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ORIGINAL ARTICLE

Immunohistochemical analysis of inflammatory infiltrate in aggressive and chronic periodontitis: a comparative study

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Abstract This immunohistochemical study evaluated the inflammatory infiltrate with its cluster differentiation markers (CD 4, CD 8, CD 20, and CD 68) in aggressive and chronic periodontitis gingival tissues in order to identify the specific cell distribution. Twenty-seven human gingival biopsies were obtained and analyzed. Fourteen patients were suffering from chronic periodontitis and six from aggressive periodontitis; seven patients with healthy gingiva were included as the control group. The specimens were immunohistochemically stained for anti-CD 4 (T helper cells), anti-CD 8 (T cytotoxic/suppressor), anti CD-20 (B plasma cells) and anti CD-68 (macrophages). Chronic periodontitis samples were mainly dominated by CD 4 and CD 8+ cells. On the contrary, in aggressive periodontitis patients all four cell types (CD 4, CD 8, CD 20 and CD 68 + cells, respectively) were remarkably increased. CD 20+ cells were significantly ($p<0.05$) more prevalent in aggressive versus chronic periodontitis. The control samples expressed lower CD 4, CD 8, CD 20 and CD 68+ cells confirming a none inflammatory state. The present study demonstrates prevalence for CD 20+ cells in aggressive periodontitis lesions. However, further studies need to be performed to confirm and identify a clear pattern of inflammatory cells and hereafter the mechanisms sustaining the disease.

Keywords Generalized aggressive periodontitis · Chronic periodontitis · Periodontal diseases · Immunhistochemistry · Inflammatory infiltrate

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Introduction

Periodontal disease is currently described by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions, American Academy of Periodontology, of particular interest of this study are the two forms: chronic periodontitis and aggressive periodontitis [1]. The latter has not been extensively studied; in particular, the data available regarding the inflammation process underlying its onset and progression are still limited [2–5].

Previous descriptions of early onset forms of periodontitis, due to significantly changed local factors and tissue reactions, have been confirmed and named as aggressive periodontitis [6]. This is an infectious disease with an abnormal host response to specific bacterial plaque [7] causing inflammation and destruction of periodontium that is characterized by rapid clinical attachment loss and alveolar bone resorption. Neutrophils and monocyte/macrophages are the first line of cellular defense in the host resistance to bacterial invasion. Consequently, several researchers have studied the role of leukocytes in the host inflammatory response to chronic periodontitis [8–14] and in aggressive periodontitis [5].

The main cells involved in the inflammatory process of periodontitis and the disease itself provide an important

model for the investigation of the pathologic potential of human cellular infiltrates [15]. Inflammatory cells contain different subsets (cytotoxic T lymphocytes, B lymphocytes, macrophages, etc.) characterized by the expression of specific cell surface antigens that allow researchers to perform immunohistochemical and quantitative studies. The cells of interest and their known markers are macrophages/histiocytes (CD 68), T helper cells (CD 4), T cytotoxic or T suppressor cells (CD 8), and B cells (CD 20).

Various studies have been carried out to investigate the proportions of lymphocyte subpopulations in the peripheral blood [1, 16, 17] as well as in biopsies of periodontal tissues [18–21]. Early on an assumption has been made that T lymphocytes were predominant in gingivitis, whereas B cell dominance developed during the formation of a periodontal lesion [22, 23]. Shortly after, Pietruska et al. [24] confirmed that B cells were more frequently observed in generalized chronic periodontitis, but they also found that T lymphocytes were more frequent in aggressive periodontitis lesions.

The limited data available on this subject lead to this human study. Therefore, the purpose of this study was to conduct an immunohistochemical evaluation of the inflammatory infiltrate with its cluster differentiation markers (CD 4, CD 8, CD 20, and CD 68) in aggressive and chronic periodontitis tissues in order to identify the specific cell distribution.

