Analysis of CD57+ natural killer cells and CD8+ T lymphocytes in periapical granulomas and radicular cysts

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Abstract: The aim of this study was to compare the number of CD57+ natural killer (NK) cells and CD8+ T lymphocytes between periapical granulomas (PGs) and radicular cysts (RCs). Twenty-fives cases of PGs and 25 of RCs were submitted to histological analysis and immunohistochemistry using anti-CD57 and anti-CD8 biomarkers. Positive cells were counted in 10 fields (400× magnification) and the median value was calculated for each case. Statistical tests were used to evaluate differences in the number of CD57+ NK cells and CD8+ T lymphocytes according to type of lesion, intensity of the infiltrate and thickness of the lining epithelium. The number of CD57+ NK cells and CD8+ T lymphocytes was higher in PGs than in RCs (p = 0.129 and p = 0.541, respectively). Comparison of the number of CD57+ NK cells in atrophic and hyperplastic epithelium revealed a larger number of cells in the atrophic epithelium (p = 0.042). A larger number of CD57+ NK cells and CD8+ T lymphocytes were observed in grade III infiltrates compared to grade I/II (p = 0.145 and p = 0.725, respectively). CD8+ T lymphocytes were more prevalent than CD57+ NK cells in most cases when PGs and RCs were analyzed separately or in combination (p < 0.0001). CD57+ NK cells and CD8+ T lymphocytes play a key role in antiviral defense and the presence of these cells supports evidence suggesting the participation of these microorganisms in the pathogenesis of PGs and RCs. The response mediated by CD8+ T lymphocytes was more frequent, indicating greater participation of the adaptive immunity in these chronic lesions.

Keywords: Radicular Cyst; Periapical Granuloma; Immunity, Cellular; Immunohistochemistry.

Introduction

Chronic periapical lesions develop after pulp necrosis as a response of the immune system to invasive stimuli in the periapical region.[1] These lesions are characterized by a mixed microbiota in which Gram-positive and Gram-negative bacteria predominate.[2] Some viruses, members of the herpesvirus family including Epstein-Barr virus (EBV), human cytomegalovirus (HCMV) and human herpesvirus 6, have also been associated with the resident microbiota of periapical lesions such as periapical granulomas (PGs) and radicular cysts (RCs).[3,4]

These herpesviruses are related to various mechanisms involved in the dysregulation of the immune response in periapical lesions which, according to some authors, increases the pathogenicity of these lesions.[5,6] The first line of defense of the immune system against viral invasion is mediated by natural killer (NK) cells.[7] These cells are actively involved in the host immune defense through the production of cytotoxic granules, activation of the Fas and TRAIL death receptors, and the production of a variety of cytokines that orchestrate the innate and adaptive immune responses by their action on T cells.[8] Despite recent advances, studies on the importance of NK cells for the host defense against viral infections are still limited. Although these cells are considered components of the innate immunity, some specific types of NK cells have been shown to proliferate in response to certain viral infections, including infection with HCMV.[9,10]

Several markers that could be used to better characterize the different phases of NK cell maturation are being studied. Based on the expression of CD56, two subsets of these cells have been identified: CD56bright and CD56dim. CD56bright NK cells do not express CD57 and are more frequent in secondary lymphoid organs. These cells are characterized as an immunomodulatory subset since they express high levels of cytokines (interferon γ [IFN-γ] and tumor necrosis factor [TNF]) and contain very few cytolytic granules. On the other hand, CD56dim cells are CD57^{+} NK cells frequently found in peripheral tissue and are characterized by marked cytotoxic activity as a result of the high expression of granzymes and perforins that destroy infected cells. In addition, CD57^{+} CD56dim NK cells produce increased levels of IFN-γ upon target cell recognition. Within this context, some studies claim that CD57^{-} CD56bright NK cells are precursors of CD57^{+} CD56dim cells.[9,10] Thus, CD57 is a very useful marker of NK cell maturation and activation and has been used in experiments to consistently detect these cells.[2,10]

