Biosynthesis of immunoglobulin isotypes in human periapical lesions

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Periapical lesions from five patients were incubated with radioactive amino acids (^{14}C isoleucine and ^{14}C lysine). Immunoglobulin synthesis was detected by autoradiography of an immunoelectrophoretic pattern. IgG and IgA were synthesized in vitro by lymphocytes from all of the periapical lesions studied. IgG was the predominant class of immunoglobulin present, followed by IgA. There was no evidence of the biosynthesis of IgM or the third component of complement (C3).

Several recent investigations have suggested the possibility of local antibody production in periapical granulomas cysts. Morse and others[1] stained sections of 33 periapical lesions from endodontically treated teeth with methyl green pyronine to determine the presence of plasma cells. They suggested that positive staining indicated immunoglobulin production in these cells. Kuntz and Genco[2] used an immunofluorescent antibody technique to demonstrate immunoglobulins and complement in periapical granulomas. In another study, Kuntz and others[3] observed IgG, IgA, and IgM both extracellularly and within cells resembling plasma cells. The third component of complement (C3) was seen in vessel-like structures in many periapical lesions,[3] but Morton and others[4] failed to demonstrate this protein in their studies. In similar studies by Toller and Holborrow[5] on periapical cyst walls, an immunofluorescent antibody technique was used to demonstrate IgA and lesser amounts of IgG and IgM in the lesions. Naidorf[6] studied three periapical lesions by microscopic examination and subjected them to electrophoretic and single-radial immunodiffusion analyses. He showed the qualitative presence of IgG, IgA, and IgM immunoglobulins in two periapical granulomas; however, whether these immunoglobulins were derived from a serum or produced by local synthesis could not be determined.

This study was undertaken to examine further the local synthesis of immunoglobulin in periapical lesions. The incorporation of ^{14}C-labeled amino acids is a convenient marker of de novo biosynthesis. Immunochemical methods were used for separation and detection of the various immunoglobulin isotypes.

MATERIALS AND METHODS

A modification of the method of Hochwald and others[7] was used to study immunoglobulin synthesis in periapical lesions. Biopsy specimens of periapical lesions were obtained from five patients treated at the graduate endodontic clinic, school of dental medicine, University of Pennsylvania. Each specimen was obtained from teeth treated previously with gutta-percha root canal fillings.

The tissue samples were placed immediately in a test tube containing 10 ml of ice-cold Hank's balanced salt solution (free Ca†† and Mg††). Under aseptic conditions, the tissues were washed free of blood clots and minced with a scalpel and forceps.

Fragments of the periapical tissue were placed in 1 ml of modified RPMI-1640 media that had been supplemented with 1.0 μCi ^{14}C isoleucine, 1.0 μCi ^{14}C lysine, and 25 μg gentamycin. Approximately 100 mg (wet weight) of tissue was placed in each tube.

Controls consisted of tissue speci-

mens that were frozen immediately after the preparation just described. Experimental samples were incubated for 48 hours at 37 C with continuous rotation. Incubation was terminated by freezing the sample. The samples and controls were then subjected to two freeze-thaw cycles, followed by centrifugation at 750 g for ten minutes. The supernatant fluid was dialyzed against .05 M borate buffered saline solution, pH 8.4, for seven days, or until all free radioactive amino acids were removed, then concentrated (10×) using a filter with a 25,000 molecular weight (mol wt) cutoff (Minicon).

Concentrated samples were then mixed with carrier serum (1:5 dilution of normal human serum) and subjected to immunoelectrophoresis[8] in agarose gel (Sea Kem) according to standard procedure.

Precipitin lines were developed by addition of various antiserums. Monospecificity of immunoglobulin class-specific and C3 antiserums used in this study was determined by both adsorption and competitive inhibition. Five antiserums were used: rabbit anti-human γ chain, rabbit anti-human μ chain, rabbit anti-human α chain, rabbit anti-whole serum, and rabbit anti-human C3. The slides were washed in 0.015 M in phosphate-buffered saline solution (pH 7.8) for 72 hours, with three buffer changes, and were allowed to air dry.

