Apical lesions contain elevated immunoglobulin G levels

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Portions of periapical lesions were obtained at the time of apicoectomy from teeth without obvious periodontitis. The tissue samples were weighed, their density determined and homogenized. The homogenate was then assayed for IgG content by rocket immunoelectrophoresis, and the IgG levels were expressed as μg of IgG per mg of tissue. Of the 16 lesions examined, we found an average of 11.0 μg/mg of tissue IgG compared with 3.8 μg/mg in clinically noninflamed control tissue. The mean IgG concentration of eight of the samples that were diagnosed as chronic apical granulomas by the oral pathology service was 16.0 μg/mg. The elevated levels of IgG in periapical granulomas contribute evidence that immunopathologic events participate in the genesis of these lesions. The exact mechanisms that account for the pathogenesis of chronic periapical lesions are not well understood. Although it seems likely that the bacteria that invade deep carious lesions are the cause of most of the granulomatous lesions, microbiologic studies have frequently failed to demonstrate bacterial presence in these lesions.[1-3] Recently, increased attention has been focused on the possible roles of immunopathologic mechanisms in the genesis of these lesions.[4,5]

Histopathologic examination shows the typical picture of chronic inflammation in the granulomatous lesions: macrophages, lymphocytes, plasma cells, and a few polymorphonuclear leukocytes (PMNL) are all present. The presence of plasma cells clearly implies the local synthesis of immunoglobulins. Several studies have indeed demonstrated the presence of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin E (IgE) in these lesions.[6-8] We are unaware, however, of any studies in which immunoglobulin concentrations have been accurately measured in periapical lesions.

MATERIALS AND METHODS

Portions of apical lesions from teeth without periodontal involvement were obtained during routine surgery and placed into 1.5 ml plastic test tubes. The tubes were immediately stoppered and frozen. The tissue samples were maintained at −20 C until analysis.

Then, the samples were thawed and weighed. The density of the samples was determined by measuring how much saline solution was displaced in a small glass test tube relative to a fine mark scribed with a diamond pencil. Displacement was measured with a microsyringe. This volume was divided into the weight of the tissue to give the sample density (ρ).

After the density was determined, the samples were placed into glass microhomogenizers. For each mg of tissue, 10 μl of diluent was added. The sample diluent consisted of the electrophoresis buffer (19 mM succinic acid, 97 mM boric acid, 31 mM Tris Base, pH 5.6) diluted 1:8 with distilled water. The homogenized tissue was transferred to centrifuge tubes and centrifuged for 20 minutes at 1900 × g to pellet the tissue debris. The supernatant was applied to commercially prepared electroimmunoassay plates (Worthington Diagnostics, Freehold, NJ) and electrophoresed at a constant voltage of 12 V/cm for 90 minutes. Samples and immunoglobulin standards were run in duplicate, and 1:5 dilutions of the samples were also run on each plate.

After electrophoresis, the plates were stained with dichrome stain (10 mg% napthol blue-black, 20 mg% thiazine red R, and 210 mg% mercuric chloride in 5% acetic acid) and subsequently destained in 5% acetic acid. The plates were allowed to dry, and the rocket lengths (Fig 1) were measured with a clear plastic reticle calibrated in 0.5 mm increments. The IgG concentration in the sample was then read from a computerized table, supplied by the manufacturer, relating the average standard rocket length to the appropriate standard curve. The average length of the replicate standard and sample rockets were used for these calculations.

The density (found to be 1.0 mg/μl) was measured to calculate the total homogenate volume. We decided to calculate this value because