With the establishment of an infection, a specific immune response develops. CD8^{+} T lymphocytes play a key role in this type of response.[11] Together with CD4^{+} T lymphocytes, these cells represent the most frequent population in periapical lesions and are important for the immunogenic mechanisms involved in their pathogenesis.[12,13,14,15] According to Slots et al.,[7] CD8^{+} T lymphocytes are key players in the antiviral host defense and are found in large numbers in periapical lesions. In addition to destroying infected cells, these lymphocytes produce proinflammatory cytokines such as IFN-γ, TNF-α, TNF-β, and granulocyte-macrophage colony-stimulating factor.[16]

Some studies suggest the participation of NK cells and CD8^{+} T lymphocytes in chronic periapical lesions.[2,4,17] Therefore, the objective of this study was to evaluate the presence of CD57^{+} NK cells and CD8^{+} T lymphocytes in PGs and RCs by immunohistochemistry, correlating their immunoexpression with the type of lesion, degree of inflammatory infiltration and thickness of the lining epithelium in RCs, in order to increase our understanding of the immunopathogenesis of chronic periapical lesions.

Material and methods

Human chronic periapical lesions samples

Fifty specimens, including 25 PGs and 25 RCs, obtained from the Laboratory of Oral Pathology, Department of Dentistry, Federal University of Rio Grande do Norte, were randomly selected for the study. The pathological diagnosis of the lesions was confirmed based on the combination of clinical, radiographic and histopathologic features. All PG and RC cases were obtained from patients who did not undergo previous endodontic treatment of the teeth associated with the lesions. We excluded the cases that did not present necessary clinical information, as well as the cases that did not have enough material for the morphological and immunohistochemical study. The study was approved by the Ethics Committee of the Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil (Permit No. 466/12).

Morphological analysis

For morphological analysis, the specimens were cut into 5-μm thick sections and stained with hematoxylin/eosin. The intensity of the inflammatory infiltrate was evaluated in all specimens. One microscopic field extending from the central portion

of PGs and from the subepithelial region of RCs to the periphery was evaluated. This field was divided into three parts at 400× magnification and the lesions were classified as follows, adapted from Tsai et al.[18]: grade I (mild), inflammatory infiltrate restricted to the first part; grade II (moderate), inflammatory infiltrate extending to the second part; grade III (intense), inflammatory infiltrate extending to the third part. The lining epithelium of RCs was evaluated using the criterion suggested by Moreira et al.,[19] in which RCs containing 2 to 10 layers of cells in their greatest length are classified as atrophic and those containing more than 10 layers of cells are classified as hyperplastic.

Immunohistochemical methods

The paraffin-embedded specimens were cut into 3-µm thick sections. The antigen retrieval was performed according to the following specifications: Citrate, pH 6.0, Pascal, 37°C, 3 min. The specimens were submitted to immunohistochemistry by the streptavidin-biotin method optimized by LSAB amplification system (Streptavidin biotin-labeled primary mouse antibodies, DAKO, Carpinteria, USA). The sections were incubated for 60 min with the following primary antibodies: anti-CD57 (clone TB01, Dako), diluted 1:100, and anti-CD8 (clone C8/144B, Dako), diluted 1:500. The reaction was developed with 3,3-diaminobenzidine (Sigma Chemical Co., St. Louis, USA) as chromogen and the sections were counterstained with Mayer's hematoxylin. Sections of normal human tonsils were used as positive control for CD57 and CD8. As negative control, the sections were submitted to all steps described above but replacing the primary antibody with 1% bovine serum albumin.

