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Detection of Immunoglobulins from Explant Cultures of Periapical Lesions

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The presence of immunoreactive cells in periapical inflammatory lesions suggests that immune responses participate in the disease process. The purpose of this study was to determine the presence and concentration of immunoglobulins in the supernatant fluids of explant cultures of periapical lesions. Ninety periapical lesions that had been contiguous with the apex of a root were removed and maintained in explant cultures for 96 h. Tissue culture medium was replenished at 24, 48, 72, and 96 h. Double diffusion in agarose assays demonstrated the presence of IgG in 100% of the 24-h supernatant fluids and IgA in 65% of the 24-h supernatant fluids. However, IgM was not detected. Radial immunodiffusion assays were used to detect and quantitate IgG, IgA, and IgM in samples of 24-h supernatant fluids from 90 explant cultures. IgG was the predominant immunoglobulin followed by IgA. A radioimmunosorbent test was used to detect and quantitate IgE in samples of 24-h supernatant fluids from 90 explant cultures. Forty of the 90 supernatant fluids contained measurable IgE. All detected immunoglobulins decreased in concentration in daily supernatant fluids with time (24, 48, 72, and 96 h) in the culture.

Microorganisms have been implicated in infections of endodontic origin. As the pulpal tissue becomes necrotic and loses its blood supply, the root canal system becomes a "privileged sanctuary" for microorganisms, microbial products, and pulpal degradation products. If infected root canals are not treated, periapical inflammatory lesions of endodontic origin will eventually develop. The fundamental association of bacteria with the development of lesions of endodontic origin was demonstrated by Kakehashi et al. (1).

Microscopic examination of periapical lesions reveals the presence of granulation tissue infiltrated with cells associated with an inflammatory reaction. The granulation tissue is infiltrated with lymphocytes, plasma cells, macrophages, polymorphonuclear leukocytes, giant cells, and mast cells (2). The presence of immunoglobulins in periapical lesions has been well demonstrated (3–8). If a microorganism infecting a root canal can stimulate the presence of antibodies in periapical lesions, the antibodies should be reactive with that microorganism. While in vitro models of diseased tissues have been used to investigate periodontal diseases (9–11), the use of an in vitro explant model to assess the host immune response to periapical infections of endodontic origin has received little attention (12). Part I of these studies reports the presence of immunoglobulins in an explant culture model of periapical inflammatory lesions. Part II will describe the explant culture model of periapical inflammatory lesions for in vitro biosynthesis of IgG. Part III deals with the reactivity and specificity of antibodies in supernatant fluids of explant cultures of periapical inflammatory lesions with microorganisms implicated in infections of endodontic origin.

Toller (3) and Skaug (4) demonstrated increased levels of immunoglobulins in odontogenic cysts. Kuntz et al. (5) used an indirect immunofluorescent antibody technique and observed IgG, IgA, and IgM both extracellularly and in plasma cells of periapical inflammatory lesions. Naidorf (6) used single-radial immunodiffusion of fluids to show the presence of IgG, IgA, and IgM in two of three periapical inflammatory lesions. Jones and Lally (12) used immunoelectrophoresis of supernatant fluids from explant cultures of periapical inflammatory lesions from five endodontically treated teeth that failed to heal and demonstrated the presence of ^{14}C-labeled amino acids for IgG and IgA but not IgM.

The purpose of this study was to determine the presence and concentration of immunoglobulins in the supernatant fluids of explant cultures of periapical lesions associated with infected root canals.

MATERIALS AND METHODS

Selection of Teeth with Periapical Inflammatory Lesions

A total of 90 extracted teeth with periapical inflammatory lesions contiguous with the apical foramen was obtained from 87 patients seen in the Emergency and Oral Surgery Clinics at the University of Maryland Dental School. Fifty-three of the patients were male and 34 were females. The patients ranged in age from 14 to 80 with the average age being 39. The teeth consisted of 50 molars, 26 bicuspids, and 14 incisors with periapical lesions ranging in weight from 21 to 403 mg. Only 11 of the teeth were intact without caries or loss of coronal tooth structure. Sixty of the 90 teeth had been associated with pain. A tooth was placed in the lesion-of-endo-

dontic-origin group ($n = 59$) if the periapical inflammatory lesion was located only at the apex of the root. If the lesion extended from the root apex and was contiguous with sulcular tissue, the tooth was placed in the combined endodontal-periodontal lesion category ($n = 31$). Ten of the 11 intact teeth had combined endodontal-periodontal lesions.

