Localization of immunoglobulins and the third component of complement in dental periapical lesions

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The localization of IgG, IgM, IgA, and several complement components were studied in long-standing human periapical lesions. Sections of 38 Formalin-fixed and ten fast-frozen periapical lesions were stained with fluorescein-conjugated antibodies specific for IgG, IgM, IgA, C3, C4, and C3 proactivator (C3pa). IgG, IgA, and IgM were observed extracellularly as well as in plasma cells. Plasma cells containing IgG were most numerous and those containing IgM least numerous. Numerous non-Ig-containing round cells resembling lymphocytes also were found in most lesions. Five of ten lesions processed by fast freezing showed bright C3 staining of small vessel-like structures. This staining was blocked with unlabeled anti-C3 serums, but was not blocked by prior absorption of the anti-C3 conjugate with AB blood group substances. Deposits of C3 were also identified extracellularly in most of the lesions. C3pa and C4 were not observed in the ten lesions. These experiments suggest that reactions involving complement (C3) play a role in some periapical lesions.

It is becoming increasingly apparent that immunopathologic reactions play a significant role in many chronic bacterial disease processes. Immunologic reactions have been implicated in inflammatory oral diseases such as periodontitis, candidiasis, and recurrent herpetic and aphthous ulcers.[1,2]

Recently, immunologic reactions also have been suggested to occur in periapical lesions. Naidorf[3] extracted immunoglobulins from three periapical lesions and subjected them to electrophoretic and single radial immunodiffusion analysis. One of the lesions was diagnosed as a periapical scar; it was devoid of round cells and it yielded no immunoglobulins. The other two were diagnosed as granulomas and were shown to contain immunoglobulins.

Morse, Lasater, and White[4] stained sections of 33 periapical lesions from endodontically treated teeth with pyroninmethyl green and observed immunoglobulin-producing cells in periapical granulomas and cysts. Toller[5] also reported infiltration by pyroninophilic-staining cells of periapical cyst walls. Nordh[6] studied the serum proteins in patients with radiolucent periradicular lesions. Electrophoresis of the serum of these patients showed a lower mean value of albumin, a lower αl-globulin fraction, and a higher γ-globulin fraction as compared to control subjects. The differences may have represented a response to the chronic periradicular inflammatory lesion.

Rosengren[7] and Okada and coworkers[8] have demonstrated, in cats and rabbits, respectively, that changes in serum protein fractions such as hypoalbuminemia and hypogammaglobulinemia were induced after prolonged sensitization of the animals by antigens introduced into root canals of the teeth. In addition, local and systemic pathologic changes of various organs were induced.

Boucher, Hanrahan, and Kihara[9] studied the occurrence of C-reactive protein (CRP) by means of a semiquantitative, capillary precipitation procedure in patients with various oral diseases. Nine of ten patients with acute abscesses were found to have CRP in their serum. Of the 13 patients

with chronic alveolar abscess, four had a positive result in their CRP test. After penicillin treatment and extraction of the offending teeth in two patients with acute abscesses, the serum became negative for CRP.

Barnes and Langeland[10] have shown that serum antibody can be induced to protein antigens sealed deep in dentinal cavity preparation in monkeys. These results suggest that antigens may penetrate through a deep carious lesion to come into contact with immunocompetent cells, leading to a systemic immune response.

Immunoglobulins have been identified in human gingiva. Brandtzaeg and Kraus[11] found that in clinically healthy gingiva, many of these cells contained IgG, very few contained IgA, and only rarely were IgM-containing cells found. Brandtzaeg[12] has shown that gingival pocket fluid contains IgG, IgA, and IgM in proportions and concentration comparable to that of plasma. Genco and co-workers[13] have found deposits of immunoglobulin and complement resembling immune complexes in the gingiva of patients with periodontal disease. These authors suggest that natural antibodies to plaque organisms may react with plaque antigens that penetrate the gingiva during the course of periodontal disease. These antigen-antibody reactions may lead to activation of the complement system, resulting in increased phagocytosis, chemotaxis of neutrophils and monocytes, and increased vascular permeability. Local antigen-antibody reactions also may modify local cell-mediated reactions involving sensitized lymphocytes.

