Detection of immune complexes in human dental periapical lesions by anticomplement immunofluorescence technique

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Twenty-five human dental periapical lesions were obtained. The lesions were frozen, sectioned, and examined for the presence of immune complexes by employing an anticomplement immunofluorescence (ACIF) technique. Twenty-three of the periapical specimens were positive for antigen-antibody complexes. No staining was noted in the two lesions that were histologically diagnosed as periapical scar tissue.

As a consequence of degeneration of the dental pulp tissue, the root canal system acquires the capacity to accommodate a number of antigens. These antigens can be viable and dead bacteria, bacterial products, denatured host tissues subsequent to pulpal deterioration, and possibly materials used to medicate or fill the root canal system.[1] A continuous egress of these antigens from the root canal system into the periapical tissues can cause antibody formation. If immunoglobulins are specifically secreted against antigens present in the root canal system, interaction of antigens with antibodies can result in immunologic reactions in periapical tissues. Compatible with this hypothesis is the presence of immunocompetent cells and immunoglobulins in dental periapical lesions.[2-6] Among other immunologic reactions, it has been suggested that antigen-antibody or immune complex reactions play a role in the pathogenesis of human dental periapical lesions.[1] Separate experiments in cats have provided indirect evidence that antigen-antibody complex reactions can occur in the periapical tissues.[7-8] Malmstrom,[4] using a direct immunofluorescence technique, examined the presence of complement C3 in dental periapical lesions of patients with and without "rheumatoid disease." The complement C3 was detected in four of the eleven biopsy samples from rheumatoid patients, and this substance was seen in two of the twelve biopsy samples from patients without rheumatoid disease. Kuntz and associates[5] studied the presence of immune complexes in ten human dental periapical lesions by the direct immunofluorescence technique and reported that five lesions showed bright C3 staining of blood-vessel-like structures. However, Morton and associates,[6] using the same technique in twenty-six patients, observed immune complexes in the periapical tissue of one patient with history of lupus erythematosus and suggested that insoluble immune complexes do not play an important role in the pathogenesis of dental periapical lesions. Since the direct immunofluorescence technique is less sensitive than an anticomplement immunofluorescence technique (ACIF),[9-12] which is now used for detection of antigen-antibody complexes in virus-infected cells,[12-14] this technique was employed in the present study to determine whether immune complexes were present in human dental periapical lesions.

MATERIALS AND METHODS

Twenty-five dental periapical lesions were obtained from patients undergoing periapical surgery in the Department of Endodontics at Loma Linda University School of Dentistry. The indications for surgery were in general those recommended by Ingle and Beveridge.[15] Medical histories of all patients in this study showed that they had no contributory systemic diseases. After removal of the dental periapical lesion,

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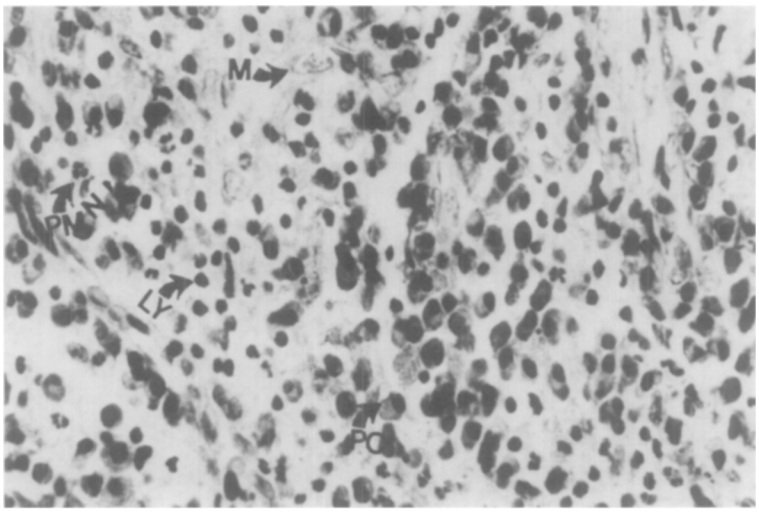

Fig. 1. Photomicrograph of an inflamed periapical specimen showing numerous inflammatory cells, such as plasma cells *(PC)*, lymphocytes *(LY)*, polymorphonuclear leukocytes *(PMN)*, and macrophages *(M)*. (Hematoxylin and eosin stain. Original magnification, ×250.)

half of the specimen was placed on a small wood block in a mounting medium,* snap-frozen in liquid in nitrogen, stored at −70° C., and sectioned later. The other half was placed in 10 percent buffered formalin for permanent fixation and subsequently sectioned at 6 microns and stained with hematoxylin and eosin for histopathologic examination.