Materials and methods

Study design and surgical procedure

Twenty-seven systemically healthy subjects were selected from the population referred to the periodontal clinic of Guarulhos University (Guarulhos, SP, Brazil), 15 men and 12 women (age, 22 to 72 years; mean, 54.3 years). The medical and dental histories were obtained and a full-mouth periodontal examination was performed. Based on these data, the periodontal diagnosis was made, and subjects who fulfilled the inclusion/exclusion criteria were invited to participate in the study. The study protocol was explained to each subject, and a signed informed consent was obtained. This study protocol was approved by Guarulhos University's Ethics Committee in Clinical Research (#205/03).

Inclusion criteria

Generalized aggressive periodontitis, chronic periodontitis, or clinical periodontal healthy subjects were diagnosed based on the periodontal classification of the American Academy of Periodontology [25]. Subjects had at least 20 teeth and needed to meet the following criteria in order to be included in this study:

Generalized aggressive periodontitis [26]

- <35 years of age;
- Minimum of six permanent incisors and/or first molars with at least one site each with probing depth (PD), clinical attachment level (CAL) >5 mm and bleeding on probing (BOP);
- Minimum of three teeth other than first molars and incisors with at least one site each with PD and CAL >5 mm;
- Familial aggregation (at least one other member of the family presenting or with history of periodontal disease).

Chronic periodontitis

- >35 years of age;
- Minimum of six teeth with at least one site each with PD and CAL >5 mm;
- At least 30% of the sites with PD and CAL >4 mm and presence of BOP.

Periodontal health

- >18 years of age;
- No sites with PD and CAL measurements >3 mm and <20% of sites exhibiting gingival bleeding and/or bleeding on probing.

In addition, all subjects needed to meet the following criteria: controlled oral hygiene, absence of any lesions in the oral cavity, and a wide band of keratinized tissue (>4 mm) and all subjects had to agree to participate in a post-operative control program.

Exclusion criteria

Exclusion criteria were pregnancy, lactation, smoking, any systemic condition that could affect the progression of periodontal disease (e.g., diabetes and immunological disorders), long-term administration of anti-inflammatory medication, excessive consumption of alcohol, localized radiation therapy of the oral cavity, and antineoplastic chemotherapy

Gingival biopsies were obtained around the teeth from either mesial or distal aspect of the periodontal lesion (AgP and ChP) including the lateral wall of the periodontal pocket during periodontal surgery to treat residual probing pocket depth or gingival sulci (PH gingival margin (esthetic

periodontal surgery)[27]. They were performed with a 15c blade. The dimensions of the gingival biopsies were around 1.5 mm in thickness and 2–3 mm in height. Seven gingival biopsies from subjects with periodontal health, 14 with chronic periodontitis, and six with generalized aggressive periodontitis were obtained.

Histological and immunohistochemical analysis

After retrieval, biopsies were immediately fixed in 10% buffered formalin. The retrieved specimens were stained with hematoxylin-eosin first and then underwent immunohistochemistry. From each biopsy, 25 sections about 3 um were prepared. Specifically, anti-CD 20 (B plasma cells), anti-CD 4 (T helper cells), anti-CD 8 (T cytotoxic/suppressor), and anti-CD 68 (macrophages) were used.

The immunohistochemical staining of CD 4 (diluted at 1:25), CD 8 (diluted at 1:100), CD 20 (diluted at 1:50), and CD 68 (diluted at 1:50) was performed using the strep-ABC (Streptavidine-Biotine-Peroxidase) method. Three-micrometer sections were cut and mounted on poly-L-lysine-coated slides. Paraffin sections were dewaxed by xylene, rehydrated and finally washed in PBS (pH 7.4) for 10 min. In order to unmask the antigens, a microwave oven and a 2.1% content of citric acid was used related to the antibodies CD 4, CD 8, CD 20, CD 68. The subsequent steps were optimized by automatic staining device (Optimax, BioGenex, San Ramon, CA, USA). Sections were incubated with primary antibody for 30 min at room temperature. Slides were rinsed in buffer, and immunoreaction was completed with the Strep-ABC-Peroxidase method, applying the "Peroxidase Detection System" kit by Novocastra (Newcastle Upon Tyne, UK) and utilizing a multi-link as a secondary biotinylated antibody. After incubation with a chromogen employing "liquid DAB substrate pack", the specimens were counterstained with Mayer's hematoxyline and cover slipped. A negative control using the secondary antibody without the primary one was used.

