Immunohistochemical Study of Presence of T Cells, B Cells, and Macrophages in Periradicular Lesions of Primary Teeth

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BACKGROUND: The periapical lesion is the result of a local inflammatory reaction caused by bacteria and its products present on the root canal. The interaction between inflammatory cells and bacteria elicit both specific and non-specific immune responses. OBJETIVE: Due to the lack of studies evaluating the role of the immune system in periapical lesions of primary teeth and considering the potentially systemic effects that these infections can cause in children, especially because of the immaturity of their immune system, we sought to evaluate the presence of T cells, B cells and macrophages on periradicular lesions in primary teeth. STUDY DESIGN: 14 periradicular lesions were analyzed. The immunohistochemistry technique was performed using CD45RO, CD20, CD68 monoclonal antibodies aiming to identify T cells, B cells and macrophages, respectively. Cells were quantified by microscopic analysis of histological sections. RESULTS: Mean percentage of positive cells CD45RO was 11.76; CD20 was 5.25; CD68 was 10.92. Our results showed that T and B cells and macrophages comprise the majority of the inflammatory infiltrate. CONCLUSION: We concluded that both humoral and cell mediated immune reactions take place in periradicular lesions of primary teeth. The immune system plays an important role on the periradicular inflammatory processes in primary teeth.

Keywords: *periradicular lesion; immunohistochemistry; B-lymphocytes; T-lymphocytes; macrophages*
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INTRODUCTION

The pulpal tissue can develop an inflammatory process when exposed to bacteria from the mouth. If untreated, this process can reach the periapical tissue. The bacteria and its products act as antigens capable of triggering an inflammatory reaction in affected periapical tissues.[1] As a consequence, the pathological root resorption appears and causes periradicular lesions.[2,3]

The periradicular tissue presents both specific and non-specific cells that are linked to the local immune response. These cells are part of the immune system, which is formed by a complex group of several cells such as phagocytes (macrophages and neutrophils), natural killer cells and lymphocytes. Their role is to recognize antigens that penetrate the body and neutralize or eliminate them.[4-8]

In the first years of life, children have an immature immune system. This causes a greater vulnerability to infectious diseases.[9] The cells capable of triggering humoral response, B cells, will not produce immunoglobulin in a level equal to adults until the age of seven. T cells, which are responsible for cellular immune response, present a diminished lymphokines production in children when compared to adults. Although the number of neutrophils and macrophages in the neonatal period is similar to adults' rates, migration of neutrophils towards aggressive agents is slower because of the lower amount of chemotactic factors. These rates will only reach normal values by the age of 5.[10]

The immaturity of both the immune system and the mechanism involved in inflammatory reactions, as described, makes the children an easy quarry for systemic dissemination of infections. The maintenance of infectious focus in children can compromise their health as a whole.[11]

The mouth is the source for a variety of antigens including numerous microorganisms for both gastrointestinal and respiratory system. Several important oral diseases

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including dental caries, gingival and periodontal diseases are consequences of an imbalance between oral microorganisms and host defense. [12]

The infection caused by cariouss lesions has been pointed as the main etiological factor for the development of periradicular lesions in primary teeth, such as abscesses and granulomas.[13,14] The infected periradicular canal is the shelter for thousands of bacteria that can only be destroyed by surrounding tissues from either the apical foramen (anterior teeth) or the furcation region (posterior teeth). [15] The host defense is based on an inflammatory reaction that will require permanent efforts to combat the aggressor in the radicular canal. This process will, uneventfully, release products from bacteria metabolism and proteolysis of necrotic tissues to the organism.

Nowadays, Pediatric Dentistry faces a challenge. Children continue to suffer from carious lesions in the mouth, which have not been treated properly due to the false impression that they will uneventfully exfoliate and therefore do not require treatment. It is been proved that these teeth are significant bacteria focuses. [13,14]

Studies have shown that the immune system is involved in periapical lesions in permanent teeth.[15-22] It is reasonable to say that this mechanism may be present in primary teeth presenting with periradicular lesions. However, no scientific proof had been produced.

The aim of the present study is to verify the participation of the immune system by quantifying the presence of T cells, B cells and macrophages in periradicular lesions of primary teeth.

