Immunoglobulins in periapical granulomas: a preliminary report

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This preliminary report presents a method for the isolation and identification of immunoglobulins and some of their components in periapical granulomas.

The periapical granuloma, one of the most common forms of dental pathosis, is characterized as a localized mass of chronic granulation tissue formed in response to infection with infiltration of chronic inflammatory cells, chiefly lymphocytes and plasma cells.[1]

While this definition is satisfactory to the pathologist, it gives the endodontist no insight into the biological significance of this lesion. Specifically, the textbook description either ignores or sketchily presents the relationship of the described cellular elements to the immune response. Recently, attention has been called to the necessity for investigations of this nature.[2]

Immunologists have suspected antigen-antibody relationships in chronic granulamatous lesions. Burnet[3] said, "It is a good rule that any local accumulation of lymphocytes in the body, especially if plasma cells are also present, is a sign that immunological activities are going on." The plasma cell, the end of the B-cell line, can be likened to the hedgehog in the aphorism, "The fox knows many things, but the hedgehog knows one big thing." The plasma cell is an efficient protein manufacturing plant that turns out only one product—an immunoglobulin of a single type. The immunoglobulin is capable of combining efficiently only with the antigen which turned on the specific precursor B cell that has the appropriate receptor sites for that antigen. This is the essence of the clonal-section theory of immunity.

Burnet[3] also noted that the interrelationship of chronic infection with immunological anomalies is one of the potentially important areas of medicine that is almost unexplored. The importance of such lesions to systemic health has been reported by Osserman and others.[4-6] They provided strong inferential evidence that long-standing chronic infections, especially those of the biliary tract, with round cell infiltration may be precursors of monoclonal plasma cell dyscrasias. From the viewpoint of the clinical endodontist, the presence of B cells and humoral antibodies suggests the possibility of clinically evident immediate reactions of hypersensitivity. On the other hand, T cells associated with cellular immunity can be involved in delayed hypersensitivities.[2]

It seems that the isolation and identification of immunoglobulins in periapical granulomas are essential preliminary steps in attacking and solving problems of this nature.

Materials and Methods

Immediately after extraction, the granulomatous masses attached to the apices of three anterior teeth were removed from the tooth structure. The granulomas were divided into two sections. The section that was closest to the apex (approximately a third of the mass) was placed in a 10% Formalin solution and sent to the pathology laboratory for examination. The distal third of the mass was rinsed in phosphate-buffered saline solution and was placed in a Pyrex culture tube* with 0.25 ml of phosphate-buffered saline solution. The sample was placed alternately in the freezing compartment of the refrigerator (−16 C) for 20 minutes and in a water bath (+52 C) for 12 minutes. After the third cycle, the tissue was crushed with a glass rod against the bottom of the tube. It then was subjected to nine additional freeze-thaw cycles so that the immunoglobulins present could be released into the solution. The suspension of fragmented cells was spun down in a centrifuge (2,000 RPM for an hour) and the antibody containing supernatant was separated.

