

Influence of surgical treatment of periapical lesions on serum and blood levels of inflammatory mediators

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Summary

Changes in the serum levels of immunoglobulin IgA, IgG, IgM, positive acute phase proteins and complement activity, as well as the lymphocyte subpopulations and the neutrophil leucocyte-related chemiluminescence in the blood of patients with apical granuloma as related to endodontic and surgical treatment were investigated. Measurements were performed on admission, and 7 days and 3 months after the treatment. Elevated IgM concentration, positive acute-phase protein levels and spontaneous whole-blood chemiluminescence were noted at admission. However, a significant decrease in the serum level of each of the six investigated acute-phase proteins, and in the spontaneous chemiluminescence of blood was observed during the 3-month follow-up period. The significant increase in serum complement activity following therapy suggests that complement fixation might have occurred in these patients. A significant increase in the ratio of early sheep erythrocyte rosette-forming lymphocytes was also observed. The results of this study provide evidence for complete recovery after elimination of local inflammation by proper endodontic treatment and apicectomy in patients with apical granuloma.

Keywords: apical surgery, inflammatory mediators, periapical periodontitis.

Introduction

Periapical disease of the teeth usually originates from pulpitis due to progression of dental caries. Chronic apical periodontitis or apical granuloma represents a local tissue injury with well-defined signs of chronic inflammation (Bergenholtz *et al.* 1983, Nilsen *et al.* 1984, Skaug *et al.* 1984). Although the primary aetiological agents of pulpal-periapical diseases are micro-organisms, it has been shown that most apical granulomas are sterile and bacteria are present only in

the necrotic area of the lesion and at the outer surface of the root (Block *et al.* 1976, Langeland *et al.* 1977). Recently, immunological reactions have been suggested as a possible mechanism in the mediation of periapical inflammation (Morse 1977, Torabinejad & Bakland 1978, Nair & Schroeder 1983). However, immune reactions are not restricted to the periapical tissues. Antigens in the root canal and in the periapical area were shown to induce systemic immunological reactions (Barnes & Langeland 1966, Okada *et al.* 1967). Moreover, potent soluble mediator substances, such as cytokines, prostaglandins and leucotrienes, can be produced by activated lymphocytes and macrophages at the site of local inflammation, inducing significant changes in remote biological systems of the body.

The aim of the present study was to investigate immunological and inflammatory changes in serum and peripheral blood of patients with periapical periodontitis related to the endodontic treatment of the affected tooth and to the removal of the periapical lesion.

Materials and methods

Patients

A total of 14 male and 22 female patients, of mean age 28.1 and 25.2 years, respectively, were recruited into the study. The patients were referred to the Stomatologic Clinic of the Medical University of Debrecen and, apart from their periapical disorder, all subjects were healthy. The lesions were at least 3 mm in diameter on periapical radiographs. The teeth were root filled and then apicected. The root canals were obturated with AH 26 and gutta-percha. Teeth were apicected because of extreme curvature in the apical third of the canal which did not allow complete instrumentation and filling procedures, or because the resolution of the large lesion did not occur sufficiently rapidly following root canal therapy (Barnes 1981, Frank *et al.* 1983). The surgical technique suggested by Barnes (1981) was used. Diagnosis was

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confirmed by histological evaluation of the lesions that had been excised.

Blood and serum sampling

Blood was drawn from the antecubital vein with the informed consent of the patients. Sera were obtained after complete clotting of the blood. For mononuclear cell separation and chemiluminescence measurements, clotting was inhibited by adding 10 U ml⁻¹ heparin. Mononuclear cells were isolated by sedimentation using a Ficoll-Isopaque (Lymphoprep) (Nycomed AS, Oslo, Norway) gradient as described by Boyum (1968).

Kinetic nephelometry measurements

Serum concentrations of immunoglobulin IgA, IgG and IgM, as well as of acute-phase proteins alpha-1-antitrypsin (AAT), alpha-2-macroglobulin (AMG), C-reactive protein (CRP), third component of the complement system (C3) and haptoglobin (HPT), were measured by rate nephelometry in the Beckman Immunochemistry System-II. All dilutions and solutions of nephelometry were prepared using a Beckman dilutor and dispenser. Antisera, calibration mixtures, buffers and solutions of Beckman Inc. were used (Beckman Instructions 1978).