Autoradiographs of the slides were prepared by inverting the slides on Kodak SB-5 film. Slides were exposed for four to six weeks. The x-ray film was then developed and fixed according to standard procedure. The arcs formed by the labeled antibody and complement were graded from minus (—) for no band visible, to three plus signs(+ + +) for a dark well-defined arc.

RESULTS

The autoradiographs of the five samples were examined for arcs that would indicate local synthesis of IgG, IgA, IgM, or C3. The results of this investigation are summarized in the Table.

IgG and IgA were synthesized in variable amounts (as evidenced by differences in arc density) in all five of the lesions studied (Fig 1-3). These immunoglobulins formed arcs on the autoradiographs of the anti-whole serum reactions, which confirmed the presence and synthesis of the two immunoglobulins. No additional arcs were noted. Evidence of synthesis of IgM (Fig 4) or C3 (Fig 5) was not detected on any of the autoradiographs examined.

In all of the periapical lesions studied, IgG appeared to be the predominant class of immunoglobulin synthesized as indicated by arc density.

DISCUSSION

The immunoglobulin synthesized in human periapical lesions resembles that of the secondary immune response; IgG and IgA are the major products. In contrast, primary immune responses are characterized by an IgM antibody

Immunoglobulin and antibody-producing cells have been shown to accumulate in chronic inflammatory foci produced by local infection of antigen or nonspecific inflammatory agents.[9] The immune response may be directed against antigens in the root canal. The antigens presumably would be bacterial; however, studies on antibody specificity have not been reported. Bacterial products, or even root canal filling materials and medicaments, may represent additional sources of antigenic stimulation.[10]

Brandtzaeg[11] has developed a model to study the local immune response to a persistent antigenic stimulus provided by insolubilized egg-white lysozyme. Two weeks after injection of 0.4 mg of polymerized antigen into the gingiva of a systemically immunized rabbit, dense infiltrates of IgG immunocytes were present around the antigenic depot. Relatively few cells contained IgA, and no IgM-containing cells were found. A similar result was obtained by Kraus and others,[12] who injected rabbit gingiva with inactivated collagenase for six weeks. It is possible, therefore, that the predominance of the IgG isotype in periapical lesions is associated with the chronicity of the lesion rather than with a pecu-

Table • Immunoglobulin and complement synthesis by human periapical lesions.

Specimen No.	Whole Serum	Autoradiography*			
		IgG	IgA	IgM	C3
1	IgG, IgA	+ +	(+)	—	—
2	IgG, IgA	+ + +	+ +	—	—
3	IgG, IgA	+ + +	+	—	—
4	IgG, IgA	+ +	(+)	—	—
5	IgG, IgA	+ + +	+ +	—	—

*The intensity of the autoradiographic line is graded as follows: — = negatives; (+) = just visible; + = clearly visible, + + + = very dark.