MATERIALS AND METHODS

Specimens

Fourteen primary molar teeth with periradicular lesion were obtained from children (aged 3-11 years old) at the Pediatric Dentistry Clinic of the Federal University of Santa Catarina state. Teeth were extracted due to advanced pathological root resorption, pulpal chamber floor perforation or large coronal destruction which made endodontic treatment unfeasible. Teeth were fixed in neutral-buffered formalin (10%).

The protocol for extraction and use of human samples was approved by the Ethics Committee of the institution .

Tissue preparation

Teeth were analyzed macroscopically.Tissue attached to the teeth´s roots was removed so that the histological sections could be made. Teeth were decalcified by a 10% ethylenediaminetetraacetic acid (EDTA) solution (pH 7,3) for 4 weeks. After that, decalcified teeth and tissue samples were washed in water for 24 hours, dehydrated in a graded series of ethanol and embedded in paraffin. Five sections of 3 μm

Table 1. Primary antibodies used to characterize inflammatory cells in periradicular lesions.

Antibody	Specificity	Dilution	Clone	Source
Mouse monoclonal antihuman CD45RO	180 kDa glycoprotein on T cells	1:500	OPD4	DakoCytomation
Mouse monoclonal antihuman CD20	33 kDa polypeptide on B cells	1:100	L26	DakoCytomation
Mouse monoclonal antihuman CD68	110kDa glycoprotein on macrophages	1:300	EBM11	DakoCytomation

Table 2. Patients data, histological diagnosis, bacteria presence and positive cell quantification for each sample.

Sample nº	Tooth	Sex	Age	Diagnosis	Infection on lesion	Immunohistochemical evaluation***		
						CD45RO	CD20	CD68
1	65	F	10y 4m	Acute abscess	No	6%	9%*	7%
2	74	M	9y 4m	Epitheliated granuloma	No	12%	6%*	6%
3	74	M	6y 1m	Granuloma	No	10%	-**	21%
4	74	M	4y 9m	Chronic abscess	Yes	15%	3%*	10%
5	85	M	6y	Chronic abscess	No	10%	11%*	6%
6	75	M	9y 1m	Chronic abscess	No	10%	7%	17%
7	85	M	6y 11m	Chronic abscess	Yes	7%	0,3%*	15%
8	85	M	9y 5m	Chronic abscess	No	11%	3%	3%
9	85	F	5y	Epitheliated granuloma	No	6%	1%*	8%
10	75	F	11y 4m	Granuloma	No	2%	0	11%
11	64	F	3y 9m	Epitheliated granuloma	No	53%*	19%*	10%
12	85	M	7y 3m	Chronic abscess	No	4%*	9%*	16%
13	65	M	6y 10m	Chronic abscess	No	-**	0	10%
14	74	F	6y 4m	Acute abscess	Yes	7%	0	13%
Mean						**11,76**	**5,25**	**10,92**
Standard Desviation						**12,87**	**5,69**	**4,98**

* focal distribution
** lose of the section
*** percentage of number of cells per 10 fields of vision.