Tissue Explant Cultures

Immediately following extraction, the tooth with the attached periapical inflammatory lesion was placed directly into a sterile 50-ml centrifuge tube (Corning, Waxford, PA) containing 20 ml of RPMI 1640 tissue culture medium (Flow Laboratories, Inc., McLean, VA) supplemented with 0.1 mg/ml gentamicin sulfate (Valley Biologics, Inc., State College, PA) plus 5 μg/ml amphotericin B (Sigma Chemical Co., St. Louis, MO) and refrigerated at 4°C. Within 60 min all specimens were taken to a laboratory and placed in a sterile 60- × 15-mm Petri dish (Falcon; Becton Dickinson and Co., Oxnard, CA) containing RPMI 1640 tissue culture medium. A #15 sterile scalpel (Martin Co., Arthur N. Thomas Dist., Philadelphia, PA) was used to remove the soft tissue lesion from the root and then used to remove blood and debris from the tissue. The tissue was blotted on the lid of the Petri dish to remove excess fluid and placed in a tared (Mettler Instrument Corp., Highstown, NJ) Petri dish, and the net weight of the tissue was determined. The tissue was placed on a sterile glass slide and a scalpel was used to divide the tissue into 25- to 35-mg segments. The segments of tissue were macerated with a scalpel to increase surface contact with the tissue culture fluid. Each piece of tissue (25- to 35-mg segments) was then placed into a separate well of a sterile 96-well microtiter plate (Flow Laboratories) to which was added approximately 250 μl per well of enriched RPMI 1640 medium. The explant tissue cultures were then incubated at 37°C in an atmosphere of 5% CO_2 and 100% humidity (Lunaire Enivronmental, Inc., Williamsport, PA). At 24-h intervals, for a period of 4 days, the supernatant fluid from each well of the microtiter plate was aspirated using a 21-gauge needle on a 1-ml tuberculin syringe (Becton Dickinson & Co., Rutherford, NJ) and replenished with fresh enriched RPMI 1640 medium. The aspirated fluid from each specimen for each day was pooled in separate 12- × 75-mm culture tubes (Falcon) and immediately frozen at −20°C.

Cytological Imprints

Twenty-five glass slides on which the tissue had been macerated were immediately sprayed with Cytology Fixative (Surgipath, Grayslake, IL) and air dried. The slides were later stained with hematoxylin and eosin to produce a cytological imprint (touch preparation) of the cells in the lesion.

Double Diffusion in Agarose Assay

Supernatant fluids collected daily (24, 48, 72, and 96 h) from 20 explant cultures of periapical lesions were allowed to react with goat anti-human γ-, α-, or μ-chain serum in a double diffusion in agarose assay. The immunodiffusion agarose slides were made by gently boiling 1 g of agarose (BBL, Cockeysville, MD) in a solution that included: 95 ml of phosphate-buffered saline (PBS), 5 ml of borate buffer containing NaCl, and 1 ml of 1% merthiolate. The agarose solution was then pipetted (5 ml per slide) onto 3- × 1-inch, 1.2-mm-thick microscope slides (American Scientific Products, McGraw Park, IL). The agarose was allowed to gel for 30 min at room temperature and then allowed to mature overnight at 4°C in covered Petri dish humidors. A 2-mm in diameter Gelman punch (Gelman Instrument Co., Ann Arbor, MI) was used to make six peripheral wells each 6 mm from central well in the agarose gels. Fifteen microliters of undiluted IgG fraction of either goat antihuman γ, α, or μ chain serum (Pel Freeze, Rogers, AK) was added to the center well. Fifteen microliters of undiluted supernatant fluids from each of the four 24-h periods was added to sequential wells around the periphery. The slides were then incubated for 24 h at room temperature in Petri dish humidors followed by further incubation at 4°C. RPMI 1640 medium and human serum containing IgG, IgA, and IgM were used as controls. The double diffusion in agarose gels were observed for the development of lines of precipitation between the center well and the peripheral wells after 24, 48, and 72 h using an illuminated magnified viewer (Cordis Corp., Miami, FL). After 72 h of incubation, the slides were immersed in several changes of PBS at room temperature over a 24-h period to remove nonprecipitated proteins. To remove the PBS, the slides were then immersed in several changes of distilled water at room temperature over a 24-h period. To dry the slides, filter paper (Will, Baltimore, MD) was cut into 3- × 1-inch strips, moistened with distilled water and laid on top of the agarose. The slides were dried overnight at 37°C in an incubator (Elconap Co., Newark, NJ) and stained by immersion in a solution of Amido Black for 10 min. The slides were rinsed five times (10 min each rinse) in a fresh acetic acid-methanol solution to remove excess stain and then allowed to air dry. After the staining procedure, the double diffusion in agarose gels were again observed for the presence of lines of precipitation between the center well and the peripheral wells.