At the analysis, each specimen was divided into three areas to allow quantification of the distribution of CD marked cells: (1) sulcular epithelium; (2) lamina propria; (3) oral gingival epithelium. The evaluations were performed in ten randomly selected high power (×40) fields for each area by a trained examiner. Quantitative analysis was performed for CD 4, CD 8, CD 20 and CD 68+ cells.

A light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera and interfaced to a monitor and personal computer was used to evaluate CD 4, CD 8, CD 20, CD 68+ cells in the areas of gingival biopsies. This optical system was associated with a digitizing pad and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc, Immagine and Computer, Milano, Italy).

Statistical analysis

Comparison among control, chronic, and generalized aggressive periodontitis groups within the same areas (i.e., sulcular epithelium, lamina propria, oral epithelium) and for each marker was determined by means of the Kruskal–Wallis test, followed by Dunn's Multiple Comparisons post-test. Differences were considered statistically significant when $p<0.05$.

Results

Hematoxylin-eosin sections were first analyzed, and it was possible to observe in the control group a healthy gingiva

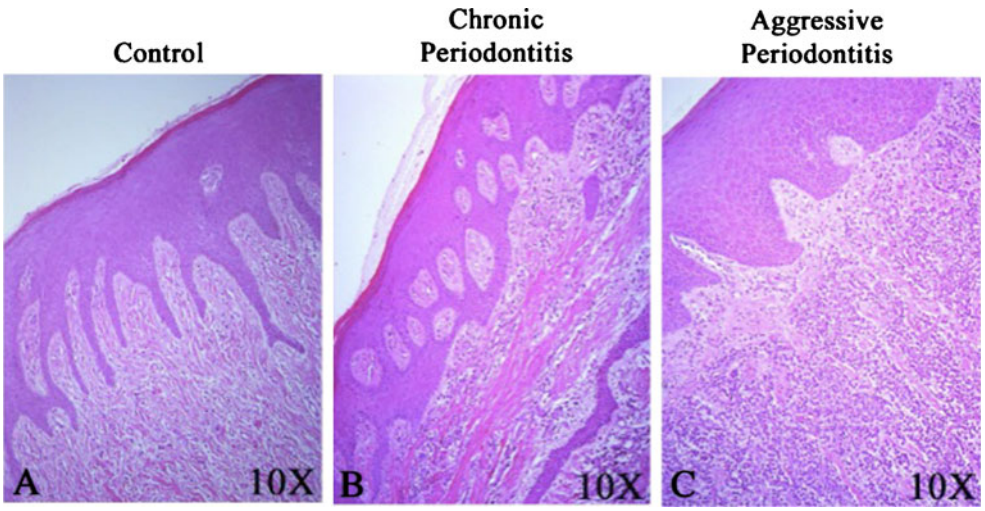

Fig. 1 Sections showing a healthy gingiva (**a**) and histological picture of chronic (**b**) and aggressive (**c**) periodontitis. H&E ×10

with a minimal inflammatory infiltrate (Fig. 1a), whilst a slight hyperkeratosis was detected in both chronic (Fig. 1b) and aggressive periodontitis (Fig. 1c). Hereafter, a precise immunohistochemical analysis of the inflammatory subpopulations followed.

The biopsies retrieved from healthy subjects depicted a slight inflammatory infiltrate was always present in the control group with CD 4+ cells representing the major component of the different subpopulations. Sulcular epithelium was constantly more affected than middle area and oral epithelium (Table 1). However, in chronic periodontitis, the sulcular epithelium exhibited in all the specimens the highest number of inflammatory cells when compared with the lamina propria and oral epithelium. CD 4 + cells were present in the highest amount followed by CD 8, CD 20 and CD 68+ cells. The main inflammatory cell types registered were CD 4 and CD 8+ cells. When looking at the lamina propria and the oral epithelium an equal distribution of the inflammatory subpopulations was observed (Table 1).