Complement is an important mediator of inflammation. Important biologic reactions associated with the complement system include immune adherence, virus neutralization, phagocytosis, anaphylatoxin production, histamine release from mast cells, leukocyte and monocyte chemotaxis, stimulation of B-lymphocytes, and cytolysis of erythrocytes and bacteria.[14] Much information has been collected that establishes the role of antigen-antibody reactions in various forms of acute and chronic inflammation. It is now recognized that the role of antigen in certain immune reactions is fulfilled once it combines with and modifies antibody, activating the first component of complement. Antibodies are ineffective in many biologic reactions unless they interact with an effector system in blood or tissue fluids, such as complement.

The purpose of this study was to determine if immunoglobulin or complement components are localized or fixed in periapical lesions, which would provide evidence for the participation of immunologic reactions in these lesions.

MATERIALS AND METHODS

Biopsy specimens of periapical lesions were taken from 48 patients treated at the State University of New York at Buffalo Dental School Clinic and in the offices of several local endodontists. At the time of surgery, the lesions were removed by gentle curettage, and representative portions of the lesions were cut into 1- to 2-mm × 5-mm fragments. Thirty-eight of the lesions were processed by the Eidelman procedure,[15] which involved immediate fixation in 10% neutral buffered Formalin at 4 C. Lesions were fixed in Formalin for four hours to eight weeks, and it was found that the amount of time that the tissues were left in Formalin at 4 C did not affect immunoglobulin staining reactions. The tissues then were placed in 30% (weight by volume) sucrose for 24 hours at 4 C, after which they were fast frozen and sectioned in a cryostat (fixed, not washed). Ten lesions were immediately frozen on dry ice at the time of surgery and then sectioned in the cryostat.

Four of the lesions were processed in three different ways. One part of each of these four lesions was immediately fast frozen, another was fixed by the Eidelman technique, and the remaining part processed by washing for 48 hours in isotonic saline solution at 4 C, after which it was processed by the Eidelman procedure. All of the processed tissues were sectioned in the cryostat at 4 μm to 6 μm.

Before staining, the sections were air-dried on the slides for one hour. Then, they were prewashed for 15 minutes in 0.15-M sodium chloride and 0.02-M sodium phosphate buffered at a pH of 7.5 (phosphate-buffered saline [PBS]), and rinsed in distilled water. After this, they were incubated with the appropriate fluorescein conjugate, washed for one hour in PBS, and rinsed in distilled water. They then were treated for 20 minutes in 95% ethanol, rinsed in distilled water, and mounted in glycerine buffered at a pH of 7.5. The slides were read within 24 hours after staining. Adjacent, or in some cases, close-by sections were stained with the following fluorescein-conjugated goat antiserums*: anti-human IgG, anti-human IgA, anti-human IgM, anti-human third component of complement (C3), anti-human fourth component of complement (C4), and anti-C3 proactivator (C3pa). Further sections were stained with hematoxylin and eosin.

Specificity of the conjugates was checked by blocking the homologous reactions with purified IgG, IgM, IgA, and unlabelled anti-C3 serums. The C3 staining also was checked by mixing the anti-C3 conjugate with AB blood group substances to inhibit possible blood group substance staining. Antigen absorption controls with the anti-IgG conjugate were carried out by mixing human γ-globulin at a concentration of 50 mg/ml with fluorescein-conjugated goat anti-human IgG. The tissue did not stain or in some cases staining was very dull. In

contrast, the anti-human IgG fluorescein conjugate similarly absorbed with equine γ-globulin stained the tissues as brightly as the unabsorbed conjugate. Similar absorption experiments also were run on IgA and IgM. A second control, using PBS on the slides instead of the fluorescein conjugate was used to determine the levels of autofluorescence. These slides were negative. Genco and associates[18] used further controls with these same conjugates.

RESULTS

Localization of IgG, IgA, IgM, and C3 was studied on sections of 16 periapical lesions processed by the Eidelman procedure. Table 1 shows the results of these experiments. IgG was observed in all lesions studied. Fluorescein-conjugated goat anti-human IgG stained tissues fast frozen and also tissues washed in PBS before fixation. The washed tissues showed less background connective tissue staining as compared with the unwashed tissues. Mononuclear cells, resembling plasma cells and containing cytoplasmic IgG, were found in all lesions (Fig 1). In all periapical lesions studied, IgA was localized in the connective tissue and in plasma cells (Fig 2). A number of lesions showed typical plasma cells that stained for IgM (Fig 3). IgG staining was the most intense, and more plasma cells stained for IgG than for IgA or IgM. However, in a few lesions, IgA-containing cells predominated. In general, IgA-containing cells were found more often than those containing IgM. Ig-negative mononuclear cells resembling lymphocytes also were observed in these lesions.