Radial Immunodiffusion

In this study, AccraAssay (ICN ImmunoBiologicals, Lisle, IL) kits for low level IgG, IgA, and IgM were used to assay the concentration of immunoglobulins in the supernatant fluids from the explant cultures of periapical lesions. Each kit contains four standards (human IgG, IgA, or IgM) each of low-level reference serum prepared from a pool of normal human sera and calibrated in mg/dl against standards obtained from the World Health Organization. A micropipette (2 to 10 μl; Brinkman) was used to place 10 μl (in duplicate) of each of the four standards and RPMI 1640 tissue culture medium in to wells of the AccraAssay plates appropriate to assay for IgG, IgA, or IgM. Ten microliters of each of the 90 (24-h) supernatant fluids was also placed into wells of the appropriate plates to assay each of the supernatant fluids for IgG, IgA, or IgM. Based on the results of the RID assays, the 10 supernatant fluids with the greatest quantity of IgG and the 10 supernatant fluids with the greatest quantity of IgA were assayed again using 10 μl from each of the daily (24, 48, 72, and 96 h) supernatant fluids. The RID plates were incu-

bated at 26°C for 80 h using the end point method. The diameters of the precipitation rings were then measured using a sidelight (Background Light AutoAssay; Giles Scientific, New York, NY) and a digital micrometer (Giles Scientific). The average of two readings was squared and the quantity of each immunoglobulin (IgG, IGM, or IgA) for each supernatant fluid was determined by using regression analysis from the calibration curves produced for each immunoglobulin. Calibration curves were produced by plotting the values for the reference standards in mg/dl versus the squares of the diameters of the precipitation rings for IgG, IgA, or IgM.

Radioimmunosorbent Test to Detect and Quantitate IgE

Because serum IgE is usually present in serum and body fluids in nanogram-per ml quantities, radioimmunoassays have been devised to detect IgE. The assay used in this study is a solid phase direct (noncompetitive) radioimmunosorbent test (RIST). In this study, supernatant fluids (24 h) from 90 explant cultures of periapical lesions were surveyed for IgE using the Phadebas IgE PRIST 180 test kit (Pharmacia Diagnostics, Piscataway, NJ). This assay uses paper discs with sheep anti-IgE covalently coupled to it as the solid phase. One anti-IgE paper disc is added to the bottom of each 12- × 75-mm polypropylene tube (Abbott, Chicago, IL). Seven standard solutions (in duplicate) of IgE (0.5, 1.0, 2.5, 7.5, 20, 50, and 100 kilounits/l) were used to prepare a standard curve. One-hundred microliters of each standard solution of IgE, 90 (24-h) supernatant fluids, and RPMI 1640 were pipetted onto the discs in the tubes. The tubes were covered with plastic film (Parafilm "M"; American Can, Greenwich, CT) and incubated at 26°C for 3 h. Following incubation, each tube was washed three times with 2.5 ml of 0.9% saline, which contained one vial of Phadebas washing solution per liter of saline. After the final wash solution was aspirated from the tubes, the tubes received 100 μl of ^{125}I-labeled rabbit anti-IgE serum. The tubes were then covered with plastic film and incubated overnight at 26°C. All the tubes except for two used to measure the total count were again washed three times with the above washing solution. The tubes were then capped and assayed for ^{125}I-labeled IgE using a 5300 Auto-Gamma RIA System (Packard Instrument Co., Downers Grove, IL).