In addition, the sulcular epithelium present in aggressive periodontitis patients demonstrated the highest values of CD 4, CD 8 and CD 20+ cells. A remarkable difference was seen when comparing CD 4 and CD 8 cells with CD 20+ cells and an even greater difference with CD 68+ cells. Lower values in inflammatory subpopulations were recorded in the lamina propria and oral epithelium (Table 1). Significant differences between the control group and chronic ($p<0.05$) and aggressive periodontitis ($p<0.001$) were recorded in the CD 4 and CD 8+ cells in all three examined gingival levels (Fig. 2a–f), while no significant differences were detected comparing chronic with aggressive groups (Table 2).

CD 20+ cell values were significantly different in all three groups at all gingival levels ($p<0.05$) with higher appearance in aggressive periodontitis; only in the sulcular epithelium no significant difference was observed between chronic versus aggressive periodontitis (Fig. 3a–c). In addition, CD 68+ cells were significantly more present in aggressive periodontitis ($p<0.01$) than in chronic periodontitis or control group (Table 2) (Fig. 3d–f).

Discussion

Periodontitis is an infectious disease in which there is an inflammation and consecutive loss of connective tissue supporting or surround the teeth (e.g., alveolar bone, gingival, etc). In the present study, subjects with GAgP and ChP were selected according to the parameters of PD and CAL, as well as age. The three common features of AgP according to the Consensus Report of the American Academy of Periodontology [25] are: otherwise clinically healthy subjects, familial aggregation and rapid attachment loss and bone destruction. In addition, the current classification [25] described that the periodontal disease in GAgP subjects should run in first molars and incisors and in three or more other teeth. The first two features and the clinical characteristics were used as inclusion criteria in the present study. However, there are no consistent means of determin-

Table 1 Mean and standard deviations of immunohistochemical analysis in the control group, generalized aggressive and chronic periodontits

	Control ($n=7$)	Chronic periodontits ($n=14$)	Aggressive periodontits ($n=6$)	p values
CD 4				
Sulcular epithelium	19.86±1.95	34.57±1.79	46.17±2.93	0.0004
Lamina propria	8.57±1.72	22.50±1.74	37.33±4.55	0.0002
Oral epithelium	1.71±0.95	24.36±2.62	34.50±3.51	0.0001
CD 8				
Sulcular epithelium	8.29±2.36	25.36±1.28	41.67±2.42	0.0002
Lamina propria	4.43±1.72	17.64±2.68	37.67±3.56	0.0001
Oral epithelium	2.29±0.95	20.07±1.64	33.50±5.72	0.0001
CD 20				
Sulcular epithelium	10.86±1.22	18.64±2.56	33.67±3.39	0.0004
Lamina propria	2.71±0.76	5.07±0.83	28.33±1.63	0.0001
Oral epithelium	1.43±0.98	4.36±0.93	28.33±1.86	0.0001
CD 68				
Sulcular epithelium	3.00±0.82	3.21±0.70	7.67±0.82	0.0006
Lamina propria	1.86±0.69	2.36±0.63	7.33±1.21	0.0001
Oral epithelium	0.86±0.69	2.93±0.62	7.50±1.05	0.0001

CD 4 (T helper cells), CD 8 (T cytotoxic/suppressor cells), CD 20 (B cells), CD 68 (macrophages/histiocytes). Kruskal–Wallis test ($p<0.05$)