Immunohistochemical analysis

After immunohistochemical staining, a trained observer analyzed the specimens throughout their extent under an Olympus CX41 light microscope (Tokyo, Japan) at 100× magnification to identify the area with the largest number of immunoreactive cells. Large CD57^{+} immunopositive cells with a cytoplasm containing several voluminous granules easily identified by light microscopy were classified as NK cells. The cytotoxic T lymphocytes were identified as small CD8^{+} cells with sparse agranular cytoplasm surrounding a spherical and intensely stained nucleus[20]. After identification of this area, the total number of immunopositive cells was counted in 10 consecutive fields at 400× magnification and the sum of these numbers was used to calculate the mean for each case. Subsequently, the median number of NK cells and CD8^{+} T lymphocytes was calculated for each group.

Statistical analysis

The results were analyzed statistically using the Statistical Package for the Social Sciences (version 17.0; SPSS, Inc., Chicago, USA). For evaluation according to the intensity of the inflammatory infiltrate, lesions with a grade I and II infiltrate were combined in a single group and compared to lesions with a grade III infiltrate. The number of CD57^{+} NK cells and CD8^{+} T lymphocytes was compared according to type of lesion (PGs or RCs), intensity of the inflammatory infiltrated (grade I/II or grade III), and type of cystic lining (atrophic or hyperplastic) by the nonparametric Mann-Whitney test. The Wilcoxon test was used to verify the distribution of cases according to the ranks of immunostaining scores for CD8^{+} and CD57^{+} in PGs and RCs. Spearman's correlation test was performed to verify possible correlations between the number of CD57^{+} NK cells and CD8^{+} T cells in periapical granulomas and radicular cysts. A level of significance of 5% ($p < 0.05$) was adopted for all tests.

Results

Clinical and radiographic aspects

The analysis of the 25 cases of PGs showed a higher incidence in female patients (n = 19; 76%), the age of the patients ranged from 15 to 57 years, with a mean age of 31,6 years, anatomically sited mostly in the anterior maxilla (n = 13; 52%), showing well delimited radiolucencies (n = 21; 84%). The 25 RCs were more prevalent in female patients (n = 16; 64%), the age ranged from 19 to 78 years, with a mean age of 36.6 years. As for the radiographic appearance and anatomical location, a radiolucent image was observed in 23 cases (92%), mostly in the anterior region of the maxilla (n = 15; 60%).

Morphological analysis

Analysis of the intensity of the inflammatory infiltrate showed a grade III infiltrate in 84% (n = 21) of the 25 cases of PGs. In RCs, a grade III infiltrate was observed in 56% (n = 14) of the cases, while a grade II infiltrate was present in 32% (n = 8) and a grade I infiltrate in 12% (n = 3). Regarding the thickness of the lining epithelium in RCs, the epithelium was atrophic in 60% (n = 15) of the cases and hyperplastic in 40% (n = 10).

Immunohistochemical analysis

CD57$^+$ cells

CD57$^+$ cells were detected in 22 (88%) cases of PGs (Figure 1A) and in 21 (84%) RCs (Figure 1B). The median number of CD57$^+$ cells was 6.5 in PGs and 2.5 in RCs. The nonparametric Mann-Whitney test revealed no statistically significant difference in CD57$^+$ immunopositivity between the two groups (p = 0.129). There was no significant difference in the number of CD57$^+$ cells according to the degree of inflammatory

infiltration (p = 0.145). The thickness of the lining epithelium in RCs was also correlated with the number of CD57$^+$ cells. The median number was 4.5 in lesions with an atrophic epithelium and 1.5 in lesions with a hyperplastic epithelium. The nonparametric Mann-Whitney test showed a significant difference in CD57 immunopositivity between these two groups (p = 0.042) (Figure 1A, B) (Table 1).