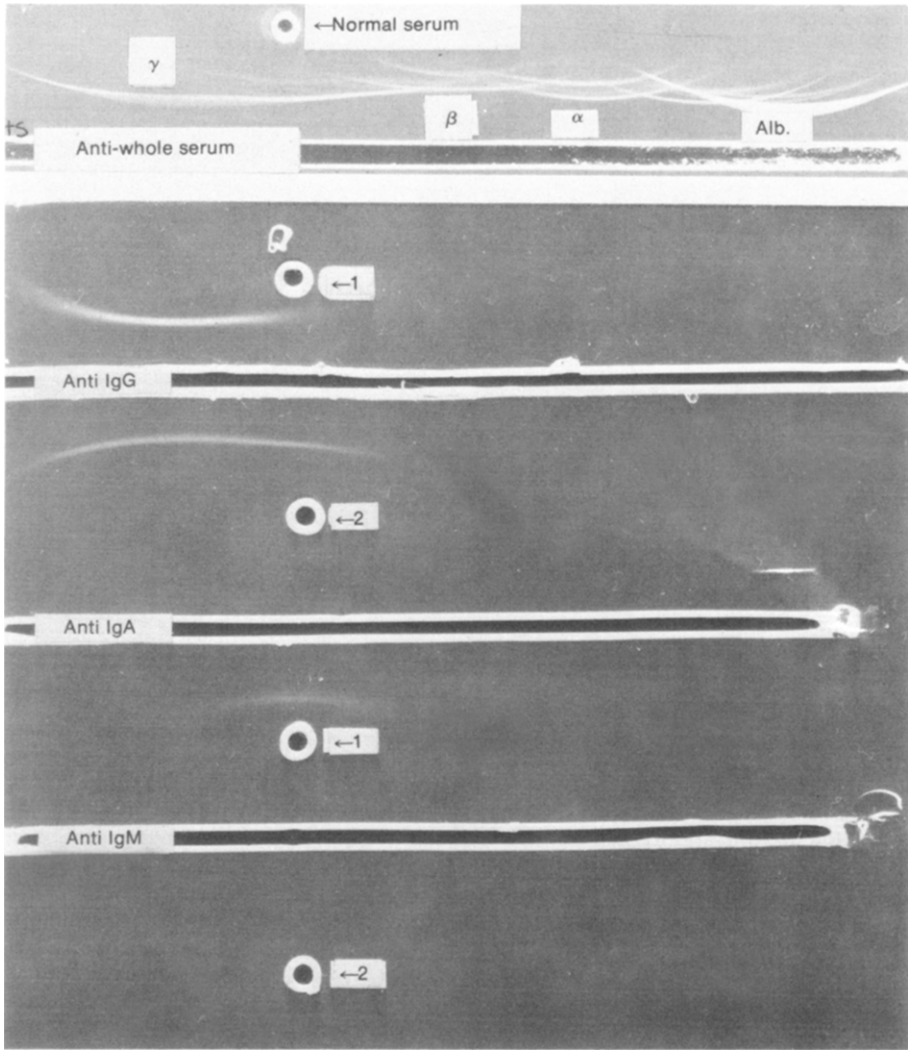

Fig 1—Immunoelectrophoresis. Normal serum reacted with anti-whole serum (top). Precipitin arcs of albumin, alpha, beta, and gamma globulins are labeled. Lower section shows extracted immunoglobulins of cases 1 and 2 reacted with anti-IgG, A, and M.

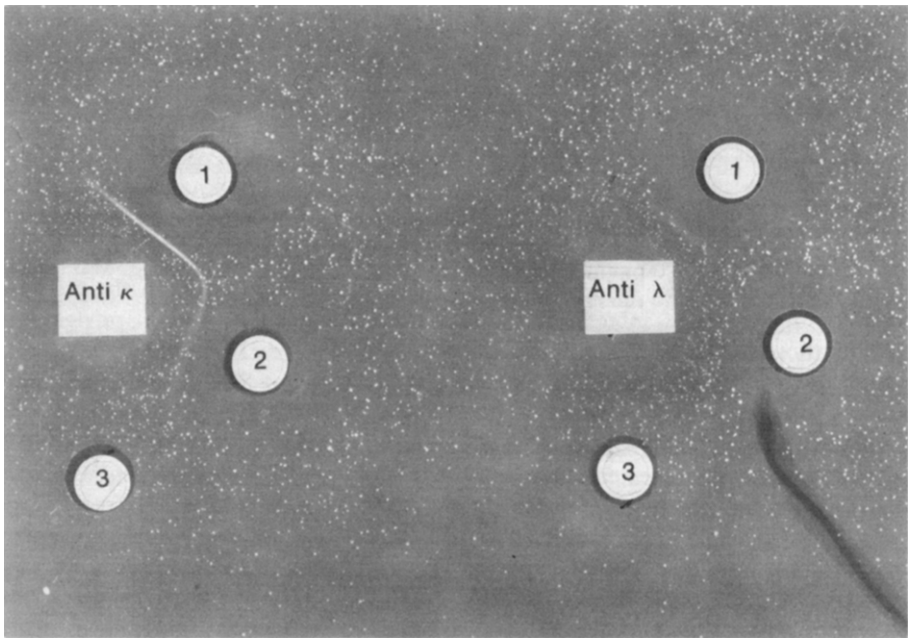

Fig 2—Extracted immunoglobulins placed in their respective wells in Ouchterlony plates and reacted with kappa and lambda antiserums. Dark line on lower right is artifact.