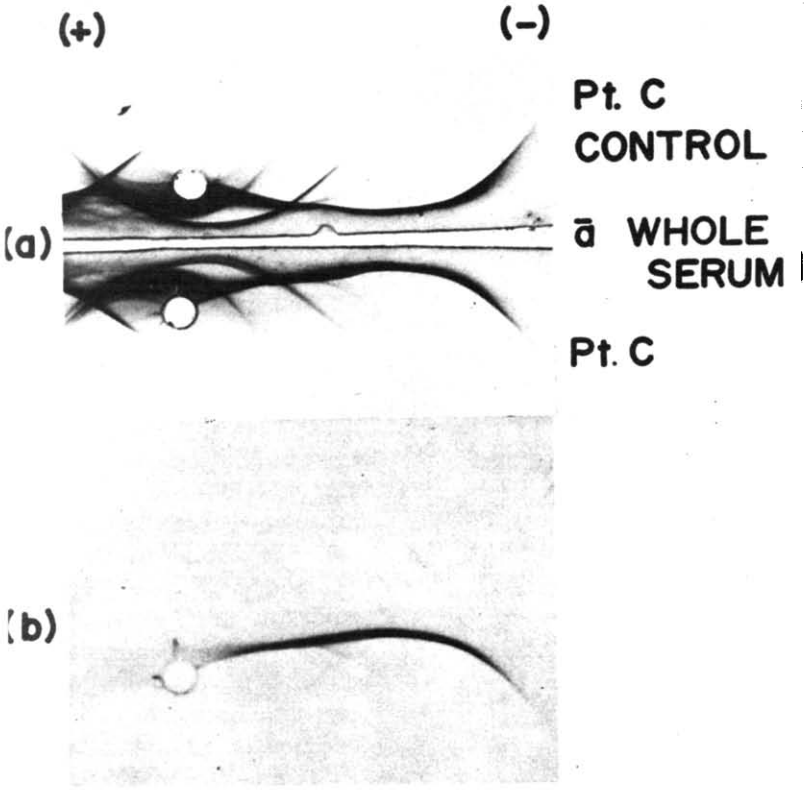

Fig 1—Synthesis of proteins in chronically inflamed human periapical tissue. (a) Immunoelectrophoresis pattern: top well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with (10×) concentrated control culture (frozen immediately after preparation) supernatant from patient C; trough, rabbit anti-human whole serum; bottom well, carrier serum (1:5 dilution of normal serum) mixed 1:1 with (10×) concentrated culture fluid (incubated 48 hrs at 37 C) from patient C. (b) Autoradiograph of (a) shows synthesis of IgG and IgA in vitro. Autoradiograph was exposed for 28 days.

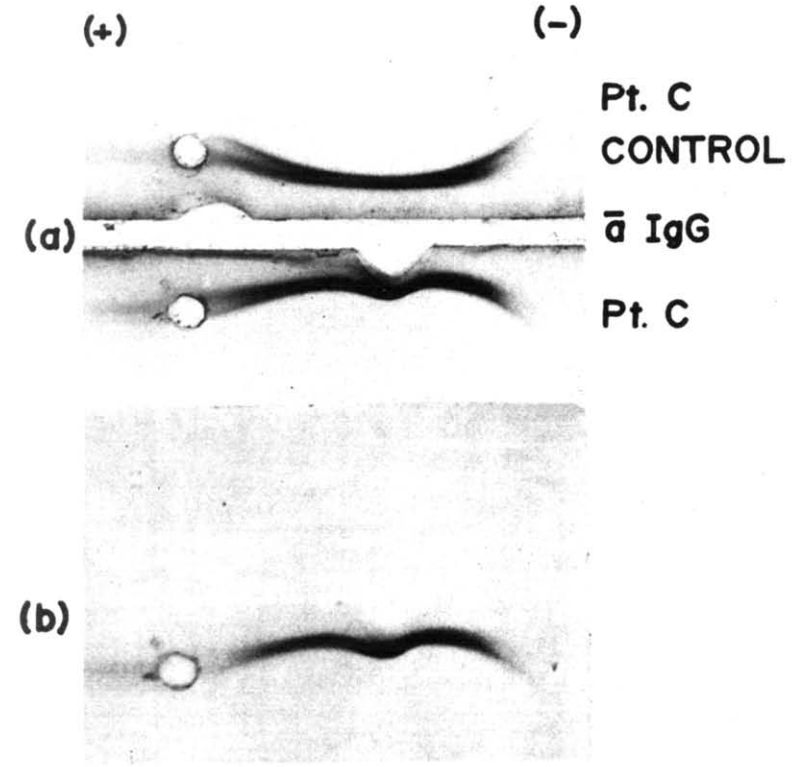

Fig 2—Synthesis of IgG antibody in chronically inflamed gingival tissue. (a) Immunoelectrophoresis pattern: top well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with (10×) concentrated control culture (frozen immediately after preparation) supernatant from patient C; trough, rabbit anti-human γ chain antiserum; bottom well: carrier serum (1:5 dilution or normal serum) mixed 1:1 with (10×) concentrated culture fluid (incubated 48 hrs at 37C from patient C. (b) Autoradiograph of (a) shows synthesis of IgG in vitro. Autoradiograph was exposed for 28 days.