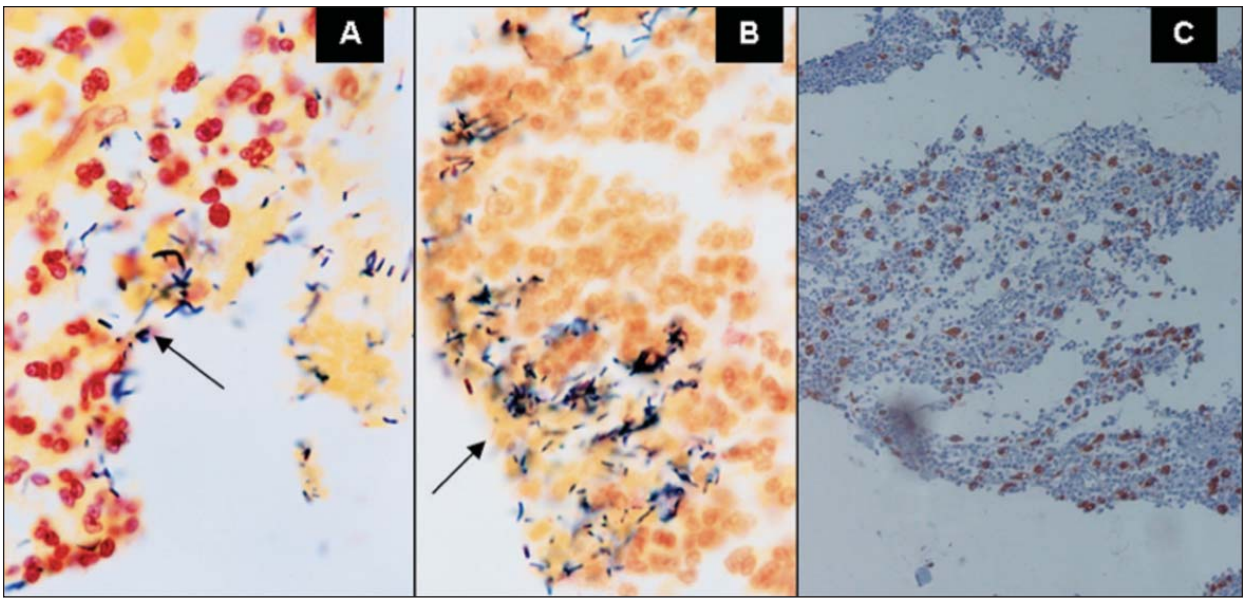

Figure 1. (A,B) Histological section of lesion (acute abscess) stained by BB technique showing bacteria (↗) on the lesion. Original magnification X330. (C) Immunohistochemical staining of CD68+ cells diffusely distributed. Original magnification X33.

thickness were obtained from each block and then mounted on a slide, separatelly. One section was stained with hematoxylin-eosin[23] (HE) for histopathological diagnosis. Another section was stained with Brown-Brenn (BB) technique to demonstrate the presence of bacteria.[24] The other four sections were mounted on Silane-coated glass slides (S3003, Dako Cytomation, Carpinteria, CA, USA) and processed in the standard manner for immunohistochemistry, using an immunoperoxidase method.

Immunohistochemistry

Sections were deparaffinized in xylene and rehydrated in descending concentrations of ethanol, followed by incubation on 3% hydrogen peroxide solution for 20 minutes and washed in distilled water to eliminate endogenous peroxidase activity. Then, sections were immersed in 0,01 M citrate buffer solution (pH 6.0) for 45 minutes at 95-98°C, to expose the antigens. After being washed with distilled water, the sections were incubated in 10mM phosphatase-buffered saline (PBS) solution (pH 7.2-7.4) (SIGMA CHEMICAL CO, St. Louis, MO, USA).

For each monoclonal antibody (Table 1) one section was performed. The monoclonal antibody solution was applied to the sections and the slides were maintained in a wet chamber for 12 hr at 2-8°C. Sections were then washed with PBS twice, for 5 minutes each at room temperature followed by incubations with anti-IgG/IgM secondary antibody conjugated with a peroxidase labelled polymer (EN VISION, Dako) for 1 hr at room temperature and washed again twice with PBS for 5 minutes each, at room temperature. The sections were submitted to a chromogenic reaction with a solution containing 0.03% 3.3-diaminobenzidine diluted in imidazole buffer (pH 7.2) and 0.3% hydrogen peroxide (Dako). After staining, the sections were counter-stained with Harris' hematoxylin, dehydrated in ascending concentrations of ethanol solutions, cleared in xylene and mounting in Entellan® (MERCK, Darmstadt, Germany). A lymph node was used as a positive control. Negative control was obtained by suppressing the application of primary antibodies. Stained cells were identified by the appearance of a brown ring surrounding the cellular membrane.

Evaluation of immunostaining

All sections were examined in a Olympus Bx 40 light microscope. The number of stained cells was counted in 10 consecutive microscopic high-power fields using a Muller eyepiece graticule at X100 magnification. Fields were selected by choosing the most representative region of each specimen, which was the place presenting the most cellular inflammatory infiltrate. Results were expressed as the mean percentage of positive cells which was calculated as the proportion of the total number of inflammatory cells.[19,22]

The distribution of stained cells was examined at X20 and X40 magnification and classified as diffuse or focal.

RESULTS

Results are sumarized inTable 2.

A total of 14 periradicular lesions of upper and lower molar primary teeth were examined. The patients consisted of 9 males and 5 females with a mean age of 7.3 (ranged from 3.9 to 11.4 years). Their histological diagnosis was acute abscess (n=2), chronic abscess (n=7), granuloma (n=2) and epitheliated granuloma (n=3). Three pulps were contaminated by bacteria (Fig. 1).