CD8$^+$ T lymphocytes

Analysis of the immunoexpression of CD8$^+$ T lymphocytes revealed the presence of these cells in 25 (100%) PGs (Figure 1C) and in 23 (92%) RCs (Figure 1D). The median number of CD8$^+$ T lymphocytes was 84.5 in PGs and 70.5 in RCs. The nonparametric Mann-Whitney test showed no significant difference in immunostaining for CD8$^+$ T lymphocytes between lesions (p = 0.541) or between the different degrees of inflammatory infiltration (P=0.725). Regarding the association between CD8$^+$ T lymphocytes and epithelial thickness of RCs, the median number of these cells was 70.5 in lesions with atrophic epithelium
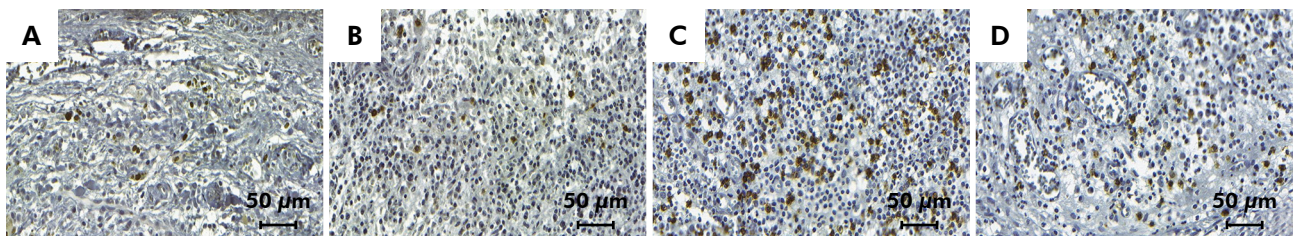

Figure 1. Immunoexpression of CD57 and CD8 in periapical lesions. (A) Photomicrograph showing weak immunostaining for CD57$^+$ cells in periapical granuloma. (B) Photomicrograph showing weak immunostaining for CD57$^+$ cells in radicular cyst. (C) Immunostaining for CD8$^+$ cells in periapical granuloma. (D) Immunostaining for CD8$^+$ cells in radicular cyst (Pannoramic Viewer 1.15.2, 50 μm).

Table 1. Median number of CD57$^+$ cells in periapical granulomas and radicular cysts and differences according to type of lesion, degree of inflammatory infiltration and epithelial thickness in radicular cysts.

Variable	n	Median	Q_{25}–Q_{75}	Mean rank	Sum of ranks	U	P
Lesion							
Periapical granuloma	25	6.5	1.0–13.95	28.62	715.5	234.5	0.129
Radicular cyst	25	2.5	0.75–6.25	22.38	559.5		
Inflammatory infiltrate							
Grade I/II	12	2.0	1.0–3.5	20.17	242.0	164.0	0.145
Grade III	38	4.5	1.0–14.37	27.18	1,033.0		
Lining epithelium							
Atrophic	15	4.5	1.0–14.0	15.43	231.5	38.5	0.042
Hyperplastic	10	1.5	0.3–3.0	9.35	93.50		

and 72.25 in lesions with hyperplastic epithelium, with no statistically significant difference between these two groups (p = 0.397) (Figure 1C, D) (Table 2).

The Kolmogorov–Smirnov test revealed absence of a normal distribution for the number of CD57^{+} NK cells (p < 0.0001) and CD8^{+} T lymphocytes (p = 0.035).

The number of CD8^{+} T lymphocytes and CD57^{+} cells in PGs and RCs was also analyzed. The Wilcoxon test showed a larger number of CD8^{+} T lymphocytes in most cases when PGs and RCs were analyzed separately or in combination. All tests had p < 0.0001. (Table 3) Spearman's correlation test disclosed no significant correlation between the number of CD57 and CD8 T cells in periapical granulomas (r = 0.311; p = 0.130) and radicular cysts (r = 0.267; p = 0.197).

