

# Immunonopatogenesis of Chronic Periapical Lesions

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## Summary

*Chronic periapical lesion is a result of the mutual activity of microbiota in the root canal and multilateral response of the host to infection. Nonspecific, acute and chronic inflammations, including humoral and cellular immunological responses, participate in the occurrence, development and perpetuation of these lesions. Biopsy samples of periapical tissue were taken by surgical procedure in 34 patients, with the object of verifying immunoglobulin classes G, A and M. The study involved determination of total proteins by Lowry's method. Concentrations of IgG, IgA and IgM were determined by the method of radial immunodiffusion. Their correct amount was obtained by placing them in relation to the verified amount of total proteins. The study revealed different percentages of the share of immunoglobulins, classes G, A and M, and diversity in their values.*

*IgG was found in 100% of cases, IgA in 76% and IgM in 44%. Mean value of the quantity of IgG amounted to 385.2 mg/g protein, ranging in value from 27.3 to 826.0 mg/g protein, IgA 51.0 mg/g protein, ranging from 0 to 336.8 mg/g protein, and IgM 20.3 mg/g, ranging from 0 to 156.2 mg/g protein.*

*The results obtained on the basis of this study indicate the local synthesis of immunoglobulins in chronic periapical lesions, and the involvement of nonspecific inflammatory and specific immunological reactions, which together are responsible for the pathogenesis of these lesions.*

**Key words:** *periapical lesion, immunoglobulins G, A and M*

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## Introduction

Chronic periapical lesions are a consequence of an untreated acute inflammatory process, and at the same time the protective reaction of the organism which was unsuccessful in neutralising the harmful factors which subsequently perpetuate the inflammatory process. The inflammatory process in periapical chronic lesions is still not fully understood, although four main biochemical factors are known:

1. kinin system
2. vasoactive amines
3. complementary system
4. metabolites of arachidic acid.

Two main vasoactive amines are involved in the inflammatory reaction; histamines and serotonin. In the kinin system the responsible factors, and mediators of the inflammation, are kinin and lys-bradykinin (1). The complementary system is the main effector system of humoral type immunity. It comprises 15 albumins which are activated by classic or alternative means. The biological effects of the complementary system are:

- a) anaphylotoxic effects
- b) chemotoxic effects
- c) immunoatherention
- d) cytolyse effects.

Enzymatic oxidation of arachydonic acid leads to release of mediators of inflammation, i.e. immunomodulators, the main representatives of which are leukotriene and prostaglandin. The main sources of leukotriene are mastocytes, macrophages, basophilic and polymorphonuclear leukocytes.

Chronic periapical lesion is defined as the protective reaction of the organism, represented by newly created granulational tissue, permeated with inflammatory cells; leukocytes, T and B lymphocytes, macrophages, mastocytes, plasma cells. According to M. Kuo (2) macrophages are very important in the development of the periapical lesion. Their two basic functions are phagocytosis and cytokin production. Interleukin, one of the cytokins, is produced, apart from mononuclear phagocytes, by epithelial, endothelial cells, fibroblasts, B lymphocytes and some lines of T lymphocytes. The ability of the phagocytose macrophages is related to microorganisms and their decayed products, and antigen-

-antibody complex. Macrophages also represent antigens of T lymphocyte substances, and thus induce the commencement of immunological reactions by their effect on B lymphocytes and their transformation into plasma cells, which are responsible for the formation of antibodies. Accumulation of T lymphocytes is confirmed by chronic periapical lesions, particularly those colonised by streptococci (3).

T lymphocytes appear in the form of two subpopulations of opposite regulative function: T-helper and T-killer of cytotoxic cells. T-helper lymphocytes stimulate the transformation of B lymphocytes into plasma cells. The plasma cells are in turn responsible for the formation of antibodies - immunoglobulins of different classes.

