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Histological and immunohistochemical study on the apical granuloma

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Abstract

Introduction: Periapical granuloma is one of the most frequent periodontal pathology and belongs to the group named as apical periodontitis. *Materials and Methods*: Out of 78 of diagnosed granulomas, we selected samples that we analyzed histologically and immunohistochemically. *Results*: The histopathological aspect has been dominated by the presence of mononuclear cells of the lymphocyte and plasma cells type, showing the chronic aspect of the apical lesion. Also, we noticed that in the apical granuloma macrophages occur most often. This density of macrophages explains cellular and tissular disruption that occur in apical region of the tooth under the influence of bacterial flora that reached this area, as they have the role to phagocyte pathogens and cell and tissue residues that result from bacterial aggression. The reaction of the plasma cells, determined by their number, has been always associated with the age of the granulomas, and it is more intense in old, neglected granulomas, compared to recent granulomas. *Conclusions*: The number and type of immunity cells varies in the apical granuloma accordingly to the age of granuloma.

Keywords: apical granuloma, histopathological exam, immunohistochemistry, immune response.

Introduction

Chronic periapical lesions represent a frequent complication related either to a badly done endodontic treatment as well as to a not treated or badly treated cavity that complicates with pulpal and then periodontal pathology. Often, they pass as chronic disease with little to no perspective of conservative treatment [1–3].

Periapical lesions are classified depending on their histological structure, radiological dimension and clinical manifestations, such as periapical granuloma, periapical condensation, radicular cysts and others [4, 5].

The periapical granuloma has a complex structure, resulting of proliferative processes as well degenerative and infiltrative manifestations. There are described four areas between the dental root and the alveolar bone: first is an area of necrosis and even infection; second, there is a layer of contamination, where the first reactions of defense occur, with vasodilatation and inflammatory exudate; third, the area of irritation, where cellular multiplication occurs; fourth, the area of stimulation where the toxins determine a reaction from osteoblasts [6].

Clinicians are still making efforts to create a treatment that can be effective and heal these kinds of lesions [7], but in order to achieve that multiple studies of the complex structure in different stages of development of the granuloma must be undertaken still. This is the purpose of our study, to determine the status of various lesions, correlating the histological, immunohistochemical (IHC) founding with clinical and radiological data.

Materials and Methods

The study involved 78 cases of periapical granuloma, diagnosed as such considering the clinical and radiological assessment. The patients were selected from private practices during one year. Of the 78 periapical granulomas, 32 were a complication of an untreated complicated caries and the rest have been complication of an endodontic treatment that was not correctly made. The age of the patients was between 26 and 57 years old. In all cases, the treatment was tooth extraction and cleaning the socket with a curette, removing all damaged tissue. All samples were fixed in 10% formalin, for 48–72 hours. For the histological study, the fragments were embedded in paraffin and colored with the Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome stainings. For the IHC study, from the biological material embedded in paraffin, we made serial sections that were collected on slides covered with poly-L-lysine and dried in a thermostat at 37^0C, for 24 hours. After that, we deparaffinized and hydrated the sections. The exposure of the antigens of interest was

done by boiling the sections in sodium citrate, pH 6, for 21 minutes, in a microwave (seven cycles for 3 minutes). After cooling down the slides, they were rinsed with tap water and washed with distilled water for 15 minutes. The endogenous peroxidase was blocked by incubating the sections in 3% hydrogen peroxide for 30 minutes, followed by a wash with distilled water for 10 minutes and wash in 1% phosphate-buffered saline (PBS) for 5 minutes. Afterwards, we blocked the specific sites with 2% skimmed milk for 30 minutes. The sections were incubated with primary antibodies for 18 hours (overnight), in a refrigerator, at 4^0C. Next, we applied the secondary biotinylated antibody for 30 minutes, at room temperature, and then we washed the sections with 1% PBS (three baths for 5 minutes), and then we applied Streptavidin–Horseradish peroxidase (HRP) 30 minutes, at room temperature, followed by washing the slides with 1% PBS 3×5 minutes. The signal was detected using 3,3'-Diaminobenzidine (DAB) (Dako) and the reaction was stopped by washing the sections in 1% PBS, when the IHC reaction was intense enough. Latest, we did the contrasting with Mayer's Hematoxylin, dehydration with alcohol, the clarifying with xylene and mounting the slides with a DPX medium (Fluka).