A second experiment was conducted using the five supernatant fluids, which gave the highest concentrations of IgE in the experiment described above. In this experiment the concentration of IgE was determined for each daily (24, 48, 72, and 96 h) supernatant fluid.

RESULTS

Microscopic examination of 25 cytological imprints made from the periapical inflammatory lesions in this study demonstrated the presence of lymphocytes and plasma cells in all lesions.

When the daily supernatant fluids (24–96 h) from 20 specimens (nos. 51 to 70) were allowed to react with goat anti-human γ-chain serum by double diffusion in agarose, lines of precipitation were observed 100% of the time with only the 24-h supernatant fluids (Fig. 1). The percentage of supernatant fluids producing lines of precipitation then decreased with each day to 10% for the 96-h specimen (Fig. 2). When the supernatant fluids were allowed to react with goat anti-human α-chain serum, lines of precipitation were observed only with supernatant fluids harvested at 24 h (65%) and 48 h (20%) (Fig. 2). No line of precipitation could be observed when the daily supernatant fluids were allowed to react with goat anti-human μ-chain serum.

Studies using RID were undertaken to determine the levels of immunoglobulins in the supernatant fluids from the explant cultures of 90 periapical lesions. RPMI 1640 by itself did not produce a detectable precipitation ring with any of the RID assays and so was considered a negative control. Calibration standards made from human sera, which all produced precipitation rings were the positive controls. Ten of the 90 supernatant fluids assayed did not produce a detectable precipitation ring with the low-level IgG RID plates. Of the 80 supernatant fluids with a detectable precipitation ring, 20 were within the assay range of the standards (80 to 480 mg/dl) in the kit. The highest concentration of IgG was 335 mg/dl. The average concentration of the 20 samples within the

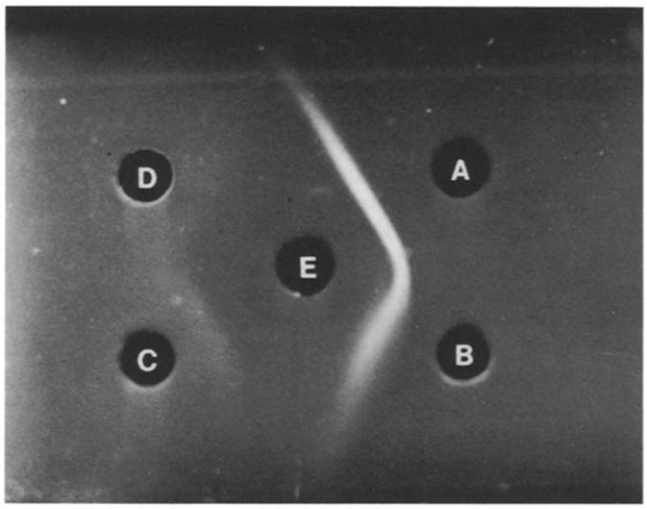

FIG 1. Photomicrograph showing lines of precipitation between wells in agarose when daily supernatant fluids (24 to 96 h) were allowed to react with goat anti-human γ-chain serum by double diffusion. *A*, 24-h supernatant fluid; *B*, 48-h supernatant fluid; *C*, 72-h supernatant fluid; *D*, 96-h supernatant fluid; and *E*, goat anti-human γ-chain serum.