Discussion

Periapical lesions develop in response to microbial infections in the root canals. After pulp necrosis, microorganisms stimulate the migration of immune system cells to the periapical region where these cells trigger a series of reactions that involve the recruitment of other inflammatory cells and the release of proinflammatory and immunoregulatory cytokines and chemokines.[14,21] In this respect, an increasing number of studies have demonstrated the important role of viruses in the etiopathogenesis of periapical lesions, especially symptomatic and large lesions, but their exact role in the formation and development of these alterations remains uncertain.[2,3,4] A better understanding of the cytotoxic response involved in the defense against these microorganisms is therefore extremely important.

This study evaluated the presence of CD57^{+} NK cells and CD8^{+} T lymphocytes in the pathogenesis of PGs and RCs since they are important cells involved in the defense against intracellular microorganisms. CD57 was first identified in NK cells, but its expression was later also observed in CD8^{+} T cells. CD8^{+}/CD57^{+} T cells are considered senescent cells whose function is not fully understood, while in NK cells the acquisition of CD57 confers a greater cytotoxic potential to the cell.[2,10] In the present study, cells exhibiting immunostaining for CD57 associated with the cell morphology findings (large CD57 immunopositive cells with a cytoplasm containing several voluminous granules) were defined as NK cells.

Table 2. Median number of CD8^{+} T lymphocytes in periapical granulomas and radicular cysts and differences according to type of lesion, degree of inflammatory infiltration and epithelial thickness in radicular cysts.

Variable	n	Median	Q_{25}–Q_{75}	Mean rank	Sum of ranks	U	P
Lesion							
Periapical granuloma	25	84.5	28.6–120.4	26.76	669.0	281.0	0.541
Radicular cyst	25	70.5	29.75–121.75	24.24	606.0		
Inflammatory infiltrate							
Grade I/II	12	62.0	28.75–118.12	24.21	290.5	212.5	0.725
Grade III	38	79.95	28.8–123.35	25.91	984.5		
Lining epithelium							
Atrophic	15	70.5	49.5–85.0	11.93	179.0	59.0	0.397
Hyperplastic	10	72.25	9.62–159.92	14.6	146.0		

Table 3. Distribution of cases according to the ranks of immunostaining scores for CD8 and CD57 in periapical granulomas and radicular cysts (Wilcoxon test).

Lesion	CD8 < CD57	CD8 > CD57	CD8 = CD57	p
Periapical granuloma	2 (8%)	23 (92%)	0 (0%)	< 0.0001
Radicular cyst	1 (4%)	23 (92%)	1 (4%)	< 0.0001
Periapical granuloma + radicular cyst	3 (6%)	46 (92%)	1 (2%)	< 0.0001

CD57^{+} cells exert immunoregulatory functions, producing cytokines such as IFN-γ, the main cytokine responsible for the activation of macrophages that destroy phagocytosed microorganisms. In addition, these cells play an effector role in direct cell lysis and antibody-dependent cell-mediated cytotoxicity.[8,22,23] Immunohistochemical analysis of CD57^{+} NK cells showed a small number of stained cells in the two types of lesions. This number was larger in PGs (median 6.5) than RCs (median 2.5), but the difference was not statistically significant (p = 0.129). Kettering and Torabinejad[24] analyzed 10 periapical lesions and detected CD57^{+} NK cells by immunohistochemistry in all samples, but their numbers varied widely from one lesion to another. The authors concluded that the presence of these cells in chronic periapical lesions is related to a nonspecific reaction of the immune system to microorganisms present in the root canal system. According to Loyola et al.,[25] it is widely accepted that the formation of cystic cavities in PGs is due to necrosis; however, although little studied in periapical lesions, apoptosis is also believed to play a role in the formation of RCs. The occurrence of cell death in the process of cavitation of a PG and its progression to an RC through activation of the apoptotic cascade may explain the slow progression of these alterations.[26] Thus, the higher expression of CD57^{+} NK cells in PGs, although not statistically significant, may be related to a higher activity of these cells in early stages of chronic periapical lesions, which also contribute to the formation of RCs by inducing the apoptosis of cells infected with microorganisms.

