of the root canal system is the major cause of endodontic failures in properly treated cases (Nair *et al.*, 1990a; Sjögren, 1996; Figdor, 2002). Failures due to extraradicular actinomycosis, cystic lesions, foreign-body reaction, and scar-tissue healing are rare.

(VI) Concluding Remarks

Apical periodontitis is essentially a disease of root canal infection. Contemporary knowledge on the taxonomy of the root canal flora is based on microbial culture techniques. Careful application of molecular genetic methods in endodontic microbiology not only confirms those findings but also widens the taxonomic spectra of the endodontic flora. However, currently there is no evidence that the 'as-yet-culture-difficult' (Munson *et al.*, 2002) organisms are viable root canal pathogens (Sundqvist and Figdor, 2003). The enhanced precautions needed for useful application of molecular techniques in endodontic microbiology (Ng *et al.*, 2003) have not yet been fully appreciated by many researchers, who seem to be infatuated with the sophistication of the techniques. However, application of molecular techniques is about to herald an era of great understanding of the complex interaction between the microbial and host factors, to provide a clearer picture of the pathogenesis of the disease at the subcellular level. Because apical periodontitis

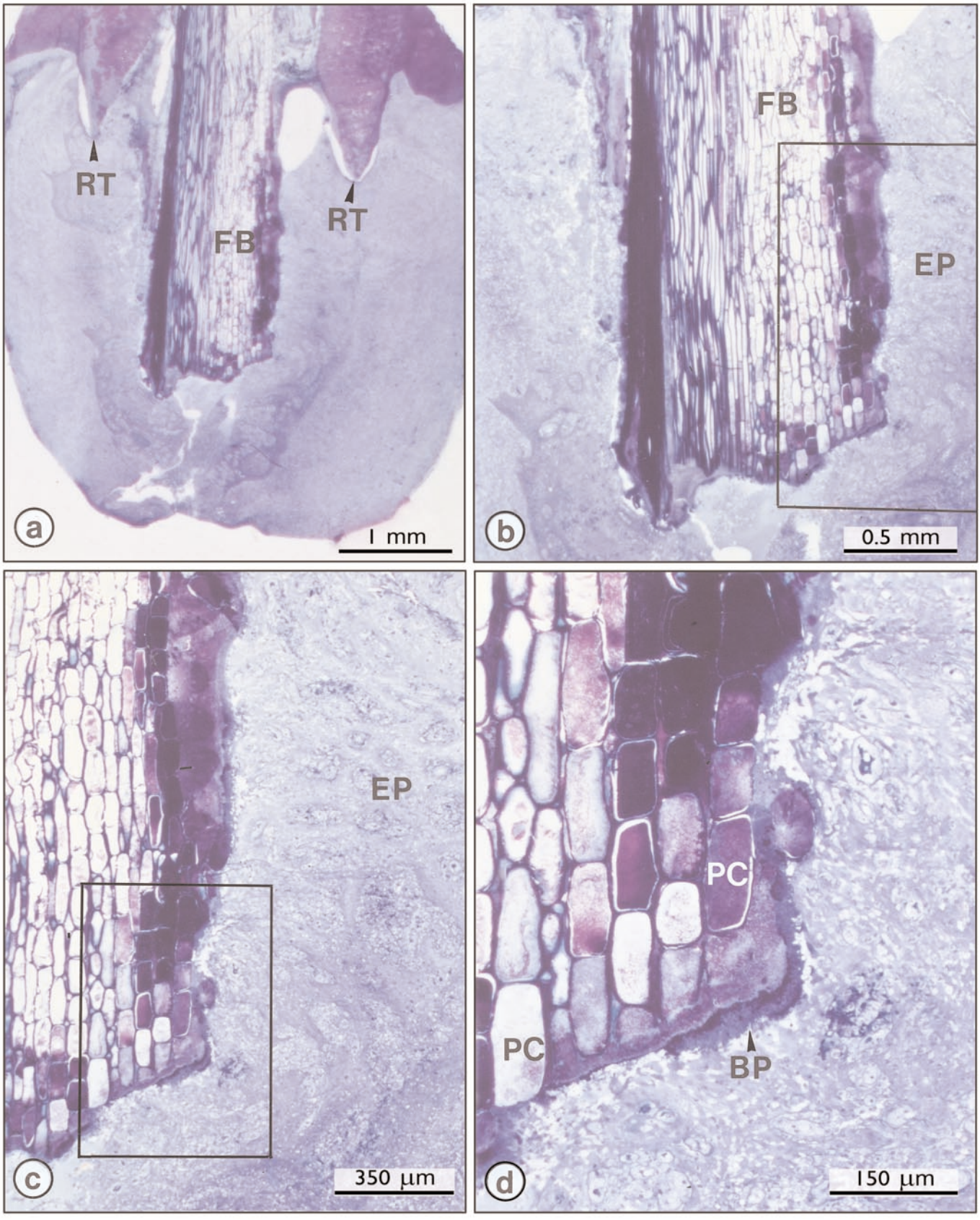

Figure 16. A massive paper-point granuloma affecting a root-canal-treated human tooth **(a)**. The demarcated area in **(b)** is magnified in **(c)** and further magnified in **(d)**. Note the tip of the paper point (FB) projecting into the apical periodontitis lesion and the bacterial plaque (BP) adhering to the surface of the paper point. RT, root tip; EP, epithelium; PC, plant cell. Magnifications: (a) 20x, (b) 40x, (c) 60x, and (d) 150x. (From Nair, 2002.)

is essentially a disease of root canal infection, the logical treatment has been to eliminate infection from the root canal and exclude further infection of the canal. Since the essential role of root canal microbes in both primary and post-treatment apical periodontitis has been well-recognized, the major thrust of treatment procedures should be with the clinical management of problems associated with the control and elimination of infection. In recent years, there has been a trend to focus on the purely mechanical aspects of treating the disease. While those are important, a clear understanding of the etio-pathogenic factors involved is necessary for the therapeutic application of intelligent solutions to solve the problem.

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