Immunohistochemical analisys showed that the mean of CD45RO+ cells was greater than other examined cells. The

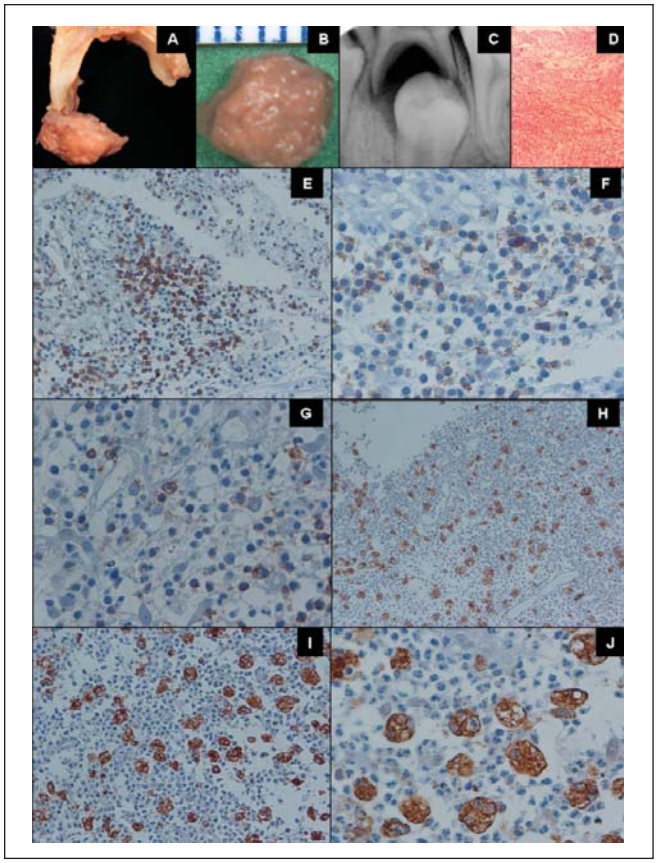

Figure 2. (A) Macroscopic aspect of the lesion attached to tooth and after being removed (B). (C) Periapical radiography of tooth (D) Histological section of lesion (granuloma) stained by HE technique. Original magnification X33. Immunohistochemical staining for CD45RO+ cells (E), CD20+ cells (F,G) focally distributed and CD68+ cells diffusely distributed (H,I,J). Original magnification X66, X132, X132, X33, X66, X132 respectively.

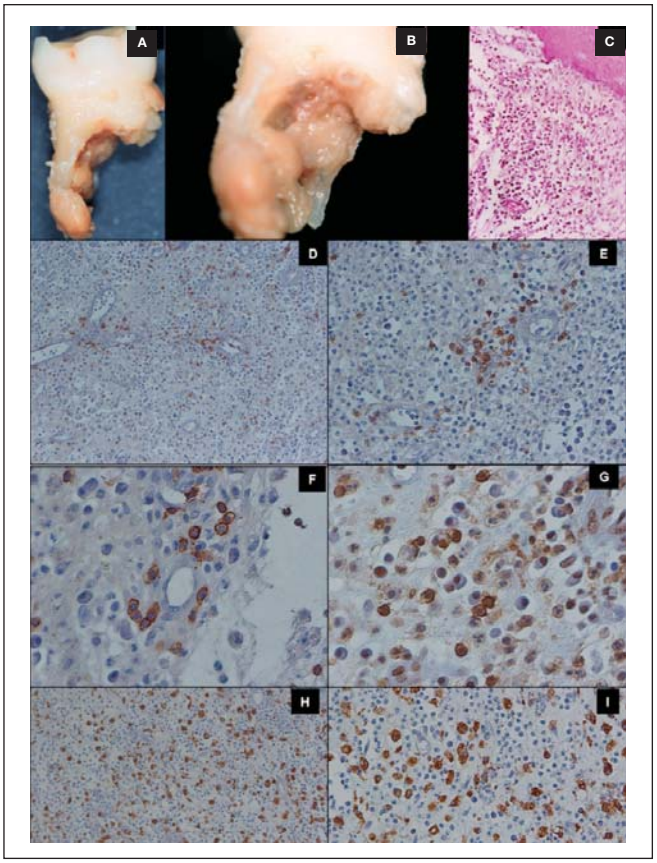

Figure 3. (A,B)Macroscopic aspect of the lesion. (C) Histological section of lesion (chronic abscess) stained by HE technique. Original magnification X66. Immunohistochemical staining for CD20+ cells (D,E,F), CD45RO+ cells (G), (F,G) CD68+ cells (H,I). Original magnification X33, X66, X132, X132, X33, X66 respectively.