The third component of complement was identified in sections from fast-frozen tissues, but not in tissues processed by the Eidelman technique (Tables 1 and 2). It appears that Formalin fixation destroyed the C3 in these lesions. The fourth component of complement was not observed in any tissue even when several different C4 conjugates were used.

Table 1 • Ig and complement in Formalin-fixed human periapical lesions.

Substance localized	No. positive	No. tested
IgG	16	16
IgA	16	16
IgM	7	14
C3	0	16
C4	0	16

Table 2 • Ig and complement in fast-frozen human periapical lesions.

Substance localized	No. positive	No. tested
IgG	10	10
IgA	10	10
IgM	7	10
C3*	10	10
C4	0	10
C3pa	0	5

*In five of ten lesions, circular structures stained for C3.

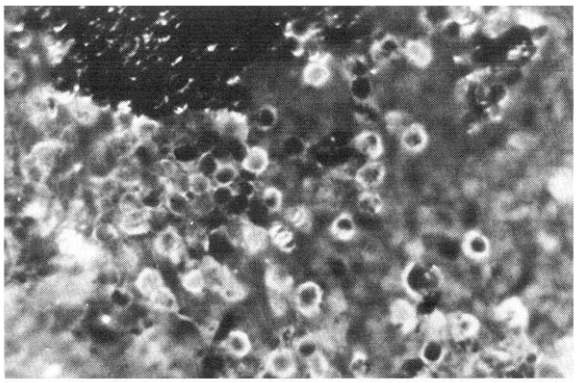

Fig 1—Section of periapical lesion stained with fluorescein-conjugated goat anti-human immunoglobulin G. Notice numerous plasma cells staining for IgG (orig mag ×480).

Table 2 shows immunoglobulin and complement localization in lesions that were fast frozen immediately after removal. IgG and IgA were localized in all lesions, and IgM was localized in seven of ten lesions. The third component of complement was localized in every lesion both intravascularly and in the form of acellular deposits in the connective tissue. In addition, in five of ten lesions, C3 stained circular structures resembling vascular channels (Fig 4,5).

The specificity of the C3 staining was studied by blocking experiments using unlabeled antiserums to C3, or purified blood group A and B substances and equine stomach sediment as a source of blood group A substance. As can be seen in Table 3 blocking with unlabeled anti-C3 sources resulted in dull or negative staining, whereas neither source of blood substances reduced the staining intensity. The proactivator for the third component of the complement system (C3pa) was not found in any of the tissues.

Histologic Findings

Sections of every lesion were stained with hematoxylin and eosin. The criteria for comparative evaluation included presence of an epithelial lining, characteristics of the stroma, vascular response, type of inflamma-

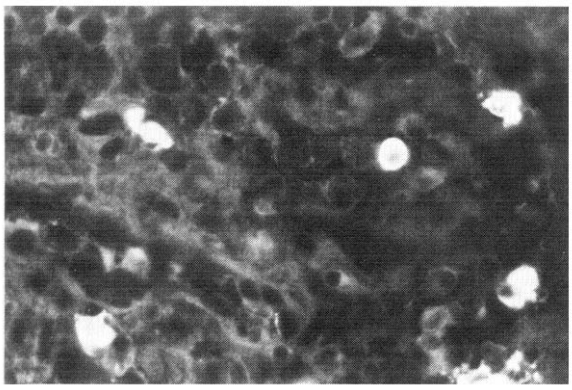

Fig 2—Section of periapical lesion stained with fluorescein-conjugated goat anti-human immunoglobulin A. Notice several cells staining for IgA (orig mag ×480).