In our study, we used the following antibodies: CD68 (monoclonal mouse anti-human CD68, clone KP1, Dako, 1:200 dilution) for the macrophages; CD79α (monoclonal mouse anti-human CD79-alpha, clone JCB117, Dako, 1:50 dilution) for plasma cells; tryptase (monoclonal mouse anti-human mast cell tryptase, clone AA1, Dako, 1:500 dilution) for the study of mastocytes; CD3 (monoclonal mouse anti-human CD3, clone F7.2.38, Dako, 1:25 dilution) for emphasizing the T-lymphocytes; CD20 (monoclonal mouse anti-human CD20cy, clone L26, Dako, 1:50 dilution) for studying the B-lymphocytes.

Results

In our study, all the patients have been informed about their diagnosis and have agreed with the treatment and also have agreed for their biological material to use for study. Clinically, in the majority of the cases, the gingiva was red or swollen at the apex of the affected tooth. If the periodontal lesion becomes chronic, the puts will emerge to the outside through an epithelial channel and its opening is visible on the gingiva around the apex of the tooth. The patients declared they had episodes of slight pain but they go by fast. The tooth had no vital signs and either presents a big cavity, with large amounts of soft dentine, either has a large filling, sometimes poorly adapted, and its color is abnormal, turning grey. We considered dental X-rays are always useful in establishing the diagnosis, as it shows clearly the periapical lesion. When the lesion appears on the radiography very well delimited, with a white stripe of tissue, the conservative treatment is no longer effective. The granuloma has to be physically removed and the bone around cleaned, therefore the treatment has to be radical, either tooth extraction or apex removal surgery. The radiography gives us information about the root canal treatment and about the root anatomy, which is useful during the extraction. On the radiography, the periapical granuloma was identified a radiotransparency at the apex of the tooth, with clear margins, very well delimited of the rest of the bone (Figures 1 and 2).

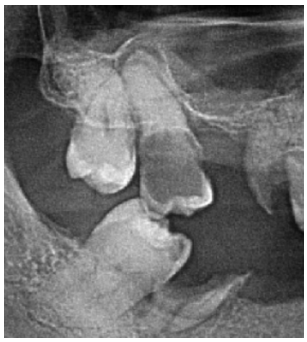

Figure 1 – ***Radiological aspect of 27. Deep cavity with extended crown disruption. Radiological transparency around the apex, apical granuloma is visible.***

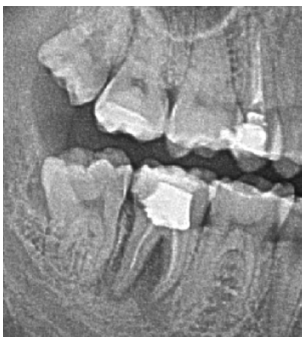

Figure 2 – ***Radiological aspect of 47. Endodontic treatment failure due to crown infiltration with incorrectly adapted filling. Radiotransparency on both roots with the presence of periapical lesion.***

Histopathologically, we have followed the modifications that occur on the apex, so we achieve a better understanding of the destructive chronic inflammatory process and therefore provide a better preventive treatment. The connective tissue was analyzed by investigating the inflammatory infiltration, and the presence of the lymphocytes, plasma cells, macrophages, and mast cells. We also appreciated the concentration of these cells as a clue about the evolution of the inflammatory status in a regressive phase or acute progressive phase.

The presence of a connective tissue with a chronic inflammation infiltrate, with no epithelium, confirms the diagnosis of conjunctive granuloma.

The microscopic aspect of the apical granuloma was extremely various from one case to another, probably due to the etiopathogenic mechanisms that led to their formation, but also due to the reaction of the immunity system specific to each patient. Most often, the histopathological (HP) aspect has been dominated by the presence of mononuclear cells of the lymphocyte and plasma cells type, showing the chronic aspect of the apical lesion (Figure 3). The macrophages, in classic HE and GS staining, showed as cells with increased dimensions, abundant cytoplasm, clearer than the lymphocytes and plasma cells, with vacuoles and cellular and tissue debris (Figure 4). For some patients, we identified extended areas of cell necrosis, associated with hemorrhagic infiltrate, more or less intense, an aspect that suggests an alteration of the circulatory local system (Figure 5). In other patients, we identified intense reactions of the conjunctive matrix, with the increase of the fibrillary collagen and even calcium salts deposits, and therefore the long evolution of the lesion is confirmed (Figure 6). In all granulomas, we identified on the border an area of higher density of the conjunctive tissue, as a reaction to limit the granuloma of the rest of the periodontal connective tissue (Figure 7).