mean ± SD of CD45RO (cell T) + cells was 11.76 ± 12.87. In the majority of the sample (n=12) these cells were distributed on a diffuse manner, on the inner area of lesion where cell infiltrate was more intense . The mean ± SD of CD20+ cells (cell B) was 5.25 ± 5.69. These cells were distributed focally (n=8), in region where the cell infiltrate was more intense. When B cells were focally distributed (3 samples) they outnumbered T cells. The mean ± SD of CD68+ cells (macrophages) was 10.92 ± 4.98. Macrophages were diffusely distributed on the lesion and placed close to neutrophils (Fig. 1,2,3,4). When the lesion was attached to the teeth odontoclasts expressing immunoreativity for CD68 were observed (Fig. 5).

DISCUSSION

The Immunohistochemical technique is a method used to visualize the cellular distribution in a tissue. Due to the variable distribution of cells in periradicular lesions, the cell distribution may vary according to the field under exam. The examined fields were chosen by selecting the area with greatest infiltrate, as previously done by Alavi *et al*.[25] and Rodini and Lara.[19] For example, one could notice that macrophages were spread on the entire lesion meanwhile other cells did not present this pattern. It is of significance to say that the obtained results do not reflect the pattern of the entire lesions.

The results confirm that the constitutive pattern of periapical lesions in primary teeth is the same as observed in permanent teeth.[15-22] Both cellular and humoral immune mechanisms are involved on the pathogenesis of the periapical lesion in primary teeth,[26,27] which is primarily formed by T cells, B cells, plasmatic cells, macrophages, neutrophils and mastocytes.[28]

As this is the first research to evaluate periradicular lesions in primary teeth using immunohistochemical techniques we have compared our findings with prior published studies regarding permanent teeth.

In the present study, the number of T cells exceeded the number of B cells in the majority of specimens. The T cells had a diffuse pattern of distribution. This result is the same as found by others authors.[2,17,25,27,29-32] Akamine *et al*.[33] reported that the greater number of T cells in the inflammatory infiltrate may occur due to its involvement in the development and progression of the lesion, while B cells main participation is on the repair process.

Johannssen[34] reported that T cells were more numerous in

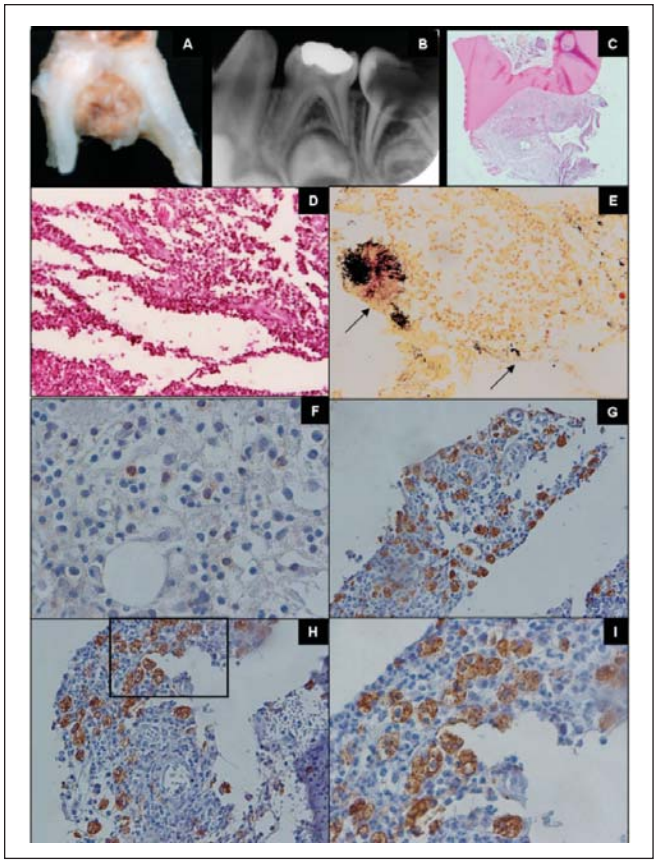

Figure 4. (A) Macroscopic aspect of the lesion attached to furcation region of the second lower molar. (B) Periapical radiography of tooth. Histological section of lesion (chronic abscess) stained by HE (C,D) and BB techniques (E) showed the presence of bacteria on the lesion. Original magnification X6.6, X66, X66 respectively. Immunohistochemical staining for CD20+ cells (F) and CD68+ cells (G,H,I). Original magnification X132, X66, X66, X132 respectively.

chronic lesions rather than acute lesions while plasmatic cells presented in a greater number is acute lesions.