The polymorphonuclear leukocytes have a protective and destructive function in nonspecific inflammatory reactions. The protective function is expressed in their ability to phagocytose. They contain lysosomal granules, in which there are numerous hydrolytic enzymes, whose release is responsible for the tissue damage. The enzymes in the lysosomal granules are collagenase, alkaline phosphatase, elastase, protease, lysosomes, which have a destructive effect on the extracellular constituents of connective tissue (4,5,6).

Mastocytes are considered potential cells in the pathogenesis of chronic periapical lesions. They are mediator cells of inflammation because they contain chemical constituents known collectively as leukotrienes: histamine, bradykinin and serotonin.

Today, increased attention has turned to the role of immunological phenomena in the occurrence, development and perpetuation of chronic periapical lesions of pulpal origin. Bacteria, decayed products of bacteria of the transformed tissue substance of the pulpal tissue in the root canal are potential antigens capable of initiating the onset of immunological reactions which spread into the periapical tissue. The immunological response is a biological phenomena which includes initial protective mechanisms, but which is also a responsible mediator of tissue damage, and in some circumstances is also responsible for the reparatory process of the tissue. Antigens or immunogens are foreign macromolecular proteins lipopolysacharides, carbohy-

drates, lipids or nucleic acids (7). The basic antigens of the cellular wall of *Streptococcus mutans*, the most frequent microorganisms in the root canal are proteins, polysaccharides, lipoteichoic acids, glucosyl transferase, glucan and peptidoglycan. Thus, it can be said that the pathogenesis of chronic periapical lesion of pulpal origin is activation of nonspecific inflammatory and specific immunological reactions. Nonspecific inflammatory reactions start by activating Hageman's factor, which involves the fibrinolytic and kinin system, and release of kinins and plasmins. They in turn stimulate the complementary system, which causes local inflammation of the connective periapical tissue. Activation of arachidonic acids, helped by phospholipase, releases the metabolites of this acid, particularly prostaglandins (PGE<sub>2</sub>), which results in bone resorption (8). At the same time specific immunological reactions of cellular and humoral type occur. Antigens of the humoral stimulation of B lymphocyte lead to the formation of immunoglobulins which also activate the complementary system, resulting in inflammation and bone resorption. The cellular form of immunological reactions through the T lymphocytes leads to a release of lymphokins, particularly osteoclast activating factor (OAF) which, together with prostaglandine, is the factor most responsible for the occurrence and development of bone resorption.

The effector cells of bone resorption are osteoclasts. Apart from prostaglandin and osteoclast activating factor (OAF) cytokins are also responsible for activation of osteoclasts, with regard to bone resorption of the periapical region, i.e. interleukin (IL-1) and tumour necrotizing factor (TNF- $\alpha$ ) and lymphotoxin.

### Aim of the investigation

The general aim of the study was the quantitative analysis of samples of tissue from chronic periapical lesions, from the immunological perspective.

Basic aims of the investigation were:

1. To determine the content of total proteins.
2. To determine the quantity of immunoglobulins, classes G, A and M
3. To determine the content of lysozymes.

4. To confirm as reliably as possible data on the quantity of IgG, IgA and IgM, and placing their concentration in relation to total proteins.
5. To elucidate a part of the etiopathogenetic mechanism of chronic periapical lesion.
6. The aim of this study was a possible amendment/supplement? to current procedures in the treatment of pulpal-periapical diseases.

### Material and method of work

Tissue samples from chronic periapical lesions were collected in 34 patients, aged 24 - 45 years, in the Department of Oral Surgery at the School of Dental Medicine in Zagreb. During the surgical procedure samples of granulation tissue were taken immediately beside the apex of the tooth envisaged for apicotomy. The samples were stored in Ependorf ampules and kept in a refrigerator at a temperature of -18°C until laboratory examination. Tissue analysis was carried out in the Department of Clinical-Laboratory Diagnostics, Clinical Hospital Centre Rebro.