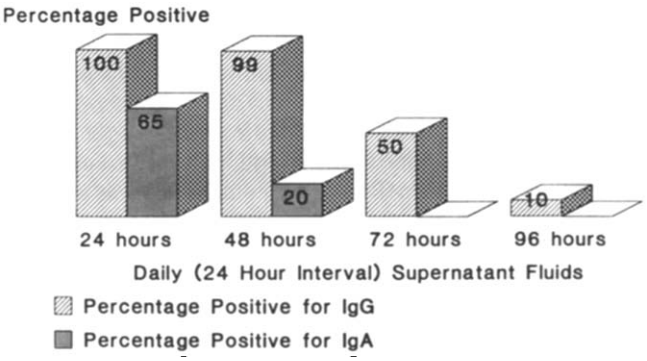

FIG 2. This graph shows the results of double diffusion in agarose of supernatant fluids collected daily (24, 48, 72, and 96 h) from 20 explant cultures of periapical lesions that were allowed to react with goat anti-human IgG (γ-chain) serum or with goat anti-human IgA (α-chain) serum.

assay range was 150.9 mg/dl of IgG. The remaining 60 supernatant fluids produced detectable precipitation rings but they were below the accuracy range of the kit. No significant relationships were found between the amount of IgG detected in the 24-h supernatant fluids and whether the patient had pain. However, a significant (analysis of variance: $F = 2.47$, $p = 0.02$) relationship was found between the level of IgG detected in the 24-h supernatant fluids and the weight of the periapical lesions used in the explant cultures. As the weight of the lesion increased so did the level of IgG.

Of 90 supernatant fluids assayed, only 16 supernatant fluids produced a detectable precipitation ring with low-level IgA RID plates. All 16 specimens were below the accuracy range of the IgA RID kit (14 to 100 mg/dl). None of the supernatant fluids produced a detectable precipitation ring with low level IgM RID plates.

The 10 supernatant fluids with the greatest quantity of IgG (24 h) and the 10 supernatant fluids with the greatest quantity of IgA (24 h) were assayed for both IgG and IgA in each daily supernatant fluid (24, 48, 72, and 96 h). The detection of IgG as evidenced by the presence of a precipitation ring was observed in 20 of 20 of the 24-h and 48-h samples of supernatant fluids, 11 of 20 in the 72-h samples of supernatant fluids, and 1 of 20 for the 96-h samples of supernatant fluids. The detection of IgA as evidenced by the presence of a precipitation ring was determined in 16 of 20 of the 24-h samples of supernatant fluids, 5 of 20 of the 48-h samples of supernatant fluids, and in none of the 72-h and 96-h samples of supernatant fluids. The amount of IgG detected in each successive daily (24, 48, 72, and 96 h) supernatant fluid from each specimen always decreased or else fell below the lower level of detection. Using the Wilcoxon signed rank test, the daily decrease in amount of IgG detected was significant ($p = 0.002$).

A RIST was used to detect and quantitate IgE in the supernatant fluids of 90 explant cultures of periapical lesions. A calibration curve using linear-log paper was constructed by plotting the percentage values obtained for the IgE standards (bound fraction/total count) against the IgE concentrations. By using the calibration curve, the concentration of IgE in kilounits/l for each of the 90 supernatant fluids from 24-h explant cultures of periapical lesions was determined. Controls in this experiment included the seven standardized serum samples with different concentrations of IgE (0.5 to 100 kilounits/l) and RPMI 1640. Forty of the 90 samples fell within the limits of accuracy of the assay (0.5 to 100.0 kilounits/l). The other 50 samples had concentrations of IgE too low for an accurate determination. With the exception of the supernatant fluid from specimen 70, the concentrations of IgE as calculated from the calibration curve ranged from 0.3 to 5.2 kilounits/l. Specimen 70 had an IgE concentration of 36.8 kilounits/l. RPMI 1640 medium, which was assayed below the range of accuracy for the RIST used in this experiment, was considered the negative control. No significant relationship was determined between the presence of IgE in the 40 specimens that had a concentration of IgE in the accuracy range and the weight of the periapical lesion, the presence of endodontic disease or endodontal-periodontal disease, or whether the patient was having pain.

An additional experiment was undertaken to determine the concentrations of IgE in daily (24, 48, 72, and 96 h) supernatant fluids from the five supernatant fluids with the highest concentration of IgE in their 24-h supernatant fluids. Controls in this experiment included the seven standardized serum samples with different concentrations of IgE (0.5 to 100 kilounits/l) and RPMI 1640. RPMI 1640 medium, which was assayed below the range of accuracy for the RIST used in this experiment, was considered the negative control. Only 4 of 20 samples assayed in this experiment were below the range of accuracy (Table 1). Similar to previous results using RID with IgG and IgA, the concentration of IgE in each successive 24-h sample from each specimen consistently decreased.