In order to appreciate the reactions of the immune system to the appearance of the granuloma, we have made several IHC stainings.

Of all the immune cells, we noticed that in the apical granuloma, macrophages occur most often (Figure 8). This

density of macrophages explains cellular and tissular disruption that occur in apical region of the tooth, under the influence of bacterial flora that reached this area, as they have the role to phagocyte pathogens and cell and tissue residues that result from bacterial aggression.

Regarding the reaction of the plasma cells, we noticed a large variety from one patient to another, as we identified apical granulomas with a moderate reaction of plasma cells (Figure 9) and granulomas with an intense reaction (Figure 10). We noticed that the reaction of the plasma cells, determined by their number, has been always associated with the age of the granulomas, and as more intense in old, neglected granulomas, compared to recent granulomas.

Regardless the age of the granuloma, we noticed the presence of a large number of mastocytes (Figure 11). They are disposed mostly around blood vessels, as non-homogenous cells, with diffuse contour, due to the mastocytes granulation processes. We believe that although they are not directly involved in the defense mechanisms of the body, mastocytes, through their mediators, contribute to the increase of the local blood flood and the cumulation of a larger number of immune cells.

Considering T- and B-lymphocytes, we noticed that they had a various reaction from one case to another and even from one area to another in the same granuloma (Figures 12 and 13). Overall, the two main types of lymphocytes had a medium reaction. We can say that the immunoreaction is extremely complex, considering both cellular and humoral immunity.

Discussions

The HP study is important because there are situations where the clinical and radiological symptomatology is similar but the HP results to be different (fibrous chronic apical periodontitis, diffuse chronic apical periodontitis, and condensed chronic apical periodontitis).

It is important to identify the factors that focus the evolution towards one of the HP forms, to be able to prevent and treat these lesions conservatory. When the inflammation is reaching a chronic status, the host always responds with vascular proliferation in order to repair the lesion, which leads to the formation of granulation tissue [8].

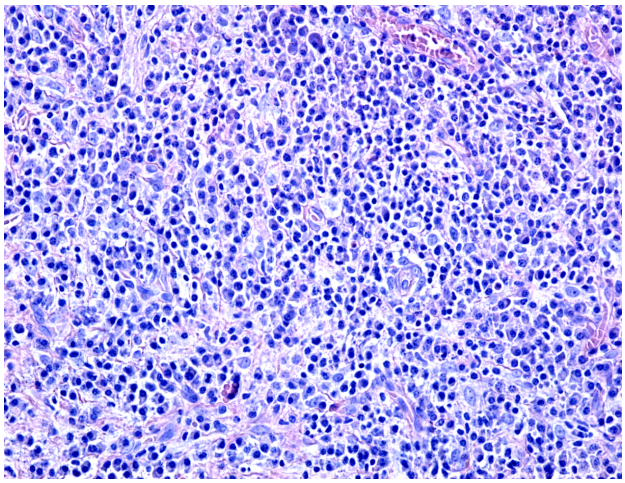

Figure 3 – ***Microscopic image of an apical granuloma consisting of lymphocyte, plasma and macrophage cells. HE staining, ×200.***

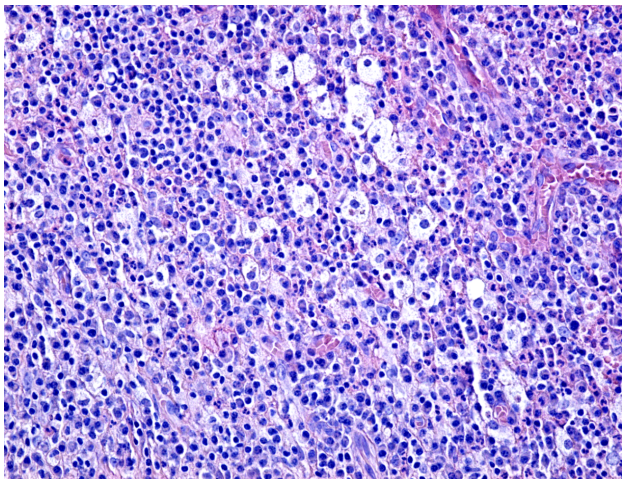

Figure 4 – ***Apical granuloma, large quantity of macrophages. HE staining, ×200.***