Determination of ceruloplasmin (CER) oxidase activity

CER oxidase activity, which is related to the serum concentration of the acute-phase protein CER, was measured by a manual kinetic method (Boyett *et al.* 1976). Briefly, 50 µl of serum were incubated with 950 µl of o-dianisidine substrate (2.04 µmol l⁻¹ o-dianisidine (Fluka, Ronkonkoma, NY, USA) in 0.1 mol l⁻¹ sodium acetate buffer, pH 5.0) at 30°C for 5 min. Changes in optical density with time were measured at 435 nm. The results were expressed in International Units (IU), representing µmol of substrate consumed min⁻¹.

Determination of serum complement activity

Serum complement activity was determined according to the classical haemolytic method of Meyer (Lachmann & Hobart 1978). Complement activity was expressed in haemolytic units (HU).

Determination of T-lymphocyte subpopulations by the E-rosette technique

T-lymphocytes of mononuclear cell suspensions were determined by the sheep erythrocyte rosette-forming cell (E-rosette) method (Stites 1976). Early E-rosettes, representing 'active' T-lymphocytes, were counted after incubation for 1 h. Late E-rosettes, representing the total number of T-lymphocytes, were counted after overnight incubation at 4°C in a 0.5% sheep erythrocyte suspension in phosphate-buffered saline solution (PBS) (GIBCO BRL, Gaithersburg, MD, USA) at pH 7.4.

Determination of lymphocyte subpopulations by immunofluorescence

T-lymphocytes were labelled by an indirect immunofluorescent method using anti-human, mouse monoclonal antibodies: anti-Leu-1 (CD5[pan-T]-marker) (Becton-Dickinson, Mountain View, CA, USA) T4 (CD4[helper/inducer]-marker) (Coulter Diagnostics, Hialeah, FL, USA), and T8 (CD8[suppressor/cytotoxic]-marker) (Coulter Diagnostics, Hialeah, FL, USA) and FITC-conjugated goat anti-mouse IgG⁺ as the second-step reagent. Labelled cell suspensions were evaluated using a fluorescence-activated cell sorter (FACS) (Coulter Diagnostics, Hialeah, FL, USA). Immunoglobulin isotype identical, non-reactive mouse monoclonal antibodies were used to determine background fluorescence. B-lymphocytes were stained by a direct immunofluorescent method using a FITC-labelled polyclonal mouse anti-human immunoglobulin Fab₂ serum ('B-cell marker') (Ortho Pharmaceutical, Raritan, NJ, USA). Fluorescence was evaluated using a FACS.

Measurement of neutrophil leucocyte-dependent chemiluminescence in whole blood

Spontaneous chemiluminescence (S) of diluted whole blood was measured in a Nuclear-Chicago ISOCAP 300 liquid scintillation counter at room temperature after adding 10⁻⁴ mol l⁻¹ Luminol (Sigma Chemical Co, St Louis, MO, USA) as amplifier to the suspension. In each case, parallel samples containing 1 mg Mannozyim (Human, Godollo, Hungary), which stimulates chemiluminescence further, were also measured. Values for derived counts per min (dpm) were expressed per 10⁶ neutrophil leucocytes.

Table 1. Immunological and inflammatory changes in serum of patients with apical periodontitis related to treatment