Anticomplement immunofluorescence (ACIF) procedure

Guinea pig complement† and the IgG fraction of goat antiserum to guinea pig complement‡ (C3 fraction) were obtained from commercial sources. Following the technique described by Kettering and coworkers,[14] anti-guinea pig complement (C3) serum was conjugated with fluorescein isothiocynate and stored at 4° C. In a preliminary test, optimal dilutions of the complement and conjugated anticomplement serum were determined by titration against cytomegalovirus (CMV)-infected cells, noninfected cells, and sections of human dental periapical lesions. In the CMV-infected cells, complement at a dilution of 1:40 and anticomplement at a dilution of 1:50 or 1:60 generally gave optimal results. Sections of human dental periapical lesions gave optimal fluorescence when both complement and anticomplement were used at a dilution of 1:20. The ACIF procedure used to demonstrate the presence of immune complexes in dental periapical lesions consisted of the following steps. The dental periapical lesions stored at −70° C. were sectioned in the cryostat at 4 microns and mounted on glass slides. The sections were air dried for 30 minutes and washed in phosphate-buffered saline (PBS) for another 30 minutes to remove unbound proteins from the tissue. An optimal dilution of guinea pig complement was added to periapical sections on glass slides, and they were incubated for 20 minutes at 37° C. in a moist chamber. After a 10-minute rinse in PBS, an optimal dilution of fluorescein-conjugated anti-guinea pig complement was added, and the incubation was conducted for 20 minutes at 37° C. in a moist chamber. After a 10-minute rinse, the slides were allowed to air dry; then they were mounted in glycerine buffered at pH 9.2 and examined with a fluorescence microscope.

As positive controls, CMV-infected cells and frozen sections of diseased kidney known to contain human complement C3 were stained by the ACIF technique. As negative controls, non-CMV-infected cells and normal human skin were also stained by this technique. In addition, further sections of human dental periapical lesions which were positive when stained by the ACIF technique were also stained with different concentrations of fluorescein-conjugated goat anti-human complement C3.* The stained tissues were examined for the presence and location (intracellular, perivascular, etc.) of antigen-antibody complexes.

RESULTS

Histologic examination of tissue samples showed that, of the twenty-five dental periapical specimens, twenty-three contained inflammatory cells, such as lymphocytes, plasma cells, polymorphonuclear (PMN)

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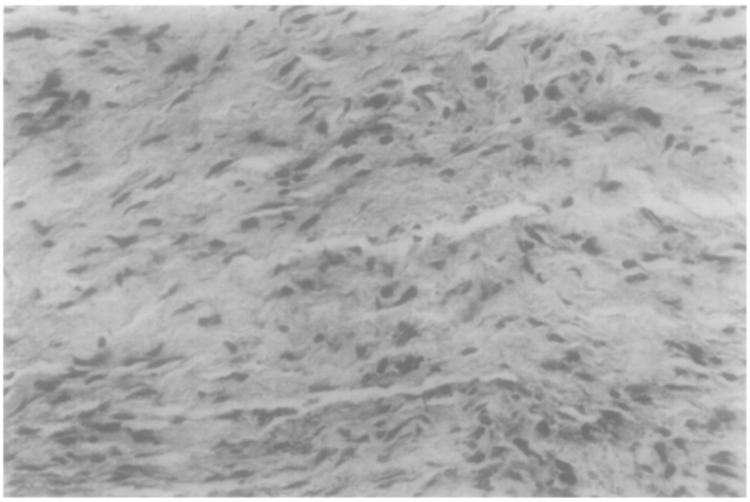

Fig. 2. Photomicrograph of periapical scar tissue showing fibrous connective tissue, fibrocytes, and a few inflammatory cells. (Hematoxylin and eosin stain. Original magnification, ×250.)