The biopsy samples were homogenised in 0.5 ml PBS buffer (NaCl 8.09/L; KCl 0.2 g/L; Na<sub>2</sub>HPO<sub>4</sub> 1.15 g/L; KH<sub>2</sub>PO<sub>4</sub> 0.2 g/L). The homogenates obtained were well mixed in vortex and centrifuged at 3500 revolutions a minute for 10 minutes. After which total proteins, lysozyme, immunoglobulins G, A and M were determined in supernatants. Total proteins were determined by Lowry's method, lysozyme by a method based on the lysa cell *hierococcus lysodeikticus* suspended in agar gel, and immunoglobulins by the method of radial immunodiffusion on LC plates (Boehring). The values of lysozymes and immunoglobulins in tissue homogenates are expressed according to gram proteins.

### Results

The results of this study of periapical tissue from chronic periapical lesions relate to the following variables:

1. Confirmed concentrations of total proteins.
2. Confirmed quantity of lysozymes.

### 3. Confirmed immunoglobulins, classes G, A and M.

Total proteins determined in each sample of tissue were used to determine the quantity of lysozymes and immunoglobulins, classes G, A and M, from their concentrations:

$$\frac{\text{Concentrations of IgG, IgA, IgM/l}}{\text{proteins in g/l}} = \text{quantity of IgG, IgA and IgM mg/g}$$

$$\frac{\text{Concentrations of lysozymes in mg/l}}{\text{proteins in g/l}} = \text{quantity of lysozymes in mg/g proteins}$$

By placing the obtained concentrations of lysozymes and immunoglobulins G, A and M, expressed in mg/l for each of the analysed samples of tissue in relation to total proteins, accurate data on their values were obtained. The authors did not find a similar method for analysing IgA, IgG and IgM in available literature. In 34 patients with chronic periapical lesions, the mean value of the quantity of total proteins amounted to 1.29 g/l, range 0.16-6 g/l, mean value of the confirmed lysozymes amounted to 15.5 mg/g protein, range 0.5-75.0 mg/g protein.

In the 34 biopoints? IgG was found in 100% of cases, IgA in 76% and IgM in 44% (Table 1).

The mean value of the quantity of IgG amounted to 385.2 mg/g protein, range 27.3-826.0 mg/g protein, IgA 51 mg/g protein, range 0-336.8 mg/g protein and the mean value for IgM amounted to 20.3 mg/g protein, range 0-156.2 mg/g protein (Table 2).

In 42% of the samples of tissue from chronic periapical lesions the values of lysozyme ranged from 0-7.5 mg/g protein. In 23% values ranged from 7.5-150 mg/g protein, in 13% 15.0-22.5 mg/g, in 3% 22.5-30.0 mg/g, in 13% 30-37.5 mg/g, in 3% 73.5-45.5 mg/g, and in 3% 75.0 - 82.5 mg/g protein (Histogram 1).

The quantity of IgG ranged from 0-900 mg/g protein. In 26% of cases the quantity of IgG ranged from 0-75 mg/g, in 9% 50-225 mg/g, in 3% 300-375 mg/g, in 17% 375-450 mg/g, in 12% 450-525 mg/g, in 6% 525-600 mg/g, in 9% 600-675 mg/g, in 6% 675-750 mg/g, in 6% 750-825 mg/g and in 3% 825-900 mg/g. Thus in 65% the quantity of IgG ranged from 300-900 mg/g protein (Histogram 2).

IgA was found in 53% of the biopsy samples from chronic periapical lesions, ranging in value from 0-30 mg/g protein, in 9% 30-60 mg/g, in 21% 60-90 mg/g, in 6% 90-120 mg/g, in 6% 120-150 mg/g, in 3% 150-180 mg/g, and in 3% of cases 330-360 mg/g protein (Histogram 3).

The share of IgM was predominant, ranging from 0-15 protein in 70% of cases, in 9% 15-30 mg/g protein, in 6% 30-45 mg/g, in 3% 45-60 mg/g, in 3% 60-75 mg/g, and in 3% 90-105 mg/g and 135-150 mg/g protein, and in 3% values ranged from 150-165 mg/g protein (Histogram 4).