B cells were more commonly found in focal patterns as some authors[2,17,30] have reported. Lukic *et al*. [30] studied 20 granulomas and developed a criterion to separate infiltrates according to the density and distribution pattern of cells. They observed that T cells are mainly located in the diffuse mononuclear infiltrate, while B cells are mostly seen in the focal mononuclear infiltrate. It is important to say that the focal infiltrate is related to a secondary humoral immune response, which is induced by pulpal and bacterial antigens of the radicular canal. Our study had 3 cases in which B cells were more numerous than T cells. In such cases, the B cells were distributed focally.

In all specimens, both B and T cells were localized on the inner portion of the lesion and rarely were visualized in the outer regions, near the fibrotic tissue. These findings are in agreement with other author's reports.[2,17] This pattern of distribution may be related to the opening of the radicular canals' apical foremen, through which the traffic of bacteria or it's metabolism products may occur. Philippi *et al*.[21] concluded that the fibrotic capsule, a region placed more distantly of the primary aggression, has cells related to remodeling rather than immune system related cells.

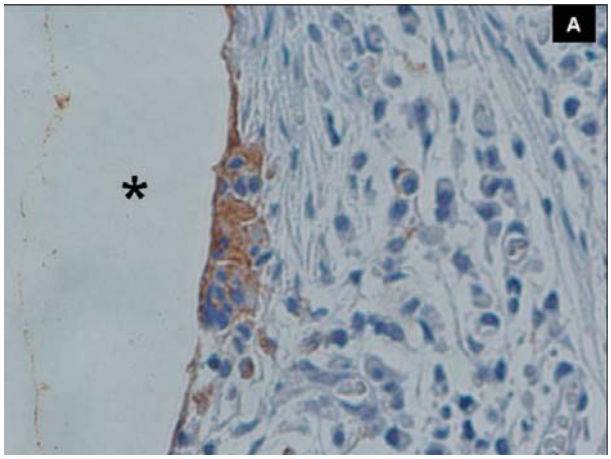

Figure 5. Immunohistochemical staining for CD68+ cells. Section showed the presence of odontoclasts expressing CD68 near root surface (*). Original magnification X132.

Accordingly to what has been said by others authors,[2,19] our specimens presented macrophages spread on the lesion as a whole. The presence of macrophages is extremely important not only for the protective response but also for the development and maintenance of the inflammatory reaction. The activated macrophage constitutes the first line of defense, by phagocytosis and the production of prostaglandins as a reaction for the presence of bacteria on the infected canal. Cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF-α) stimulate bone resorption and contribute to the expansion of the lesion. Furthermore, the macrophage, which is an antigen-presenting cell, activates T cells that will either start an immune response themselves or activate B cells in order to produce antibodies.[31]

CD68+ multinucleated cells suggest that they may contain odontoclasts precursors as stated by Angelova *et al*.[35] Multinucleated cells are present in physiological root resorption of primary teeth.[14] The presence of CD68+ multinucleated cells confirms that these cells are also present in pathological root resorption of primary teeth and are linked to the rapid root destruction we observed radiographically. Root resorption also occurs in permanent teeth, however it takes longer. It is important to emphasize that once the periapical involvement has been confirmed it is important to provide treatment as soon as possible so that we can diminish or even completely avoid sequels to the stomatognathic system and children's general health. T cells also contribute to the root resorption by secreting cytokines.

It is important to emphasize that the presence of periradicular lesions in primary teeth undoubtedly triggers a local inflammatory response in the children's organism. However, systemic involvement caused by bacteria located on perirapical lesions has been reported. Malmstrom and Natvig[36] showed that the reumathoid factor contained in plasmatic cells was found in periapical lesions of children with rheumatoid disease. Laine *et al*.[37] state that these lesions

may constitute a bacteremia focus, specially in imunnocompromised patients. Radics *et al*.[38] suggest that the periapical granuloma may compromise the general health by secreting pro-inflammatory cytokines. These cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor (TNF) trigger the acute phase characterized by fever, and hemo sedimentation speed. We are in complete agreement with the cited authors and we emphasize the importance of further studies in order to clarify the relation between these infectious focuses and the systemic modifications they promote.