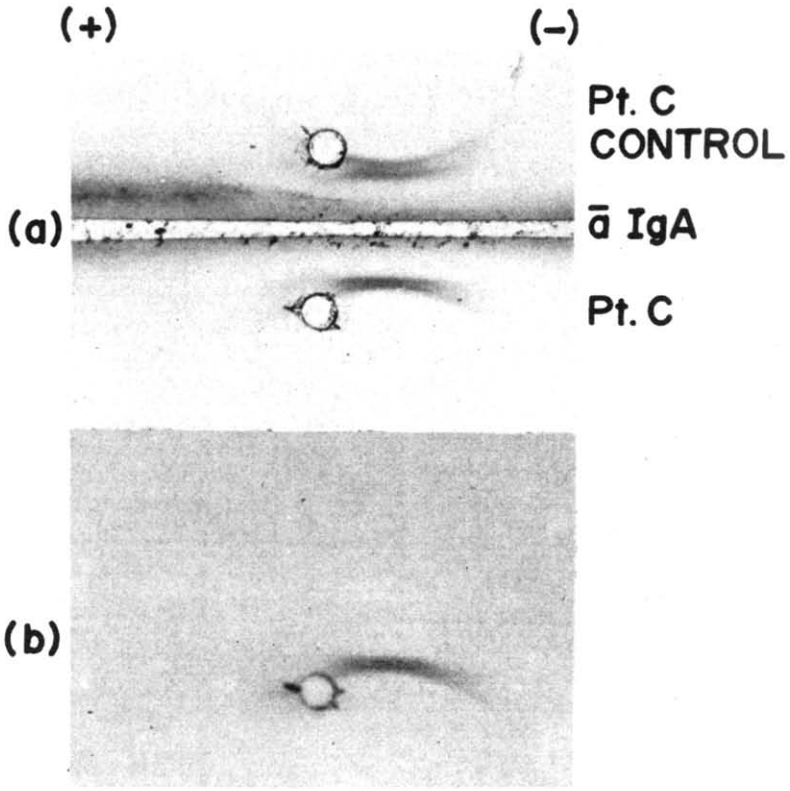

Fig 3—Synthesis of IgA antibody in human periapical lesions. (a) Immunoelectrophoresis pattern: top well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with (10×) concentrated control (frozen immediately after preparation) supernatant from patient C; trough, rabbit anti-human α chain antiserum. Bottom well, carrier serum (1:5 dilution of normal serum) mixed 1:1 with (10×) concentrated culture fluid (incubated 48 hrs at 37C from patient C. (b) IgA antibody in vitro. Autoradiograph was exposed for 28 days.