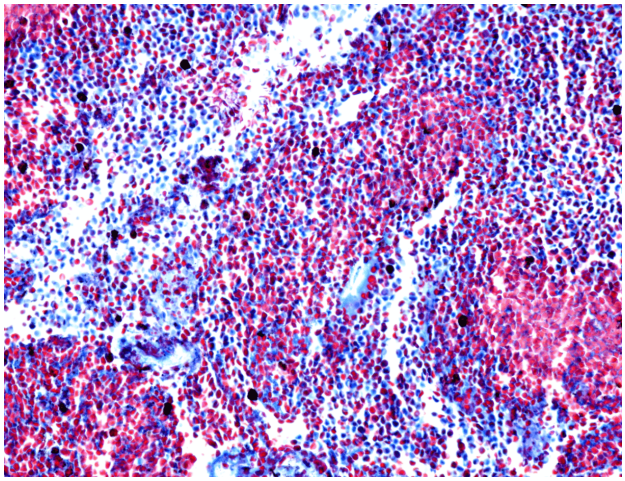

Figure 5 – ***Apical granuloma with massive hemorrhagic infiltrate and areas of necrosis. GS trichrome staining, ×200.***

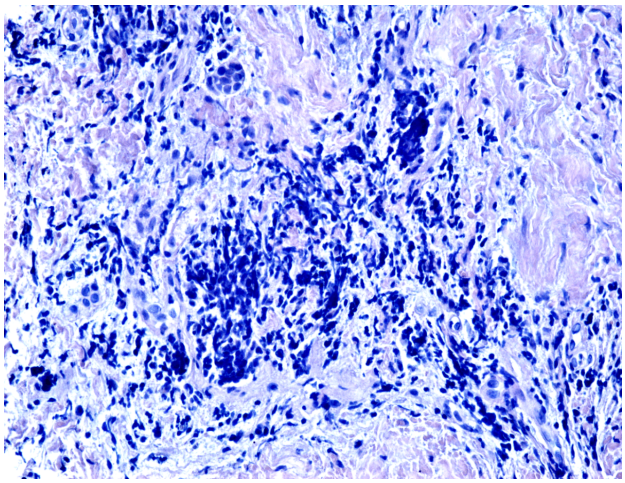

Figure 6 – ***Apical granuloma, characterized by the presence of a low inflammatory infiltrate associated with thick collagen fibers and microcalcifications. HE staining, ×200.***

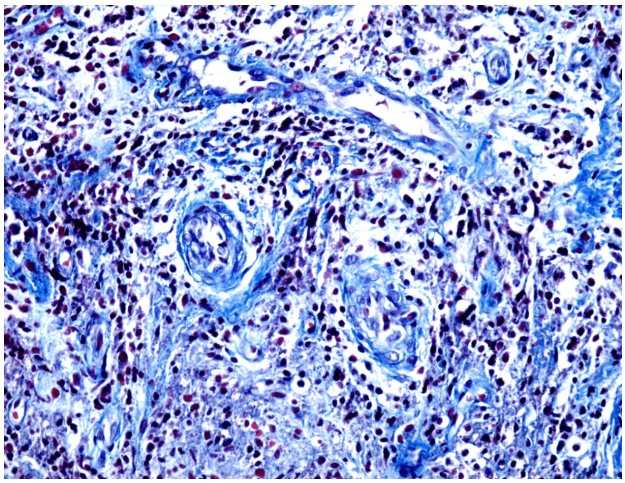

Figure 7 – ***Microscopic image at the periphery of the granuloma where the thickening of the collagen fibers is noted to delineate the granuloma from the rest of the periodontal connective tissue. GS trichrome staining, ×200.***