## Discussion

Periapical diseases are closely associated with pulp tissue diseases. Apart from the direct effect of microorganisms, their metabolic products, toxins, enzymes and products of decayed pulpal and periapical tissue can act as antigens and induce immunological responses of cellular and humoral type. Thus, together with nonspecific inflammatory reactions they have a significant role in the pathogenesis of pulpal periapical diseases.

The lysozymes analysed in this study were found in all samples of chronic periapical lesions. As they stimulate the effect of immunoglobulins their non-specific protective ability can be assumed as a nonprotein which merges with local immunological reactions in the periapical tissue (10).

The findings of inflammatory components in chronic periapical lesion are clearly diverse. Granulation tissue from a chronic periapical lesion is permeated with different chronically inflamed cells, although in the same way with immunocompetent cells and immunoglobulins of different classes.

The first direct evidence of the presence of immune components in periapical lesions was demonstrated by the presence of immunoglobulins in these lesions (11). Mathews (12) used immunofluorescence method to analyse quantitative relations between cells which produce IgG, IgA, IgM, IgE. His results indicated the predominance of IgG in 81%, IgA in 11%, and IgM in 0.2%. In this study the results obtained by the method of radial immunodiffusion show a similar percentage of distribution. Cryoscopical cuts of analysed biopsies

from chronic periapical lesions also showed the predominance of IgG positive cells (66.65%) in relation to IgA (24.75%), and positive cells (14.59%). IgA and IgM were not found in some samples in this study, and it can be assumed that the concentrations in these classes of immunoglobulins were so small that the method applied was unable to detect them. The results of this study show that IgG exceeds the values of IgA and IgM, which is in agreement with the findings of Pulver (13) and Kuntz (14). The mean values for IgG of 385 mg/g protein, IgA 51.0 mg/g, and IgM 20.3 mg/g suggest that in chronic periapical lesions permanent immunological response is involved, i.e. the presence of immunoglobulins is not a consequence of systemic production of antibodies, but rather a local synthesis of the same, and local, induction of the immunological response.

Former investigations on the immunological status of chronic periapical lesions have been based on qualitative analysis. The data in the present study are quantitative and consequently more reliable, because the confirmed concentrations of immunoglobulins are placed in relation to the total determined proteins, found in the periapical lesion.

The predominance of IgG, with 100% share in the biopsy samples, and the highest values of these immunoglobulins in the results of this study, indicate that immunological reactions of over sensitivity, type II and III, can occur in chronic periapical lesions, i.e. cytotoxic and antigen-antibody reactions of over sensitivity. This could explain the damage to periapical tissue, both connective and bony tissue. Namely, after the formation of immune complexes antigen-antibodies, activation of the complementary

system occurs, leading to tissue damage and perpetuation of the inflammatory process.

IgA has the function of blocking, i.e. erasing, the antigenic effect, by preventing the release of inflammatory factors. However, significantly lower quantities of IgA in relation to IgG may mean that the protective potential of IgA is overcome by destructive mechanisms, associated with the activation of the complementary system of immunoglobulin G.

The removal of antigenic material from the root canal system presupposes interruption of the flow of the same into the area of the periapex and consequent tissue damage. This in turn creates the conditions for reparation of both connective and bony tissue.

After obturation of the root canal self-healing of the periapical tissue occurs, withdrawal and final disappearance of the periapical lesion, monitored by regular X-ray examinations.

## Conclusions

The root canal system represents a warehouse of antigenic material. This assumes the possibility of antigenic challenge, by the formation of antibodies in the pulpal-periapical complex. In the periapical region the share of immunoglobulins, classes A, G and M, has been confirmed in different percentages and quantities, suggesting a local periapical synthesis of IgG, IgA and IgM. Based on the confirmed predominance of IgG, the possibility of reactions of type II and III over sensitivity in the etiopathogenesis of chronic periapical lesions can be concluded.