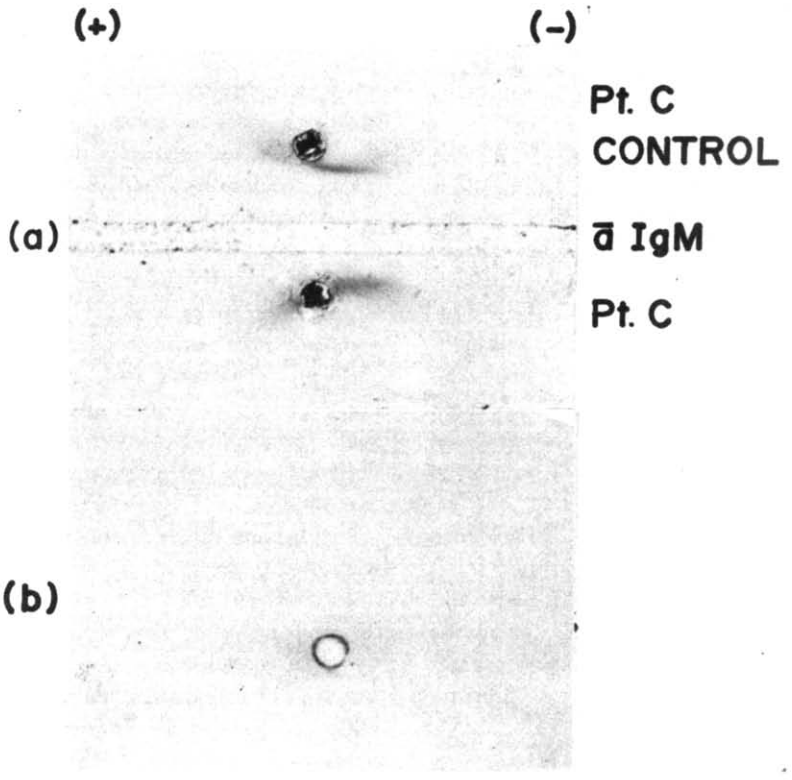

Fig 4—Synthesis of IgM in human periapical lesions. (a) Immunoelectrophoresis pattern: top well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with (10×) concentrated control culture (frozen immediately after preparation) supernatant from patient C; trough, rabbit anti-mouse μ chain antiserums; bottom well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with 10× concentrated culture fluid (incubated 48 hrs at 37 C from patient C. (b) Autoradiograph of (a). No synthesis of IgM was noticed. Autoradiograph was exposed for 44 days.

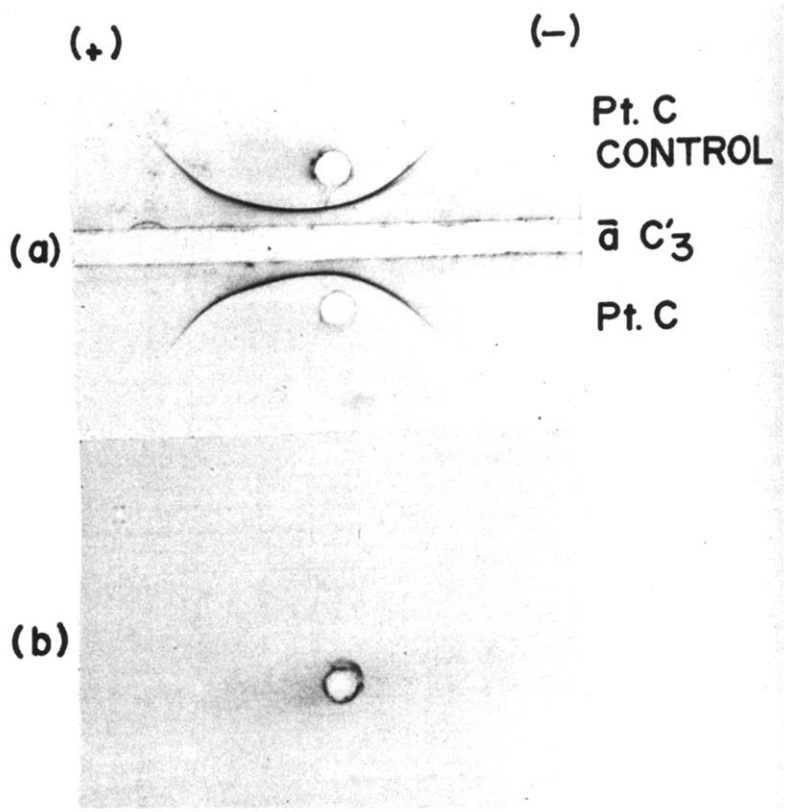

Fig 5—Synthesis of C3 in human periapical lesions. (a) Immunoelectrophoresis pattern: top well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with (10×) concentrated control culture (frozen immediately after preparation) supernatant from patient C; trough, rabbit anti-human C3; bottom well, carrier serum (1:5 dilution of normal human serum) mixed 1:1 with (10×) concentrated culture fluid (incubated 48 hrs at 37 C) from patient C. (b) Autoradiograph of (a). No synthesis of C3 was noticed. Autoradiograph was exposed for 42 days.

liarity of the initiating antigenic stimulus.