Our findings allow us to state that care provided to patients who have endodontically compromised primary teeth must be comparable to that provided to adult patients. This statement is true not only by the fact that children possess an immature immune system[9,10] but also by the fact that they usually present more than one teeth affected by periapical lesions. If these teeth remain in the mouth they will, eventfully, cause local and systemic responses such as bone and dental resorption, damage to the permanent teeth, lack of appetite, low weight, fever and disability. The general population, especially parents, must be instructed to prevent these conditions and seek for help as soon as possible when symptoms appear. Further studies are necessary to provide new insights and scientific proofs regarding this issue so that one can improve the care provided to children who have endodontically compromised primary teeth and are exposed to its systemic consequences.

CONCLUSION

The immune system plays an important role on the periradicular inflammatory processes in primary teeth. Our results showed that the T and B cells and macrophages comprise the majority of the inflammatory infiltrate. These findings indicate that both humoral and cell-mediated immune reactions are present in human periradicular lesion of primary teeth.

REFERENCES

1. Kaneko T, Okiji T, Kan L, Takagi M, Suda H. Ultrastructural analysis of MHC class II molecule-expressing cells in experimentally induced periapical lesions in rat. J Endod, 27: 337–42, 2001.
2. Susuki T, Kumamoto H, Ooya K, Motegi K. Immunohistochemical analysis of CD1a-labeled Langerhans cells in human dental periapical inflamatory lesions—correlation with inflamatory cells and epithelial cells. Oral Diseases, 7: 336–43, 2001.
3. Cardoso M, Rocha MJC. Identification of factors associated with pathological root resorption in traumatized primary teeth. Dent Traumatol. In press.
4. Takahashi K. Microbiological, pathological, inflammatory, immunological and molecular biological aspects of periradicular disease. Int Endod J, 31: 311–25, 1998.
5. Siqueira JF. and Dantas CJS. Molecular and cellular inflamation mechanism. Medsi, Rio de Janeiro, p. 238, 2000.
6. Delves PJ, Roitt IM. The Immune System.1. N Engl J Med, 343(1): 37–50, 2000.
7. Delves PJ, Roitt IM. The Immune System.2. N Engl J Med, 343(2): 108–17, 2000.
8. Gorczynski R. and Stanley J. Clinical immunology. Reichmann and Affonso Editors, Rio de Janeiro, p. 355, 2001.
9. Carneiro-Sampaio MMS. and Grumach AS. Pediatric allergy and immunology. 2.ed. Savier, São Paulo, p. 3–27, 1992.
10. Igal LH. and Ron Y. Immunology and inflammation. Basic mechanisms and clinical consequences.McGraw Hill, New York, p. 563–71, 1994.
11. Brook I. Anaerobic infections in children. Adv Pediatr, 47: 395–437, 2000.
12. Stites DP, Terr AI. and Parslow TG. Clinical Immunology. 9.ed. Guanabara-Koogan, Rio de Janeiro, p. 689, 2000.
13. Serratine ACP. Intensity, localization and consequences of infection in deciduous molar with caries lesions, bone radioluscency in furcation region, floor perforation of pulp chamber: microscopic optic evaluation. [Doctoral thesis]. Florianópolis: Faculty of Odontology of Federal University of Santa Catarina; 2002.
14. Bolan M, Rocha MJC. Histopathological study of physiological and pathological resorptions in human primary teeth. Oral Surg Oral Med Oral Pathol, 104: 680–5, 2007.
15. Marton, IJ, Kiss C. Characterization of inflammatory cell infiltrate in dental periapical lesions. Int Endod J, 26(2): 131–6, 1993.
16. Yanagisawa S. Pathologic study of periapical lesions 1. Periapical granulomas: clinical, histopathologic and immunohistopathologic studies. J Oral Pathol, 9: 288–300, 1980.
17. Piattelli A, Artese L, Rosini S, Quaranta M, Musiani P. Immune cells in periapical granuloma: morphological and immunohistochemical characterization. J Endod, 17(1): 26–9, 1991.
18. Wallstrom JB, Torabinejad M, Kettering J, Mcmillan P, Linda L. Role of T cells in the pathogenesis of periapical lesions: a preliminary report. Oral Surg Oral Med Oral Pathol, 76(2): 213–8, 1993.
19. Rodini CO, Lara VS. Study of expression of CD68+ macrophages and CD8+ T cells in human granulomas and periapical cysts. Oral Surg Oral Med Oral Pathol, 92: 221–7, 2001.
20. Liapatas S, Nakou M, Rontogianni D. Inflamatory infiltrate of chronic periradicular lesions: na immunohistochemical study. Int Endod J, 36: 464–71, 2003.
21. Philippi CK, Rados PV, Sant'ana Filho M, Barbachan JJD, Quadros OF. Distribuition of CD8 and CD20 lymphocytes in chronic periapical inflammatory lesions. Braz Dent J, 14(3): 182–6, 2003.
22. Rodini CO, Batista AC, Lara VS. Comparative immunohistochemical study of presence of mast cells in apical granulomas and periapical cysts: Possible role of mast cells in the course of human periapical lesions. Oral Surg Oral Med Oral Pathol, 97: 59–63, 2004.
23. Volnei WG, Siqueira WC. Basic histotechnology. 2.ed. Manual, Brasília, p. 138–44, 1981.
24. Ribeiro FC. Bacterial arrangement in the hard structure of non-vital teeth with necrotic pulp and apical granuloma. [Master thesis]. Bauru: Faculty of Odontology of University of São Paulo; 1982.
25. Alavi AM, Gulabivala K, Speight PM. Quantitative analysis of lymphocytes and their subsets in periapical lesions. Int Endod J, 31: 233–41, 1998.
26. Torabinejad M, Kettering JD. Identification and relative concentration of B and T lymphocytes in human chronic periapical lesions. J Endod, 11(3): 122–5, 1985.
27. Stashenko P, Yu SM. T helper and T suppressor cell reversal during the development of induced rat periapical lesions. J Dent Res, 68(5): 830–4, 1989.
28. Stashenko P, Yu SM, Wang CY. Kinetics of immune cell and bone resorptive responses to endodontic infections. J Endod, 18(9): 422–6, 1992.
29. Barkhordar RA, Desouza YG. Human T-lymphocyte subpopulations in periapical lesions. Oral Surg Oral Med Oral Pathol, 65(6): 763–6, 1988.
30. Lukic A, Arsenijevic N, Vujanic G, Ramic Z. Quantitative analysis of the immunocompetent cells in periapical granuloma: correlation with the histological characteristics of the lesions. J Endod, 16(3): 119–22, 1990.
31. Kawashima N, Okiji T, Kosaka T, Suda H. Kinetics of macrophages and lymphoid cells during the development of experimentally induced periapical lesions in rat molars: a quantitative immunohistochemical study. J Endod, 22(6): 311–6, 1996.