The extracted immunoglobulins were then subjected to immunoelectrophoretic (IEP) and single radial immunodiffusion (immunoplate [IP]) analyses. Ouchterlony plates were used to detect the kappa (κ) and lambda (λ) light chain portions of the immunoglobulin molecules using monospecific anti-κ and anti-λ antiserums. Standard methods as described by Isobe and Osserman[7] and Ouchterlony[8] were used.

Results

Three cases were studied and samples of the findings were photographed (Fig 1,2). Tables 1 and 2 summarize the results of the cases that were analyzed. The histological report categorized cases 1 and 2 as granulomas and case 3 as an apical scar (Fig 3-5).

Discussion

Nature provided a control in the form of an apical scar in the three cases studied. The study was double-blind because the pathologist was unaware of the immunologist's findings and vice-versa. The two lesions containing lymphocytes and plasma cells were diagnosed as granulomas by the pathologist. They were shown by immunological studies to have produced antibodies in the IgA, IgG, and IgM categories. The polyclonal nature of these two granulomas is further emphasized by the presence of both κ and λ chains. The lesion that was diagnosed as an apical scar was virtually devoid of the presence of round cells; it yielded no immunoglobulins.

Despite the limited number of cases, the methodology that was described should prove useful in studying the immunological aspects of periapical lesions. Refinements of technique, further studies of this nature, and research into the relationship of the periapical immunoglobulins to canal antigens are in progress.

Table 1 • Results from immunoelectrophoresis and immunoplate analysis (single radial immunodiffusion). Not all methods were used in all cases.

Case	IgG	IgA	IgM	κ	λ
1	+++(IEP)	++(IEP)	+(IP) −(IEP)	Not done	+(IEP)
2	+++(IEP)	−(IEP) +(IP)	−(IP) −(IEP)	−(IEP)	−(IEP)
3	−(IEP)	−(IEP) −(IP)	−(IP) −(IEP)	−(IEP)	−(IEP)

Table 2 • Results by Ouchterlony for κ and λ light chains.

Case	κ	λ
1	+++	+
2	++	−
3	−	−

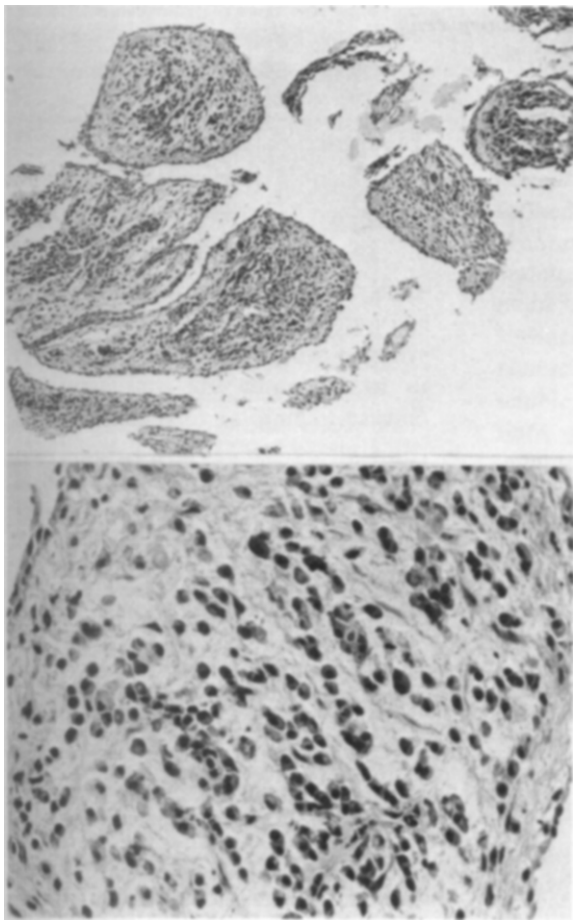

Fig 3—Tissue from apex of maxillary canine (top, orig mag X90; bottom, X375). Note presence of epithelial lining in some areas (early cyst) and rather typical infiltration of chronic inflammatory cells (lymphocytes and plasma cells).