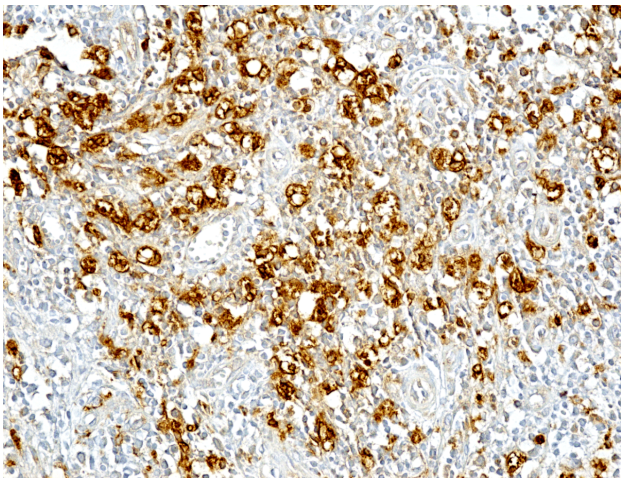

Figure 8 – ***Apical granuloma with intense reaction of macrophage cells. Anti-CD68 antibody immunostaining, ×200.***

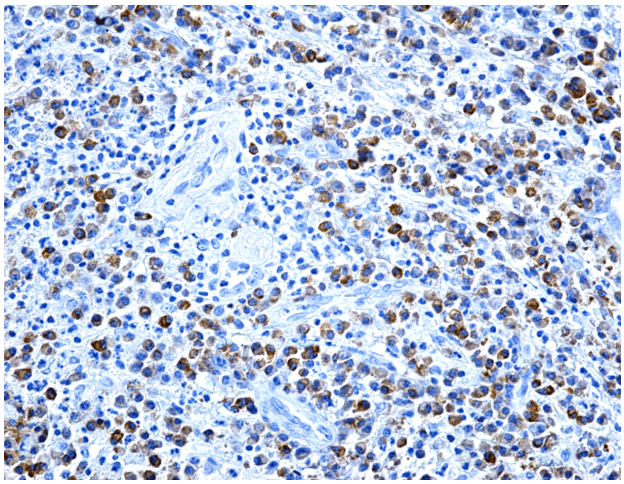

Figure 9 – ***Apical granuloma with moderate reaction of plasma cells. Anti-CD79α antibody immunostaining, ×200.***

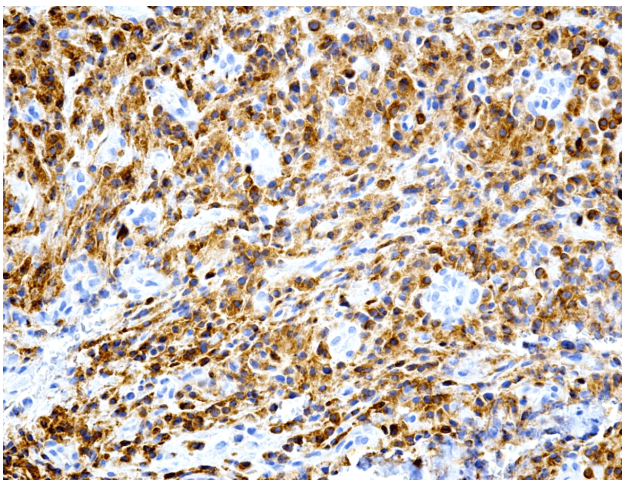

Figure 10 – ***Intense reaction of the plasma cells in a very old apical granuloma. Anti-CD79α antibody immunostaining, ×200.***

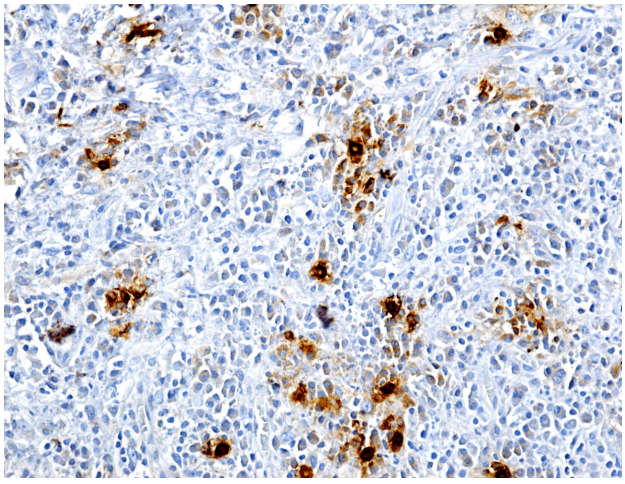

Figure 11 – ***Relatively many mast cells diffused in the structure of the apical granuloma. Anti-tryptase antibody immunostaining, ×200.***