32. Matsuo T, Ebisu S, Shimabukuro Y, Ohtake T, Okada H. Quantitative analysis of immunocompetent cells in human periapical lesions: correlations with clinical findings of the involved teeth. J Endod, 18(10): 497–500, 1992.
33. Akamine A, Hashiguchi I, Toriya Y, Maeda K. Immunohistochemical examination on the locatization of macrophages and plasma cells in induced rat periapical lesions. Endod Dent Traumatol, 10: 121–8, 1994.
34. Johannessen AC. Esterase-positive inflammatory cells in human periapical lesions. J Endod, 1986; 12(7): 284–8.
35. Angelova A, Takagi Y, Okiji T, Kaneko T, Yamashita Y. Immunocompetent cells in the pulp of human deciduous teeth. Arch Oral Biol, 49: 29–36, 2004.
36. Malmstrom M, Natvig JB. IgG rheumatoid factor in dental periapical lesions of patients with rheumatoid disease. Scand J Rheumatol, 4(4): 177–85, 1975.
37. Laine M, Venta I, Hyrkas T, Ma J, Konttinem YT. Chronic inflammation around painless partially erupted third molars. Oral Surg Oral Med Oral Pathol, 95: 277–82, 2003.
38. Radics T, Kiss C, Tar I, Marton IJ. Interleukin-6 and granulocyte-macrophage colony-stimulating factor in apical periodontitis: correlation with clinical and histologic findings of the involved teeth. Oral Microbiol Immunol, 18: 9–13, 2003.