Protein	Reference value	Sampling time		
		Before treatment	7 days after treatment	3 months after treatment
AAT (g l ⁻¹)	1.1–2.9	3.0 ± 1.0 ^{ab}	2.3 ± 1.0 ^a	2.1 ± 0.7 ^b
AMG (g l ⁻¹)	1.0–2.8	2.8 ± 1.0 ^{cd}	1.8 ± 1.1 ^c	1.8 ± 0.7 ^d
C3 (g l ⁻¹)	0.8–1.8	1.7 ± 0.4 ^e	1.5 ± 0.4	1.4 ± 0.4 ^e
CER (IU l ⁻¹)	80–140	80 ± 30 ^{af}	140 ± 50 ^f	140 ± 30 ^g
CRP (mg l ⁻¹)	> 5.0	6.6 ± 4.2 ^h	6.4 ± 6.7	3.9 ± 1.8 ^h
HPT (g l ⁻¹)	0.5–2.2	1.0 ± 0.3 ⁱ	0.9 ± 0.4	0.7 ± 0.4
IgA (g l ⁻¹)	1.2–6.7	2.1 ± 0.6	2.2 ± 0.6	2.2 ± 0.6
IgG (g l ⁻¹)	6.4 ± 17.8	12.3 ± 3.0	11.9 ± 2.7	11.8 ± 3.0
IgM (g l ⁻¹)	0.4–1.5	1.9 ± 0.9	1.9 ± 0.8	2.1 ± 0.7

*Statistically significant differences between the corresponding groups ($P < 0.05$).

Statistical evaluation of the data

Mean values ± SD for each investigated parameter were calculated at each sampling time, i.e. before any treatment, and 7 days and 3 months after endodontic therapy and surgical removal of the granuloma. Statistically significant differences were determined using Student's *t*-test, after checking the normal distribution of the data with Geary's test and comparing SD values using the *F*-test.

Results

Evaluation of serum proteins

Serum levels of nine proteins, three immunoglobulins and six positive acute-phase compounds were determined during the 3-month study period. The results are summarized in Table 1. Mean serum levels of AAT, AMG, CRP, C3, CER and IgM were slightly elevated or at the upper limit of the normal range on admission of patients, compared to the reference values. Serum levels of AAT, AMG and CER decreased significantly 7 days after treatment. The concentrations of the other proteins investigated did not change significantly at this sampling time. Each acute-phase protein investigated showed a significant decrease in serum level 3 months after treatment, while serum concentrations of immunoglobulins remained unchanged.

Evaluation of serum complement activity

A significant increase in serum complement activity was observed 3 months after treatment of the patients,

Table 2. Change in complement activity in serum of patients with apical periodontitis related to treatment

Reference value	Sampling time		
	Before treatment	7 days after treatment	3 months after treatment
20–40	20.55 ± 6.64*	19.44 ± 7.43	25.27 ± 8.65*

All values are expressed in haemolytic units (HU).

*Statistically significant differences ($P < 0.05$).

Table 3. Percentage changes in lymphocyte subpopulations in mononuclear cell suspension of patients with apical periodontitis related to treatment

Lymphocyte subpopulation	Sampling time		
	Before treatment	7 days after treatment	3 months after treatment
Late E-rosettes	52.69 ± 7.71	50.05 ± 8.43	49.52 ± 7.02
Early E-rosettes	9.38 ± 5.46*	8.02 ± 4.58	12.19 ± 4.57*
CD5 positive T-cells	75 ± 12	ND†	ND
CD4 positive T-cells	50 ± 10	ND	ND
CD8 positive T-cells	30 ± 10	ND	ND
Smlg positive B-cells	13 ± 8	ND	ND

*Statistically significant difference ($P < 0.05$).

†Not determined.

but values remained within the normal range at each sampling time (Table 2).

Evaluation of lymphocyte subpopulations

The ratio of total T-lymphocytes as determined by either the E-rosette method or immunofluorescent labelling with anti-Leu-1 as well as the ratio of helper/inducer-, cytotoxic/suppressor T-lymphocyte subpopulations and B-lymphocytes before treatment of patients was similar to that found in mononuclear cell suspensions separated from healthy control subjects. Therefore, expensive immunofluorescent investigations were omitted at subsequent sampling times. However, the ratio of early E-rosettes increased significantly 3 months after treatment, although this value also remained within the normal range. The ratio of late E-rosettes did not change during the 3-month follow-up period. The results are summarized in Table 3.