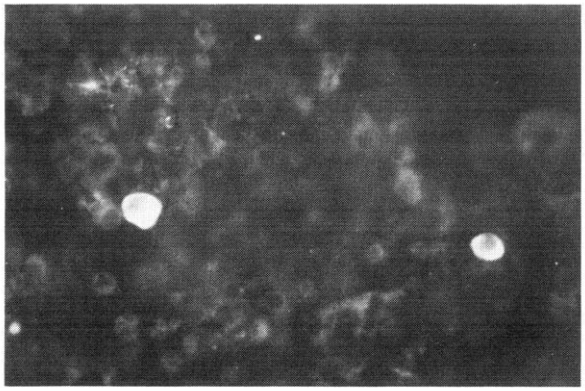

Fig 3—Section of periapical lesion stained with fluorescein-conjugated goat anti-human immunoglobulin M. Notice two plasma cells staining for IgM (orig mag ×480).

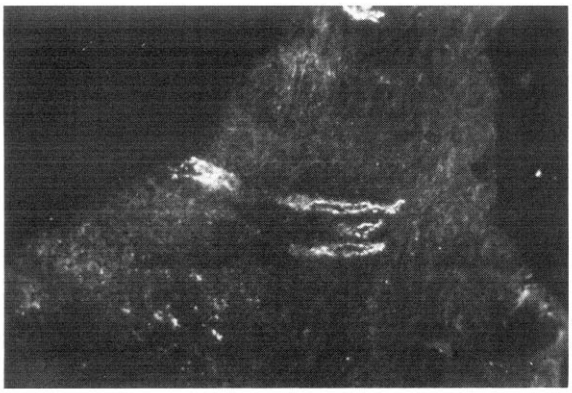

Fig 4—Section of periapical lesion stained with fluorescein-conjugated goat anti-human complement 3. Notice three vessel-like structures stained for C3. Clinically, this lesion was removed from periapical area of tooth whose root canal had been filled by silver cone technique three years previously (orig mag ×100).

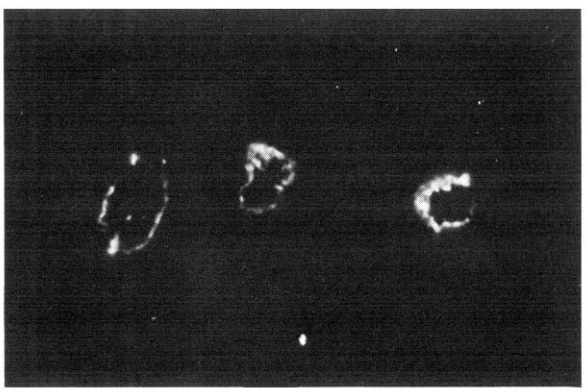

Fig 5—Section of periapical lesion stained with fluorescein-conjugated goat anti-human complement 3. Notice three vessel-like structures straining for C3 (orig mag ×480).

tory infiltrate, predominant cells, and inclusions. A cyst was diagnosed when an extensive epithelial lining associated with a definite connective tissue wall was observed.

Several specimens possessed an epithelial lining. In most cases, the lining was hyperplastic, reticulated, or both. In no instance was keratinization observed. The characteristics of the stroma were consistent with the degree of inflammation. Rarely, a mature collagen matrix was observed. More often, the stroma was edematous and fibroblast nuclei were elongated and compressed with indistinct cytoplasmic boundaries.

The vascular response was characteristic of a chronic inflammatory process. In most specimens, this was one of delicate endothelium-lined spaces (Fig 6). The inflammatory infiltrate often assumed a perivascular distribution and was composed of mixtures of lymphocytes, plasma cells, and macrophages. It should be noted that four of the five lesions in which circular structures were observed staining for C3 were diagnosed as cysts. The fifth was of questionable diagnosis but had some of the features of a cyst.

DISCUSSION

These experiments showed that IgG-containing plasma cells predominate over those containing IgA or IgM, a feature commonly observed in inflammatory lesions other than those associated with mucosal surfaces in which IgA cells may predominate.

We have demonstrated C3 in globular structures (deposits) or deposits on circular structures in fast-frozen sections. Either type of localization may have resulted from binding of C3 to immune precipitated complexes. The C3 binding to the circular structures occurred in the absence of detectable immunoglobulin binding, hence C3 in these circular structures

may represent intracellular C3, which was being stored or synthesized by the staining cells. Alternatively, C3 may have bound to receptors on the vascularlike structures. Gelford, Frank, and Green[16] have recently described a receptor for C3 in the human renal glomerulus. The globular C3 staining appeared extracellular and is more likely to be associated with immune complexes. As a consequence of the binding of the third component of complement, biologically active fragments such as C3a may be released, leading to increased vascular permeability and chemotaxis.