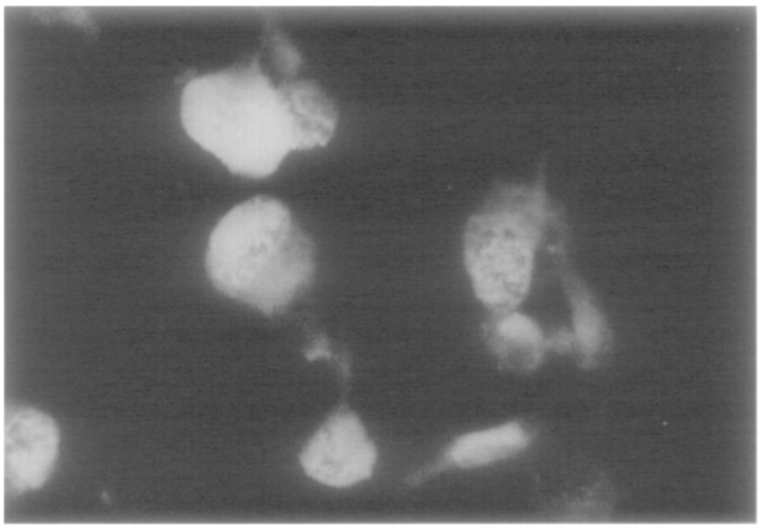

Fig. 3. ACIF staining of CMV antigen in nuclei of infected cells. (Original magnification, ×400.)

leukocytes, and macrophages (Fig. 1). Two biopsy specimens were fibrotic, with very few inflammatory cells, and were diagnosed as periapical scar tissue (Fig. 2).

CMV-infected cells and tissue sections from diseased kidney showed positive fluorescence staining by the ACIF technique (Fig. 3). Twenty-three of the dental periapical specimens were positive when stained for guinea pig complement C3. However, no staining was noted in the two lesions which were histologically diagnosed as periapical scar tissue. When the third component of guinea pig complement (C3) was present, the fluorescence was presented by distinct globules or packets in the cytoplasm of cells, presumably macrophages or PMN leukocytes, and adjacent to vascular channels (Figs. 4 and 5). In contrast, noninfected cells and normal human skin did not show similar deposits of complement C3. Furthermore, periapical sections stained with fluorescein conjugated with goat anti-human complement C3 showed no specific staining for complement C3.

DISCUSSION

Several methods are available for the detection of immune complexes. The presence of these substances in serum, tissue fluids, cells, or tissues can be revealed by (1) ultracentrifugation; (2) appearance of breakdown products of C3 fragments; (3) precipitation with Clq component of complement[16]; and (4) direct, indirect, and anticomplement immunofluorescence tech-

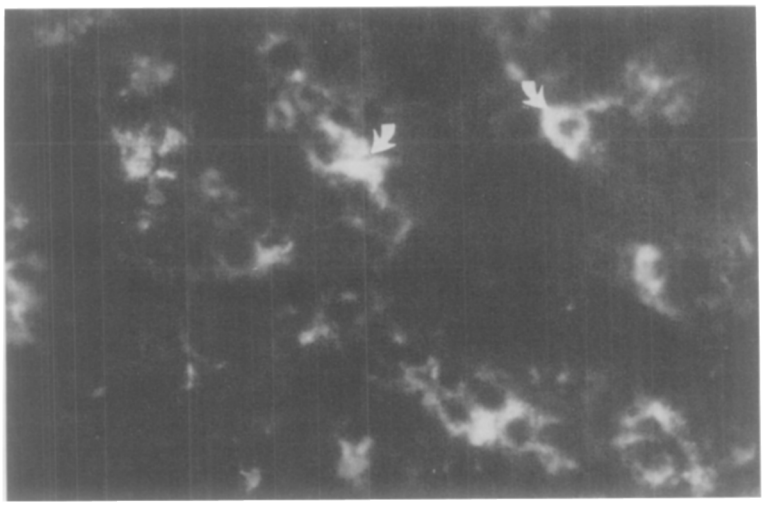

Fig. 4. ACIF staining of dental periapical specimens showing cytoplasmic location of complement (C3)-bound complexes. (Original magnification, ×400.)