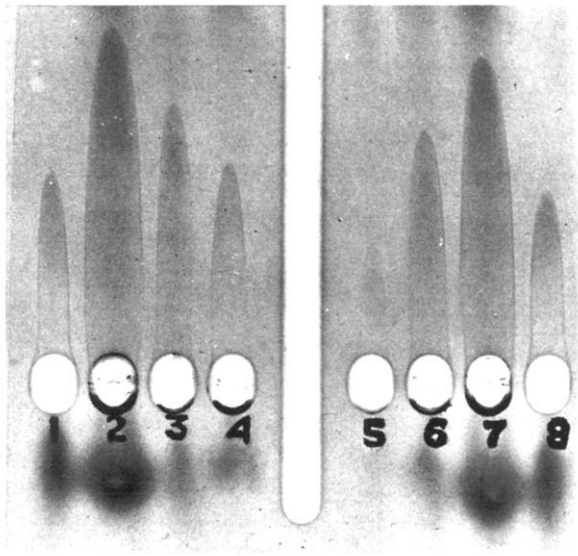

Fig 1—Typical rocket electrophoresis patterns used for quantitation of IgG levels in apical lesions. IgG concentration is proportional to length of rocket. Samples are as follows: 1 and 8, IgG standard; 2 and 7, patient "J" (undiluted homogenate); 3 and 6, patient "K" (undiluted homogenate); 4, patient "J" (homogenate diluted 1:5); 5, patient "K" (homogenate diluted 1:5).

the amount of homogenate that adhered to the walls of the homogenizer was a large and variable percentage of the total homogenate volume. Thus, a simple measurement of the homogenate volume would not have been accurate.

Knowing the IgG concentration of the samples, the tissue weight and density, and the volume of diluent added, we could then calculate the IgG content of the tissue sample with the following formula: μgIgG/mg tissue = $Cs \times (D + \frac{W}{p})/W$. Where Cs is the measured IgG concentration in the sample in μg/ml; D is the volume of diluent added in μl; W is the sample weight in mg; and p is the sample density in mg/μl.

We also obtained four pieces of noninflamed oral mucosa during routine endodontic and other surgical procedures. These tissue samples were analyzed in the same manner described and served to provide estimates of the IgG concentration of noninflamed tissue.

RESULTS

Immunoglobulin concentrations were measured by rocket electrophoresis. Typical patterns can be seen in Figure 1. Although duplicate samples show slightly different rocket lengths, the actual variation in calculated IgG levels between replicate samples was less than 5%.

IgG concentrations in the apical lesions ranged from 2.1 to 28.3 μg/mg of tissue (Table 1), with a mean of 11.0 and standard error of 2.2 μg/mg (Table 2). By comparison, the mean IgG concentration in the control tissues (n = 4) was 3.6 ± 0.2 μg/mg (Table 2). Of the 16 apical lesions we analyzed, only four had IgG concentrations that were not significantly higher than the control IgG levels (Table 1). The significance of comparisons between individual IgG concentrations and the control level was determined by assessing whether the sample IgG was in the range of the mean control IgG ± 2 standard deviations, thus giving a 95% confidence level.

We were able to obtain histopathologic diagnoses on eight of the lesions. All eight of these lesions proved to be chronic apical granulomas. The mean IgG concentration found in these eight lesions was 16.0 ± 3.4 μg/mg of tissue (Table 2). All of the lesions that were diagnosed as chronic apical granulomas had IgG concentrations significantly

Table 1 • IgG concentrations of periapical lesions.

Sample	Histopathologic diagnosis	IgG (μg/mg tissue)
A	ND*	7.0
B	ND	13.9
C	Chronic granuloma	18.3
D	ND	3.8†
E	ND	5.6
F	ND	3.4†
G	Chronic granuloma	5.6
H	Chronic granuloma	15.6
I	Chronic granuloma	5.3
J	Chronic granuloma	21.1
K	Chronic granuloma	6.1
L	ND	4.5†
M	Chronic granuloma	28.3
N	ND	7.0
O	Chronic granuloma	27.9
P	ND	2.0†
Q	Noninflamed gingiva	3.6
R	Noninflamed gingiva	3.8
S	Noninflamed oral mucosa	3.3
T	Noninflamed gingiva	4.3

*Not determined.
†Indicates values not significantly higher than control levels (at 95% confidence level).

Table 2 • Mean IgG concentrations.

Group	IgG (μg/mg tissue)*
All periapical lesions assayed	11 ± 2.2
Lesions diagnosed as chronic granulomas only	16 ± 3.4
Control tissues	3.8 ± 0.2

*± standard error

higher than levels in the control tissues.