In the present study, analysis of the association between the number of CD57^{+} NK cells and the inflammatory infiltrate showed a larger number of cells in lesions with a grade III infiltrate (median 4.5) compared to lesions with a grade I/II infiltrate (median 2.0), but the difference was not statistically significant (p = 0.145). It is known that some cytokines produced by inflammatory cells during infection, especially IL-12, activate CD57^{+} NK cells.[22] IL-12 induces the production of IFN-γ, thereby increasing the cytotoxic capacity of CD57^{+} NK cells.[27] Sabeti et al.[6] analyzed the presence of EBV and HCMV and the expression of different cytokines, including IL-12, in periapical lesions. Using polymerase chain reaction (PCR), the authors observed significant correlations between infection with EBV and HCMV and the expression of some cytokines such as IL-12. It may thus be suggested that the larger number of CD57^{+} NK cells associated with a more intense inflammatory infiltrate is due to greater antigen stimulation as a direct result of viral infection and the consequent increase in the virulence of bacteria present in the endodontic infection.[3,5]

Regarding the thickness of the lining epithelium in RCs, a significantly larger number of CD57^{+} NK cells was observed in lesions with cystic atrophic epithelium (median 4.5) compared to those with hyperplastic epithelium (median 1.5) (p = 0.042), corroborating the study of Moreira et al.[19] It is believed that CD57^{+} NK cells are involved in proapoptotic mechanisms related to the death of cystic epithelial cells.[4] The main function of mature CD57^{+} NK cells is cytotoxic activity. This cytotoxicity is considered to be fast, very potent and has multiple facets. CD57^{+} NK cells can induce apoptosis via two main mechanisms: the release of granzymes and perforins by granules towards the target cell and death signaling through TNF receptor family members.[28] Thus, the larger number of CD57^{+} NK cells in atrophic lesions suggests that these cells play an important role in apoptosis of the cystic epithelium, participating in the homeostatic control of the lesion.

Cell death mediated by CD8^{+} T lymphocytes mainly occurs in cells infected with intracellular microorganisms or in tumor cells. These cells can exert cytotoxic or suppressor functions. The development of cytotoxic CD8^{+} T lymphocytes is triggered by the recognition of antigens associated with MHC class I on the target cell by naïve CD8^{+} T lymphocytes, an event that causes cell death mediated mainly by the release of cytotoxic granules.[29,30]

CD8^{+} T lymphocytes are key players in the antiviral host defense and are found in larger numbers in periapical lesions.[7] These cells seem to exert some immunoregulatory activity in these lesions, participating in their stabilization. However, the exact mechanism of how this occurs has not yet been fully clarified, but is probably related to the release of cytokines such as IL-6, IL-10 and TGF-β whose main function is to inhibit the inflammatory response.[4,17]

In the present study, strong immunoexpression of these lymphocytes (cells with sparse agranular

cytoplasm surrounding a spherical and intensely stained nucleus) was observed in both PGs (median 84.5) and RCs (median 70.5), but no significant difference was observed between lesions (p = 0.541). According to Harari et al.,[29] the primary function of CD8^{+} T lymphocytes is the detection and elimination of cells transformed or infected by viruses. These lymphocytes induce lytic activity by two well-established mechanisms: the release of cytotoxic granules and receptor-dependent cell death, which is activated by the interaction of CD95 (Fas), TNF-α or TRAIL with ligands present on the surface of CD8^{+} T lymphocytes. In this respect, Lin et al.[31] defend that tissue destruction of infected cells in PGs is related to cystic formation that culminates in the formation of RCs, since one of the main consequences of viral infection is the induction of a cytotoxic response by the organism mediated mainly by CD8^{+} T lymphocytes.