Table 4. Changes in spontaneous (S) and Mannozyim-induced (M) chemiluminescence in whole blood of patients with apical periodontitis related to treatment

	Controls	Sampling time		
		Before treatment	7 days after treatment	3 months after treatment
Values are expressed in dpm (derived counts per minute $\times 10^{-3}$).	S 26.41 \pm 15.76 ^a	61.79 \pm 39.6 ^{a,b}	46.94 \pm 26.50 ^a	25.59 \pm 11.75 ^a
*-Statistically significant differences between the corresponding groups ($P < 0.05$).	M 89.80 \pm 19.56	122.43 \pm 62.68	96.97 \pm 36.17	80.85 \pm 13.50

Evaluation of whole-blood chemiluminescence

Spontaneous chemiluminescence (S) was significantly higher in patients before any treatment than in normal control subjects. This elevated S decreased significantly during the follow-up period. Chemiluminescence could be further stimulated by Mannozyim at each sampling time (M). The results are shown in Table 4.

Discussion

The results of this study represent the first longitudinal investigation of various serum and blood parameters of patients with chronic apical periodontitis as related to root canal and surgical treatment. The observations described here demonstrate the significance of periapical periodontitis, i.e. a localized chronic inflammatory lesion, in inducing a disturbance in the immunological and inflammatory system of the host. The elevated serum IgM concentration and the significant increase in the ratio of early ('active') E-rosettes after proper treatment indicate the effect of local immunoreactions taking place at the periapical lesion on both systemic humoral and cellular immunity. The latter finding may be of particular interest in view of the fact that about 10% of the total T-lymphocytes of the body recirculate through the periapical lesion without being involved in local reactions (Skaug *et al.* 1984). Similarly, the complement system, a major component of the body's non-specific defence mechanism, was also affected: a significant increase in serum complement activity could have been shown during the 3-month period, suggesting that complement fixation in untreated periapical periodontitis is of biological importance.

Acute-phase reaction is a well-known consequence of various tissue injuries due to the release of interleukin(IL)-1 and IL-6. Serum levels of five out of six positive acute-phase proteins were slightly elevated in patients with untreated apical granuloma. Three out of six proteins showed a significant decrease in serum level

7 days after treatment, and all six showed such a decrease 3 months after combined root canal and surgical treatment. The change was most apparent in the case of C-reactive protein (CRP), with a 41% decrease after 3 months of therapy, compared to the pretreatment serum concentration. In addition to their indicator function, acute-phase proteins are assumed to participate in the process of inflammation and tissue repair.

The results of whole-blood chemiluminescence measurements suggest that neutrophil leucocytes may also be involved in the disease mechanism in periapical periodontitis. Spontaneous chemiluminescence (S) was significantly higher in untreated patients than in normal control subjects, providing *in-vivo* evidence of a metabolically and functionally activated state of neutrophils in these patients. Such an activated state can be related to continuous release of antigens, complement fragments, immunocomplexes and cytokines from the periapical lesion (Sipka *et al.* 1987, Vadas *et al.* 1987).

The elevated S decreased significantly following appropriate treatment. Chemiluminescence could have been further stimulated by Mannozyim (M) at each sampling time, if the possibility of an intrinsic granulocyte defect in this disease is excluded (Sipka *et al.* 1986).

Conclusions

These results show that patients with periapical periodontitis undergo complete recovery after appropriate root canal treatment and apicectomy. Moreover, the study design provides a useful model for investigating interactions between a chronic local stomatological inflammation and the immunological and inflammatory homeostasis of the host related to root canal and surgical treatment.