DISCUSSION

Zmener and Dominguez (13) recently showed that histological sections of periapical lesions had a cell population similar to that seen in cytological imprints but with the addition of the connective tissue stroma. This correlation suggests that cytological imprints may be used for rapid evaluation of the cellular constituents of periapical inflammatory lesions and used in investigations where tissue sections cannot be microscopically evaluated. Microscopic evaluation of the 25 cytological imprints of periapical inflammatory lesions used in the explant cultures in this study demonstrated the presence of lymphocytes and plasma cells in all of the lesions.

When the daily supernatant fluids of 20 explant cultures of periapical inflammatory lesions were allowed to react with goat anti-human γ-chain, α-chain, or μ-chain serum in a double diffusion in agarose assay, IgG was the predominant immunoglobulin detected. Because the release of immunogens from infected root canal systems into periapical tissue is a chronic event, the predominance of IgG would seem to be consistent with the presence of a chronic infection. IgA was detected in 65% of the day 1 supernatant fluids while IgM was not detected in any supernatant fluid. IgM has been

TABLE 1. Concentration* of IgE in five daily (24, 48, 72, and 96 h) supernatant fluids from explant cultures of periapical lesions

Specimen No. (h)	Concentration
42–24	5.1
42–48	2.8
42–72	0.4†
42–96	0.3†
50–24	3.6
50–48	2.9
50–72	1.0
50–96	0.3†
70–24	34.0
70–48	16.5
70–72	2.9
70–96	1.2
93–24	3.8
93–48	3.4
93–72	1.3
93–96	0.4†
98–24	7.6
98–48	2.4
98–72	1.2
98–96	0.7
RPMI 1640	0.3†

* The concentration of IgE is given in kU/l. One (1.0) unit of IgE = 2.4 ng of IgE.
† Below the limits of accuracy for this assay.

demonstrated in the fluids from periapical inflammatory lesions (5, 6); however, in this study IgM could not be demonstrated in the supernatant fluids of explant cultures. Although it is possible that a small amount of IgM may be present in periapical inflammatory lesion fluids, either the amount of IgM in serum transudate or the amount of IgM being synthesized in explant cultures was so diluted with tissue culture medium that IgM could not be detected using a double diffusion in agarose assay. Our results are consistent with the work of Jones and Lally (12). They used immunoelectrophoresis and autoradiography to demonstrate the incorporation of ^{14}C-labeled amino acids into IgG and IgA but not into IgM in supernatant fluids from explant cultures of periapical inflammatory lesions which failed to heal following root canal therapy.

When low-level RID kits were used to quantitate IgG in the group of 90 supernatant fluids, 80 produced a detectable precipitation ring and 20 were within the assay range of the standards used in the kit. No significant relationship was found between the amount of IgG detected in the 24-h supernatant fluids and whether the patient had pain. A significant (analysis of variance: $F = 2.47$, $p = 0.02$) relationship was found between the level of IgG detected in the 24-h supernatant fluids and the weight of the periapical lesions used in the explant cultures. This suggests that the larger lesions are either more immunocompetent or tend to be associated with irritants that are of greater immunogenicity.

Of the 90 supernatant fluids, only 16 produced a detectable precipitation ring with low-level IgA RID plates. All 16 were below the accuracy range of the kit. None of the 90 supernatant fluids produced a detectable precipitation ring with low-level IgM RID plates.

In another experiment, explant cultures from which the 10 supernatant fluids with the greatest quantity of IgG (24 h) and the 10 supernatant fluids with the greatest quantity of IgA (24 h) were evaluated for IgG and IgA in each of the daily supernatant fluids (24, 48, 72, and 96 h). The results demonstrated that the diameter of the precipitation rings for both IgG and IgA always decreased with each additional 24-h period. This would suggest that the quantity of these immunoglobulins in periapical lesions was either diluted each 24-h period when the tissue culture medium was replenished or that the amount of biosynthesis of immunoglobulins decreased each 24 h. It is believed that biosynthesis would decrease with time in explant tissue culture because plasma cells are an end cell with an undefined survival time in explant cultures. The variation in the quantity of IgG in the supernatant fluid from day to day suggests that biosynthesis was taking place and that the decrease in IgG was not just a matter of diluting the immunoglobulin present in the lesion.