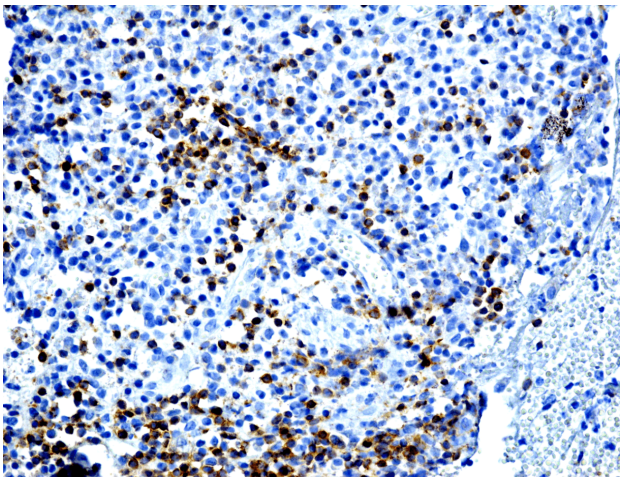

Figure 12 – ***Apical granuloma with average lymphocyte response. Anti-CD3 antibody immunostaining, ×200.***

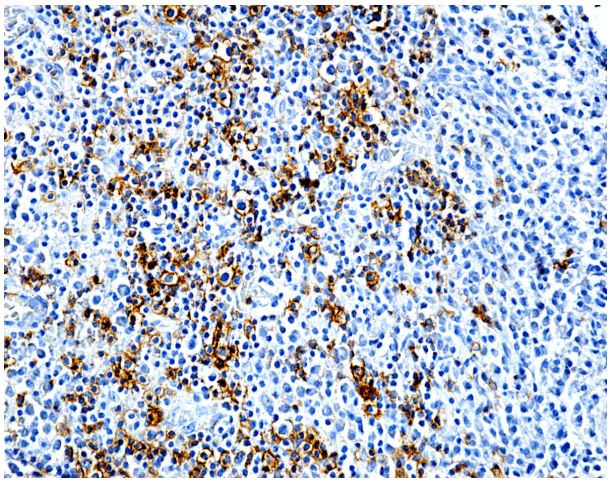

Figure 13 – ***Non-homogeneous, medium-grade B-lymphocyte reaction in an apical granuloma. Anti-CD20 antibody immunostaining, ×100.***

We must always remember to get a differential diagnosis between the apical granuloma and a cyst. Traditionally, the dentists are considering the size of the lesions as seen on the radiography, as the lesions smaller than 1 cm are granuloma and if larger are cysts. However, many specialists disagree with this protocol and some researches even prove a disagreement between the radiological diagnosis and the HP diagnosis [9].

The cone-beam computed tomography (CBCT) can provide clear information considering the diagnosis, as there are density differences between the cysts cavity and the granuloma tissue [10].

International literature gives various values for the apical granuloma frequency, having a wide range from 9.3% to 87.1% [3, 11, 12]. These values so different come from different criteria used for diagnosis, sometimes the granuloma was diagnosed as apical cyst, because under histological consideration, the granulation tissue with epithelial growth occurs in both cases.

Under IHC consideration, due to the presence of lymphocytes and plasma cells, some authors consider the apical granuloma as an immune manifestation, while the presence of macrophages indicates a non-immune apical granuloma [13]. The periapical tissues can make an immune answer, and it can be the main mechanism to defend against the infection and toxins. Cellular immunity promotes the disruption of the infected cells because the antibodies cannot attack these antigens. T- and B-lymphocytes are programmed to answer specific antigens [14], and such would explain their presence on our results. Some studies confirm that T-lymphocytes are present in a small area focused around the pathogens [15], while others suggest that these lymphocytes are diffused in the tissue [16]. Some older studies have shown a focal distribution of these lymphocytes [17]. Anti-CD3 antibody confirms the presence of T-lymphocytes in our study, in a diffused way; they are responsible for delayed hypersensitivity and cellular mediated reactions. B-lymphocytes occur in a larger number as the anti-CD20 antibody has shown us. The presence of the lymphocytes in the apical granuloma suggests that there are various aspects of the immunoreactions participating in the pathology. The specific and non-specific defense mechanisms can protect the tissues, as well as they can destroy it under certain circumstances. The main components of the adaptive immunity are B- and T-lymphocytes [18].