DISCUSSION

In this study, we found the mean concentration of IgG in all of the apical lesions was significantly higher than the IgG levels in the control tissues. The levels we found in the control tissues were in agreement with preliminary studies presented by Gross and others,[9] who found a mean IgG concentration of 3.6 μg/mg in clinically healthy gingiva.

These findings imply that immunologically mediated events can be important in the pathogenesis of apical lesions. These events could include the interaction of antibodies (IgG or IgM class) with antigens to form aggregates known as immune complexes. Such complexes can activate (or fix) complement which results in the formation of pharmacologically active peptides designated as C3a, C5a and C5b67. C3a and C5a are anaphylatoxins; that is, they can induce the release of vasoactive amines and other inflammatory mediators from mast cells and basophils. The release of these substances causes increased vascular permeability and increased local blood flow that cause an inflammatory exudate. C3a, C5a and C5b67 are also chemotactic for PMNLs that result in a local inflammatory infiltrate. The PMNLs can release a variety of lytic enzymes resulting in tissue destruction.

Activation of complement has also been implicated as a mechanism of increasing osteoclastic activity.[10] Thus, formation of immune complexes can result in localized tissue destruction, inflammation, and bone loss. Although evidence for the occurrence of immune complexes in apical lesions has recently been reported,[11] the present study is the first report that we know of in which the actual levels of the immunoglobulins necessary for immune complex formation have been accurately measured in these tissues.

It is likely that not all of the 16 lesions that we examined were chronic granulomas. We were able to obtain histopathologic diagnoses on eight of the lesions, however, and these were diagnosed as chronic granulomas. When the data from these eight specimens were analyzed, an even greater difference between the apical lesions and the control tissues was seen. The mean IgG levels of all 16 apical lesions was 2.9-fold greater than the levels in the control tissue; whereas, the mean level in the eight lesions diagnosed as granulomas was 4.2-fold greater.

Of the 16 tissue samples analyzed, only four had IgG levels that were not different from the levels in control tissue. Unfortunately, we were unable to obtain histologic diagnoses on these four samples, but they may represent noninflamed cystic lesions or apical scars.

SUMMARY AND CONCLUSIONS

The IgG contents of apical lesions were determined and compared with healthy tissues. The results of this study demonstrate that IgG levels in periapical lesions, and especially in chronic granulomatous lesions, are greatly elevated compared with the IgG concentrations of healthy tissue. This evidence additionally supports the concept that tissue destruction seen in the inflammatory apical lesion is mediated by immunologic mechanisms.

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References

1. Grossman, L.I. Bacteriologic status of periapical tissue in 150 cases of infected pulpless teeth. J Dent Res 38:101-104, 1959.

2. Shindell, E. A study of some periapical roentgenolucencies and their significance. Oral Surg 14:1057-1065, 1961.

3. Melville, T.H., and Birch, R.H. Root canal and periapical floras of infected teeth. Oral Surg 23:93-98, 1967.

4. Adamkkiewicz, V.W.; Pekovic, D.D.; and Mascres, C. Allergies of the dental pulp. Oral Surg 46:843-853, 1978.

5. Torabinejad, M., and Bakland, L.K. Immunopathogenesis of chronic periapical lesions. Oral Surg 46:685-699, 1978.

6. Pulver, W.H.; Taubman, M.A.; and Smith, D.J. Immune components in human dental periapical lesions. Arch Oral Biol 23:435-443, 1978.

7. Kuntz, D.D., and others. Localization of immunoglobulins and the third component of complement in dental periapical lesions. J Endod 3(2):68-73, 1977.

8. Naidorf, I.J. Immunoglobulins in periapical granulomas: a preliminary report. J Endod 1(11):15-18, 1975.

9. Gross, A., and others. Immunoglobulin levels in inflamed periodontal tissue. J Dent Res 56(B):90, 1977.

10. Raisz, L.G., and others. Complement dependent stimulation of prostaglandin synthesis and bone resorption. Science 185:789-791, 1974.

11. Torabinejad, M., and Kettering, J.D. Detection of immune complexes in human dental periapical lesions by anticomplement immunofluorescence technique. Oral Surg 48:256-261, 1979.