The dominance of IgG synthesis found in our study is compatible with results obtained using nonimmunofluorescent techniques in inflamed synovia, kidney, urinary bladder,[11] and gingiva.[13] The concomitant local immune response provided IgG antibodies against the infectious agent in each of these areas, and no antibody activity was detected in the minor amounts of locally synthesized IgA and IgM.[11]

The findings of our study are consistent with immunofluorescent studies of Kuntz and others[3] and Pulver and others,[14] who reported that IgG plasma cells predominate, followed by IgA-staining plasma cells. Kuntz and others[3] found IgM-containing plasma cells in seven of ten specimens examined; our study however, was not able to demonstrate de novo synthesis of IgM in periapical lesions. Two reasons that could explain this discrepancy are: (1) nonspecific immunofluorescent staining of cells could occur via the Fc receptor[15]; (2) IgM plasma cells are present in periapical lesions, but they are either not secreting antibody or are secreting antibody at such low amounts that it cannot be detected with the assay system that was used.

Production of IgA in periapical lesions appears modest. The present assay system does not permit the differentiation of the 7S monomer which is predominately found in human serum, from the 9S dimer, which is the predominant form in secretions[16]; however, experiments that will permit this differentiation are underway.

The biological significance of immunoglobulin synthesis in chronically inflamed periapical lesions remains speculative. IgG may exert a direct, protective antibacterial function or it may form immune complexes that result in complement fixation and perpetuation of the inflammatory process.

The possible role of IgA in periapical lesions is also unclear. The combination of IgA with antigen in chronic periapical lesions may be in competition with IgG and IgM, and may provide a blocking or antigen-deletion effect by preventing the release of inflammatory agents that be injurious to the host.[17] It is important to remember that although inflammation is initially conducive to local

defense, immunologically mediated attraction of polymorphonuclear leukocytes and release of imflammatory mediators may be more injurious to the host than the initial antigenic stimulus.

Biosynthesis of C3 could not be demonstrated in this study. The presence of C3 in periapical lesions has been identified in previous investigations by use of immunofluorescent techniques,[3] and local production of complement in inflamed gingiva has been shown (Lally, E.T., unpublished data). The inability to demonstrate synthesis of C3 may have been due to low levels of synthesis in the tissue cultured, or to a low specific activity of the labeled C3 component.

It is apparent from this study that periapical lesions have components of the host immune response, such as immunoglobulins derived from local synthesis. The protective and destructive aspects of chronic inflammation, and the role of this response in the resolution or perpetuation of endodontic infections, require further study.

SUMMARY AND CONCLUSIONS

Biopsy specimens of periapical lesions from five patients were examined by autoradiography to determine if immunoglobulins and complement components were synthesized in vitro from tissues taken from these lesions. Tissue fragments from the lesions were incubated with the radioactive amino acids ^{14}C lysine and ^{14}C isoleucine. Immunoglobulins synthesized de novo were detected and identified by an autoradiograph of an immunoelectrophoretic pattern.

IgG and IgA were synthesized in tissue taken from all five of the lesions. IgG was the predominant class of immunoglobulin present, followed by IgA. No traces of IgM or C3 were detected on the autoradiographs.

The following conclusions may be drawn from this study. Chronic inflammation in periapical lesions is combined with an immune response dominated by IgG synthesis. This immune response resembles that of secondary immune responses in which IgG and IgA are the major products.

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