The macrophages are one the most important cells that are found in the periapical granulomas. Their percentage in the literature is about 46% [19–21]. We identified them in large numbers as well in our study. Their role is in nonspecific immunity, specific acquired immunity and the regulation of the disruption and repair of the connective tissue. The activity of the macrophages can be determined by cytokines produced by the T-lymphocytes that are activated by the antigens and/or bacterial endotoxins. Their role has been studied for years, and it seems however that the periapical granuloma can develop independently of the T-lymphocytes under certain circumstances [22]. The macrophages are considered to be the main source of interleukin-1alpha (IL-1α), interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) cytokines that have a role in initiating and regulating the inflammation process. They can produce metalloproteinases (collagenase, elastase) and prostaglandins, all contributing in the disruptive result that is the periapical inflammation. Some studies confirm that the active production of IL-1 involves just some macrophages that in the periapical granuloma are no more than 2–3% of the entire number [23]. The macrophages presence is characteristic for the granuloma inflammation in general but they lack organization in the periapical areas.

The mastocytes play a role in allergic inflammation, anaphylactic reactions, in chronic inflammation [24, 25]. They produce a large range of mediators, creating functional reciprocal interactions between the activation of the mastocytes and the stimulation of the T-lymphocytes. The reactions mediated by the immunoglobulin E (IgE) could play an important role in the initiation and progress of the periapical lesions, if mastocytes and plasma cells containing IgE are present. IgE has an active role in the periapical granuloma pathology and the anaphylactic reactions and hypersensitivity represent an immunological active phenomenon [26, 27]. Researchers agree that the mastocytes have a contribution in the development of the fibrosis by making hyaluronic acid and intensify the collagen fibers synthesis due to heparin presence. They activate the fibroblasts through tryptase. The tryptase can activate the metalloproteinases 1 and 2, leading to extracellular matrix decompose [26]. However, there are not many studies in the literature related to the mediators released by the mastocytes and probably further studies are needed to clearly define the role of mastocytes and their mediators in the apical lesions. Based on some authors' observations, as well as our own, we can confirm that the mastocytes are involved in regulating the immune cell mechanisms in apical region, considering their interactions with other immune cells, such as T-lymphocytes and plasma cells [28].

The dentists try to clean the root canals, while performing the endodontic treatment and the purpose is

to obtain a bacteria-free area that is going to be sealed in order to prevent recontamination. However, removing the cause of the inflammation in this case is not enough, and the healing of the apex can take even months because the cells once activated in the lesion can maintain this status long after the stimuli are removed [29]. Until now, most of the times the treatment still remains radical, with the mechanical removal of the apical tissues [30, 31]. Many scientists agree that a longer evolution will lead to a reduction of the inflammatory process, and in time, there will be fewer cells, such as lymphocytes, plasma cells, macrophages and more fibroblasts [32]. The contact between the microbial factors and the defense mechanisms is always leading to a disruption of the apical region, meaning mainly the bone loss [2, 33].

Conclusions

The presence of inflammatory cells, such as B- and T-lymphocytes, macrophages, mastocytes, and plasma cells are a clear clue to the existence of an immunoreaction in the apical granuloma. The inflammation around the apex is the answer of the region tissues to chronic irritations caused by the microbial, chemical and mechanical stimuli from the endodontic level. The number of B-lymphocytes was larger, confirming the diagnosis of connective granuloma. Plasma cells were also present in high numbers on some samples, according to the status and development of the lesion. The most important role of the mastocytes in the inflammatory lesions is linked to the developing and progress of the inflammatory apical tissue. Knowing the immune processes that occur in the apical region, the dentists can perform an optimal treatment that can give in time a higher success rate of the conservative approach of this pathology.

Conflict of interests

The authors declare that they have no conflict of interests.

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