Fig. 5. ACIF staining of dental periapical specimens indicating positive vessel-like structure. (Original magnification, ×400.)

niques.[9–14] Direct immunofluorescence and ACIF techniques are among the most widely used procedures for detection of immune complexes in tissues. The basis for the development of these techniques is that antigen-antibody complexes have the capacity to bind complement fragments.[17–19] If cells or tissues which contain bound complement are treated with fluorescein-conjugated anti-C3, the most abundant complement component, the presence of fluorescence indicates the existence of immune complexes in those sites.

Since, in this study, positive reactions with negative controls (noninfected cells and normal skin) were not observed and, in addition, positive reactions with positive controls (infected cells and diseased kidney) were obtained, ACIF appeared to be a sensitive technique for the detection of immune complexes in tissues. Our findings strongly indicate that immune complexes are present in dental periapical lesions. Because other investigators have shown that periapical scar tissue has no immunologic components, such as immunoglobulin and/or complement fragments,[2, 3, 6] the absence of C3-containing cells in the two lesions diagnosed as periapical scar tissues is an additional indication of the role of immune complexes in the pathogenesis of dental periapical lesions.

The relatively low concentration of immune complexes observed by Malmstrom,[4] Kuntz and coauthors,[5] and Morton and associates[6] in dental periapical lesions may be due to one of the following: (1) complement is extremely labile[20] and it may disin-

tegrate during periapical tissue processing for the direct immunofluorescent technique, (2) the direct immunofluorescent technique may not be as sensitive as the ACIF technique for the detection of immune complexes, and (3) the concentration of human complement bound to antigen-antibody complexes in dental periapical lesions may be so low that the presence of immune complexes cannot be accurately detected by the direct technique. However, when guinea pig complement in excess is used in the ACIF technique, it binds to antigen-antibody complexes present, and these substances fluoresce when treated with conjugated anti-guinea pig complement.

The presence of immune complexes in human dental periapical lesions can partially explain how these lesions evolve and gradually enlarge. Continuous egress of antigens from the root canal system into the periapical tissues can cause antibody formation. Interaction of antigens with antibodies in antigen excess can form immune complexes in periapical tissues. In the presence of plasma, the reaction of immune complexes, platelets, and neutrophils results in release of histamine and serotonin from platelets and increases the vascular permeability.[21] Immune complexes penetrate into blood vessel walls, fix the complement system, and form factors such as C3A, C5A, and C5B, 6, 7,[16-19] which are chemotactic for PMN leukocytes in periapical tissues. When immune complexes adhere to PMN leukocytes or are phagocytized by these cells, lysosomal enzymes of PMN leukocytes are released. Lysosomes of PMN leukocytes contain a number of enzymes capable of producing tissue injury. Among the components of PMN granules are prostaglandins, cathepsins, a delayed permeability factor, kinin-forming and kinin-degrading enzymes, mast cell-rupturing factors, and proteolytic enzymes capable of hydrolyzing collagen, elastic tissue, and cartilage.[22-26]

Neutrophils are known to contain a chemotactic factor for mononuclear cells such as macrophages.[17] The cell population in antigen-antibody—mediated reactions, as in other acute reactions, changes from PMN leukocytes to monocytes. In vitro studies have shown that macrophages can also release prostaglandins, collagenase, acid hydrolase, and other enzymes by exposure to immune complexes.[17-30] Therefore, in addition to lysosomal enzymes released by PMN leukocytes, substances released from macrophages can cause tissue injury in the periapical area.

Regardless of the mediators involved, periodontal ligament destruction and bone resorption are often the final products. Although immunologic reactions in the periapical tissues start as a protective phenomenon against the antigens within the root canal system, damage to adjacent tissues is inevitable. As the result of continuous egress of antigens from the root canal system, tissue changes become more prominent and periapical lesions increase in size. Antigen-antibody complex reactions and other immunologic reactions can be stopped only if antigens within the root canal system are removed. This emphasizes the concept that complete cleaning and débridement of the root canal system is the most essential part of root canal therapy.[31-33]

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