The circular structures staining for C3 were not definitely identified, but they appeared to be blood vessels by comparison of adjacent sections stained by hematoxylin and eosin.

These results point to the possibilities of activation of the complement system in periapical lesions. Complement activation could account for the histologic observation of occasional blood vessels surrounded by neutrophils. The leukocytes may migrate to the lesions and release their histolytic lysosomal enzymes, leading in turn to tissue destruction. It has recently been shown by Hausmann and associates[17] and Raisz and associates[18] that antibodies and complement can lead to release of factor(s) that stimulate bone resorption. Because the dominating immunoglobulin in periapical lesions appears to be IgG, which can participate in complement activation, a shift in favor of the complement-fixing IgG may disturb the local immune homeostatis with aggravation and perpetuation of the inflammatory process. IgE localization was not systematically studied; however, its localization may help elucidate the role, if any, of this Ig in periapical pathosis.

Numerous nonimmunoglobulin containing cells resembling lymphocytes were observed in the periapical lesions. Farber[19] has presented evidence that there are both T and B cells in periapical lesions, with the T cells predominating. The T lymphocytes may play a role in cellular immune reactions occurring in the lesions, and B lymphocytes may give rise to more plasma cells as the lesion develops.

The periapical lesion is an easily studied model of chronic inflammation, and its study may lead to better understanding of inflammation throughout the body.

Table 3 • Specificity of C3 staining of fast-frozen human periapical lesions.

Staining reagent	Blocking reagent	Staining results: No. positive	Staining results: No. tested
F-anti-C3	Saline solution	10	10
F-anti-C3	Normal rabbit serum*	1	1
F-anti-C3	Rabbit anti-C3† (unlabeled)	0	10§
F-anti-C3	Blood group‡ A & B substances	5	5
F-anti-C3	Equine stomach sediment	5	5

*Conjugate diluted 1:1 with normal rabbit serum.
†Conjugate diluted 1:1 with unlabeled anti-C3 serum.
‡Rich sources of blood group substances.
§Two of these lesions were negative for C3, and eight produced dull staining that was noticeably less intense than that observed in unblocked experiments.

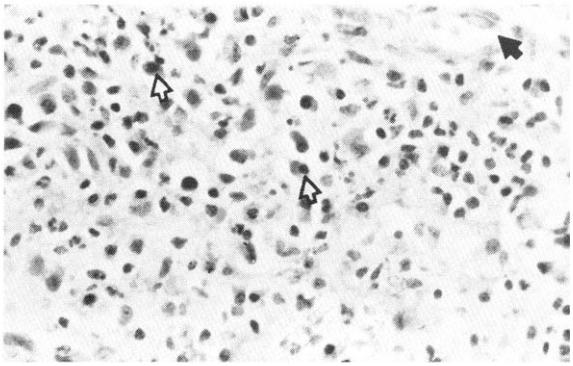

Fig 6—Section of same periapical lesion as in Figure 5. Numerous plasma cells contained in lesion have pleomorphic appearance. In some areas, typical "cartwheel" nucleus is evident (outlined arrows). Notice prominent endothelium-lined spaces (solid arrow) (H&E, orig mag ×480).

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine if there were immunopathologic reactions occurring in periapical lesions. IgG, IgA, and IgM were observed extracellularly as well as in cells resembling plasma cells. Plasma cells containing IgG were most numerous and those containing IgM least numerous. Numerous non-

Ig containing round cells resembling lymphocytes also were found in most lesions. Of the ten lesions fast frozen, five showed bright C3 staining of circular structures that may be blood vessels or endothelium-lined microcysts. This staining was blocked with unlabeled anti-C3 serums, but was not blocked by absorption of the anti-C3 conjugate with rich sources of AB blood group substances. C3pa and C4 were not detected in any of the ten lesions.

From these studies, it is clear that C3 is found on vessel-like structures in many periapical lesions. The nature of attachment of C3 is not known. In some lesions in which C3 is found fixed to tissues, this fixation appears to be immunologic, while in others, binding to circular structures occurs in the absence of detectable antibody. Complement activation resulting in a release of biologic fragments may result in inflammatory changes and possibly bone resorption in the periapical region. Further work is required to define the nature and consequence of C3 binding in periapical lesions.

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