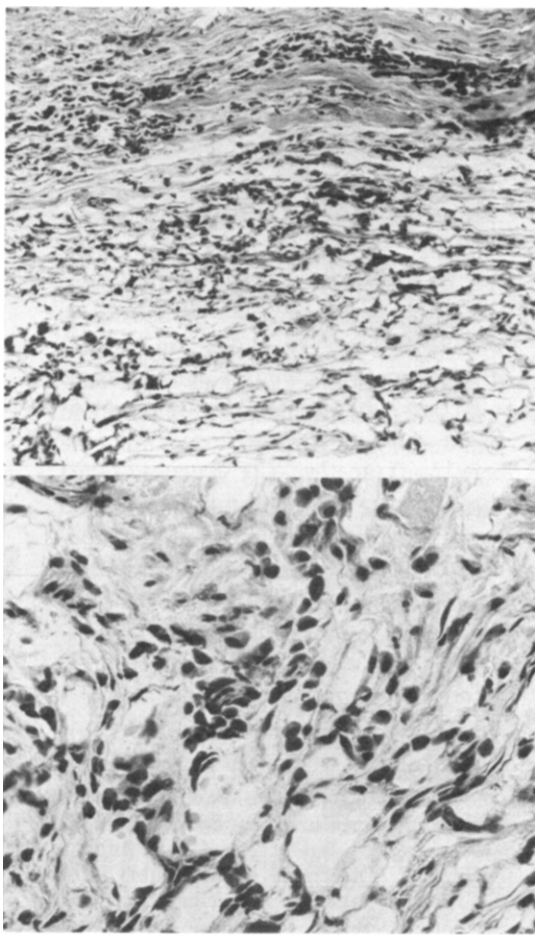

Fig 4—Tissue from apex of maxillary lateral incisor (top, orig mag X190; bottom, X275). Note presence of fat cells and infiltration by some acute but mostly chronic inflammatory cells.

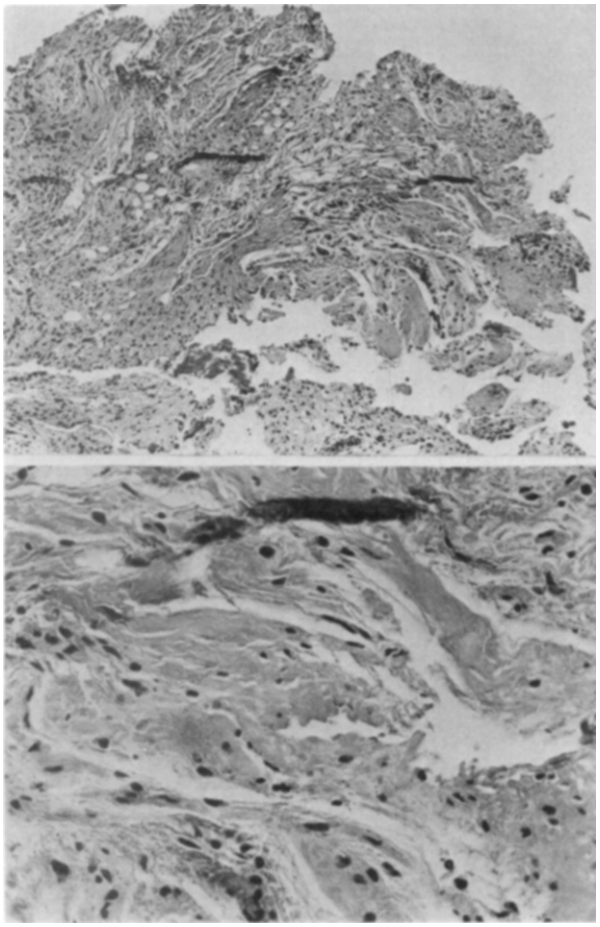

Fig 5—Tissue from apex of maxillary central incisor (top, orig mag X90; bottom, X375). Note dense collagenous tissue with presence of only occasional inflammatory cells.

Summary

Three periapical lesions were subjected to microscopic examination and immunologic analysis. One of the lesions was diagnosed as a periapical scar; it was virtually devoid of round cells and it yielded no immunoglobulins. The other two were diagnosed as granulomas and were shown to have produced immunoglobulins.

*Corning #99447.

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