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References

- BARNES G.W. & LANGELAND K. (1966) Antibody formation in primates following introduction of antigens into the root canal. *Journal of Dental Research*, **45**, 1111-1114.
- BARNES I.E. (1981) Indications for apicectomy. Operative technique. In: *Surgical Endodontics. A Colour Manual* (ed., I.E. Barnes), pp. 15-25. MTP Press, Lancaster.
- BECKMAN INSTRUCTIONS NO. 15-555624-A (1978) Beckman Instruments, Inc., Fullerton, CA.
- BERGENHOLTZ G., LEKHOLM U., LILJENBERG B. & LINDHE J. (1983) Morphometric analysis of chronic inflammatory periapical lesions in root-filled teeth. *Oral Surgery, Oral Medicine and Oral Pathology*, **55**, 295-301.
- BLOCK R.M., BUSHELL A., RODRIGUES H. & LANGELAND K. (1976) A histopathologic, histobacteriologic and radiographic study of periapical endodontic surgery specimens. *Oral Surgery, Oral Medicine and Oral Pathology*, **42**, 656-678.
- BOYETT J.D., LEHMANN H.P. & BEELER M.F. (1976) Automated assay of ceruloplasmin by kinetic analysis of o-Dianisidine oxidation. *Clinica Chimica Acta*, **69**, 233-241.
- BOYUM A. (1968) Separation of leukocytes from blood and bone marrow. *Introduction. Scandinavian Journal of Clinical Laboratory Investigation*, **21**, (Suppl. 97), 7.
- FRANK A.L., SIMON J.H.S., ABOU-RASS M.G. & GLICK D.H. (1983) Surgical Concepts. In: *Clinical and Surgical Endodontics. Concepts in Practice* (ed. L.A. Biello), p. 95. Lippincott, Philadelphia.
- LACHMANN P.J. & HOBART M.J. (1978) Complement technology. In: *Handbook of Experimental Immunology*, 3rd edn (ed. D.M. Weir), pp. 5A. 1-5A. 23. Blackwell Scientific Publications, Oxford.
- LANGELAND K., BLOCK R.M. & GROSSMANN L.I. (1977) A histopathologic and histobacteriologic study of 35 periapical endodontic surgical specimens. *Journal of Endodontics*, **3**, 8-23.
- MORSE D.R. (1977) Immunologic aspects of pulpal-periapical diseases. A review. *Oral Surgery, Oral Medicine and Oral Pathology*, **43**, 436-451.
- NAIR P.N.R. & SCHROEDER H.E. (1983) Pathogenese periapikaler Läsionen (eine Literaturübersicht). *Schweizerische Monatsschrift für Zahnheilkunde*, **93**, 935-952.
- NILSEN R., JOHANNESSEN A.C., SKAUG N. & MATRE R. (1984) *In situ* characterization of mononuclear cells in human dental periapical inflammatory lesions using monoclonal antibodies. *Oral Surgery, Oral Medicine and Oral Pathology*, **58**, 160-165.
- OKADA H., AONO M., YOSHIDA M., MUNEMOTO K., NISHIDA O. & YOKOMIZO I. (1967) Experimental study on focal infection in rabbits by prolonged sensitization through dental pulp canals. *Archives of Oral Biology*, **12**, 1017-1034.
- SIPKA S., ABEL G., CSONGOR J., NYIRKOS P. & FACHET J. (1986) Effects of Mannozyim on the chemiluminescence of phagocytes. *Acta Microbiologica Hungarica*, **33**, 263-270.
- SIPKA S., SCHROEDER I. & BRAZSNIKOVA N.A. (1987) Effect of different fractions of dialyzable leukocyte extract-transfer factor on the chemiluminescence of human phagocytes. *Allergology and Immunopathology*, **15**, 143-144.
- SKAUG N., JOHANNESSEN A.C., NILSEN R. & MATRE R. (1984) *In-situ* characterization of cell infiltrates in human dental periapical granulomas. 3. Demonstration of T-lymphocytes. *Journal of Oral Pathology*, **13**, 120-127.
- SITTES D.P. (1976) Clinical laboratory methods of detecting cellular immune functions. In: *Basic and Clinical Immunology* (eds H.H. Fudenberg, D.P. Sittes, S.L. Cladwell & J.V. Wells), pp. 375-388. Lange, Los Altos.
- TORABINEJAD M. & BAKLAND L.K. (1978) Immunopathogenesis of chronic periapical lesions. A review. *Oral Surgery, Oral Medicine and Oral Pathology*, **46**, 685-699.
- VADAS M.A., LOPEZ A.F., SHANNON M.F. & ATKINSON Y. (1987) Human myeloid growth factors. *Immunology and Cell Biology*, **65**, 141-145.

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