IgA was detected within the accuracy range of the assay in 15 of 20 of the 24-h samples and in only 5 of 20 of the 48-h samples. IgA was not detected in the 72- and the 96-h samples. The predominance of IgG over IgA and IgM in periapical lesions has been demonstrated in other studies (7, 8, 14). In this study, it is possible that the use of extracted teeth with attached periapical lesions may have selected for periapical granulomas rather than the more friable apical cysts.

Several investigations have examined the possible relationship between IgE and periapical lesions (15–17). Although the presence of IgE in periapical lesions has been well established, its role in pathogenic effects is unclear. Prior to this study, no studies had examined explant cultures of periapical lesions for the presence of IgE. When a solid phase direct RIST using ^{125}I was used in this study to detect and quantitate the IgE in 90 (24-h) supernatant fluids, the results indicated the presence of enough IgE in 40 of the 90 specimens to be within the accuracy range (0.5 to 100.0 kilounits/l) of the test kit. It would seem that either biosynthesis of IgE is taking place in the lesions or serum IgE is present locally in periapical lesions. The samples falling in the accuracy range of the assay ranged from 0.5 to 5.2 kilounits/l, with the exception of a sample of supernatant fluid from specimen 70, which had a detectable concentration of 36.8 kilounits/l in the 24-h sample. Why the concentration in this sample was so high is unknown. Although it may well be coincidental, it is interesting that this tooth with its accompanying lesion was extracted because of pain. It is possible that a local type I hypersensitivity reaction was involved in the production of pain. However, it is also possible that the patient was infected with a parasite or suffered from allergies which raised the tissue level of IgE.

Twenty-eight of the day 1 (24-h) samples from the 40 supernatant fluids with measurable IgE were associated with teeth extracted because of pain. However, 31 of the day 1 (24-h) samples from 50 supernatant fluids without measurable IgE were associated with teeth extracted because of pain. There was not a significant relationship between the presence of measurable IgE in the supernatant fluids from explant cultures of periapical lesions and the presence of pain. Twenty-one of the day 1 (24-h) samples from the 40 supernatant fluids with measurable IgE were associated with teeth having combined endodontal-periodontal lesions and 19 were associated with teeth having only an endodontic lesion. There was no significant difference in the level of IgE in supernatant fluids from either endodontic or endodontal-periodontal lesions. There was also no significant difference in the level of IgE when it was related to the weight of the lesions.

When the concentration in kilounits/l of IgE in daily (24, 48, 72, and 96 h) supernatant fluids from the five explant cultures with the highest 24-h levels of IgE was determined, the concentration of IgE in the supernatant fluids decreased each day similar to the RID results for IgG and IgA. Concentrations of IgE were still present in the accuracy range of the RIA in supernatant fluids from specimens 70 and 98 through the 96-h sample. Because the decrease from day to day is not consistently uniform, it suggests that local production of IgE may be taking place in the explant cultures of these periapical lesions and not just because of dilution of serum IgE present in the specimen. Whether the presence of IgE in periapical lesions is related to the clinical signs and symptoms of either acute or chronic lesions is debatable. Svetcov et al. (17) related patients having endodontic flare-ups to a several-fold rise in serum IgE. Nevins et al. (16) showed a significant difference in serum IgE between an experimental group with periapical lesions and a control group without endodontic disease or a history of allergies. The results of the latter study differ from a more recent study by Kettering and Torabinejad (15). They found no difference in serum level between groups similar to those used by Nevins et al. (16), but they did find a significantly higher serum level of IgE in patients with known allergies. Based on the latter study, it would seem that chronic periapical lesions do not elevate the levels of circulating IgE. More work is needed to determine if a type I hypersensitivity is involved in periapical lesions.

The informed consent of all human subjects who participated in the experimental investigation reported or described in this manuscript was obtained after the nature of the procedure and possible discomforts and risks had been fully explained.

The opinions and assertions herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

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