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 concen-
tration, positive acute-phase protein levels and sponta-
naneous 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 fol-
lowing 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, Skau et al. 1984). Although the primary aetio-
logical 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). More-
over, potent soluble mediator substances, such as cyto-
kines, 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 api-
cected. 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
suggested by Barnes (1981) was used. Diagnosis was
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⁻¹ o-dianisidine (Fluka, Ronkonkoma, NY, USA) in 0.1 mol⁻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 Mannozym (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

<table>
<thead>
<tr>
<th>Protein</th>
<th>Reference value</th>
<th>Before treatment</th>
<th>7 days after treatment</th>
<th>3 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAT (g/l)</td>
<td>1.1–2.9</td>
<td>3.0 ± 1.0ab</td>
<td>2.3 ± 1.0a</td>
<td>2.1 ± 0.7a</td>
</tr>
<tr>
<td>AMG (g/l)</td>
<td>1.0–2.8</td>
<td>2.8 ± 1.0cd</td>
<td>1.8 ± 1.1</td>
<td>1.8 ± 0.7f</td>
</tr>
<tr>
<td>C3 (g/l)</td>
<td>0.8–1.8</td>
<td>1.7 ± 0.4c</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.4d</td>
</tr>
<tr>
<td>CER (IU/l)</td>
<td>80–140</td>
<td>80 ± 30*</td>
<td>140 ± 50*</td>
<td>140 ± 30*</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>&gt; 5.0</td>
<td>6.6 ± 4.2c</td>
<td>6.4 ± 6.7</td>
<td>3.9 ± 1.8e</td>
</tr>
<tr>
<td>HPT (g/l)</td>
<td>0.5–2.2</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>1.2–6.7</td>
<td>2.1 ± 0.6</td>
<td>2.2 ± 0.6</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>6.4 ± 17.8</td>
<td>12.3 ± 3.0</td>
<td>11.9 ± 2.7</td>
<td>11.8 ± 3.0</td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>0.4–1.5</td>
<td>1.9 ± 0.9</td>
<td>1.9 ± 0.8</td>
<td>2.1 ± 0.7</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Reference value</th>
<th>Before treatment</th>
<th>7 days after treatment</th>
<th>3 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–40</td>
<td>20.55 ± 6.64*</td>
<td>19.44 ± 7.43</td>
<td>25.27 ± 8.65*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lymphocyte subpopulation</th>
<th>Before treatment</th>
<th>7 days after treatment</th>
<th>3 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late E-rosettes</td>
<td>52.69 ± 7.71</td>
<td>50.05 ± 8.43</td>
<td>49.52 ± 7.02</td>
</tr>
<tr>
<td>Early E-rosettes</td>
<td>9.38 ± 5.46*</td>
<td>8.02 ± 4.58</td>
<td>12.19 ± 4.57*</td>
</tr>
<tr>
<td>CD5 positive T-cells</td>
<td>75 ± 12</td>
<td>ND†</td>
<td>ND</td>
</tr>
<tr>
<td>CD4 positive T-cells</td>
<td>50 ± 10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD8 positive T-cells</td>
<td>30 ± 10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SmIg positive B-cells</td>
<td>13 ± 8</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*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 Mannoxym-induced (M) chemiluminescence in whole blood of patients with apical periodontitis related to treatment

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Sampling time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
</tr>
<tr>
<td>S 26.41±15.76*</td>
<td>61.79±39.6**</td>
<td>46.94±26.50*</td>
</tr>
<tr>
<td>M 89.80±19.56</td>
<td>122.43±62.68*</td>
<td>96.97±36.17</td>
</tr>
</tbody>
</table>

Values are expressed in dpm (derived counts per minute × 10⁻¹). **Statistically significant differences between the corresponding groups (P < 0.05).

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 Mannoxym 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 Mannoxym (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


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