Evaluation of the intensity of the inflammatory infiltrate showed a larger median number of CD8^{+} T lymphocytes in lesions with a grade III infiltrate (79.95) compared to those with a grade I/II infiltrate (62.0), but the difference was not statistically significant (P=0.725). According to Philippi et al.,[12] the concentration and distribution of CD8^{+} T lymphocytes are directly associated with the concentration of antigens in periapical lesions. Muglali et al.[32] emphasized the importance of proinflammatory cytokines such as IL-1 and TNF-α for the pathogenesis of periapical lesions, including the activation of osteoclasts, production and secretion of matrix metalloproteinases and stimulation of keratinocyte proliferation, as well as promotion of inflammatory cells recruitment.

As observed for CD57^{+} NK cells, the larger number of CD8^{+} T lymphocytes in more inflamed periapical lesions may be related to the exacerbation of inflammation in response to more potent antigen stimuli. In this respect, it has been suggested that herpesvirus can interact synergistically with endodontic bacteria, increasing their pathogenicity, which results in greater stimulation of the inflammatory response in the periapical region.[4] In developing countries, such as Brazil, more than 50% of the population is exposed to herpes simplex virus 1 by 5 years of age, 95% by 15 years of age, and nearly 100% by 30 years of age.[33]

Analysis of immunohistochemical staining for CD8^{+} T lymphocytes according to the thickness of the lining epithelium in RCs revealed no significant difference between lesions with atrophic and hyperplastic epithelium (p = 0.397). The behavior of periapical inflammatory lesions depends on the balance between cell proliferation and cell death. Within this context, apoptosis also participates in the maintenance of the lining epithelium thickness in RCs.[34,35] Soluk Tekkeşin et al.[36] observed overexpression of the proapoptotic molecule BAX in the epithelium of RCs. Loreto et al.[26] demonstrated the involvement of apoptosis in the pathogenesis of RCs by observing immunoexpression of TRAIL/DR5 and caspase-3 in the lining epithelium and connective tissue capsule, findings that may explain in part the indolent clinical presentation and low growth potential of these lesions. With respect to the size of the lesions, Gazidova et al[17] found a positive correlation between the levels of IL-10 and TGF-β, immunomodulatory cytokines, and the frequency of CD8^{+} T lymphocytes in chronic periapical lesions, as well as between the number of CD8^{+} T lymphocytes and lesions with larger diameters.

These findings suggest that the larger number of CD8^{+} T lymphocytes in RCs with hyperplastic epithelium and in lesions with larger diameters is related to the immunosuppressive and apoptotic capacity of these cells that act during the phase of expansion of periapical lesions, regulating their growth.

A higher intensity of CD8^{+} T lymphocytes compared to CD57^{+} cells was observed in all analyses (p < 0.0001). The larger number of CD8^{+} T lymphocytes in PGs and RCs indicates their marked involvement in these lesions, particularly due to their cytotoxic activity, destroying infected cells especially those infected with viruses. These findings also suggest an important role of the adaptive immunity in the pathogenesis of chronic periapical lesions.[12,13,14,15]

We emphasize that the integrity of the immune system is essential for the defense against infectious organisms and their toxic products. Immunodeficiency can compromise the number and activation of NK cells and CD8^{+} T lymphocytes, resulting in defects in the effector mechanisms of innate and adaptive immunity, thus leading to increased susceptibility for and difficulty in controlling infections.[37,38]

Further research should examine the presence of viruses directly (e.g., real-time PCR or DNA hybridization) in specimens and then establish their association with CD57^{+} NK cells and CD8^{+} T lymphocytes.

Conclusions

NK cells and CD8^{+} T lymphocytes are key players in the antiviral host defense and their presence in RCs and PGs supports evidence of viruses participation in the pathogenesis of these lesions. The response mediated by CD8^{+} T lymphocytes was found to be more frequent, thus indicating greater participation of the adaptive immunity in the pathogenesis of chronic periapical lesions. In addition to their direct involvement in cytotoxic mechanisms, CD57^{+} NK cells and CD8^{+} T lymphocytes may also participate in the control of cystic growth through their immunomodulatory activity.

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