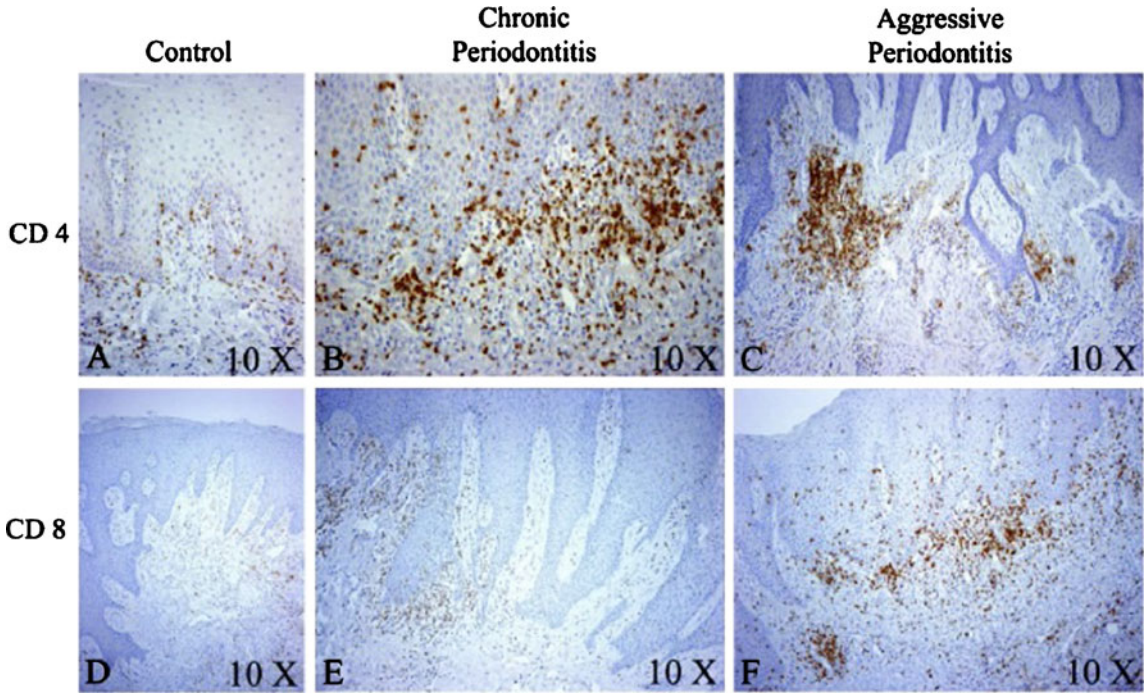

Fig. 2 Immunohistochemical images showing CD 4 and CD 8 expression pattern in control (**a**, **d**), chronic (**b**, **e**), and aggressive (**c**, **f**) periodontitis. CD4 and CD 8 staining (PAP) ×10

ing the rate of attachment loss while selecting subjects for cross-sectional studies. This situation was well elucidated in recent studies [26, 28]. Alternatively, as done in the present study, one would include younger subjects with severe disease, and estimate rapid destruction by setting the earliest starting point of disease around puberty. Therefore, although it is understood that GAgP may affect individuals at any age; the present study used age-related definitions

Table 2 *p* values of the statistical analysis by Kruskal–Wallis test, ($p<0.05$) for the immunohistochemical stained and detected markers for each area in the control group, chronic, and generalized aggressive periodontitis

	Sulcular epithelium *p* value	Lamina propria *p* value	Oral epithelium *p* value
CD 4			
Control vs. chronic periodontitis	$p<0.05$ *	$p<0.05$ *	$p<0.01$ *
Control vs. aggressive periodontitis	$p<0.001$ *	$p<0.001$ *	$p<0.001$ *
Chronic periodontitis vs. aggressive periodontitis	$p>0.05$	$p>0.05$	$p>0.05$
CD 8			
Control vs. chronic periodontitis	$p<0.05$ *	$p<0.01$ *	$p<0.01$ *
Control vs. aggressive periodontitis	$p<0.001$ *	$p<0.001$ *	$p<0.001$ *
Chronic periodontitis vs. aggressive periodontitis	$p>0.05$	$p>0.05$	$p>0.05$
CD 20			
Control vs. chronic periodontitis	$p<0.05$ *	$p<0.05$ *	$p<0.05$ *
Control vs. aggressive periodontitis	$p<0.001$ *	$p<0.001$ *	$p<0.001$ *
Chronic periodontitis vs. aggressive periodontitis	$p>0.05$	$p<0.05$ *	$p<0.05$ *
CD 68			
Control vs. chronic periodontitis	$p>0.05$	$p>0.05$	$p<0.05$ *
Control vs. aggressive periodontitis	$p<0.01$ *	$p<0.001$ *	$p<0.001$ *
Chronic periodontitis vs. aggressive periodontitis	$p<0.01$ *	$p<0.01$ *	$p<0.01$ *

CD 4 (T helper cells), CD 8 (T cytotoxic/suppressor cells), CD 20 (B cells), CD 68 (macrophages/histiocytes)

*$p<0.05$, significance of difference within the group was tested using Dunn's multiple comparison post-test

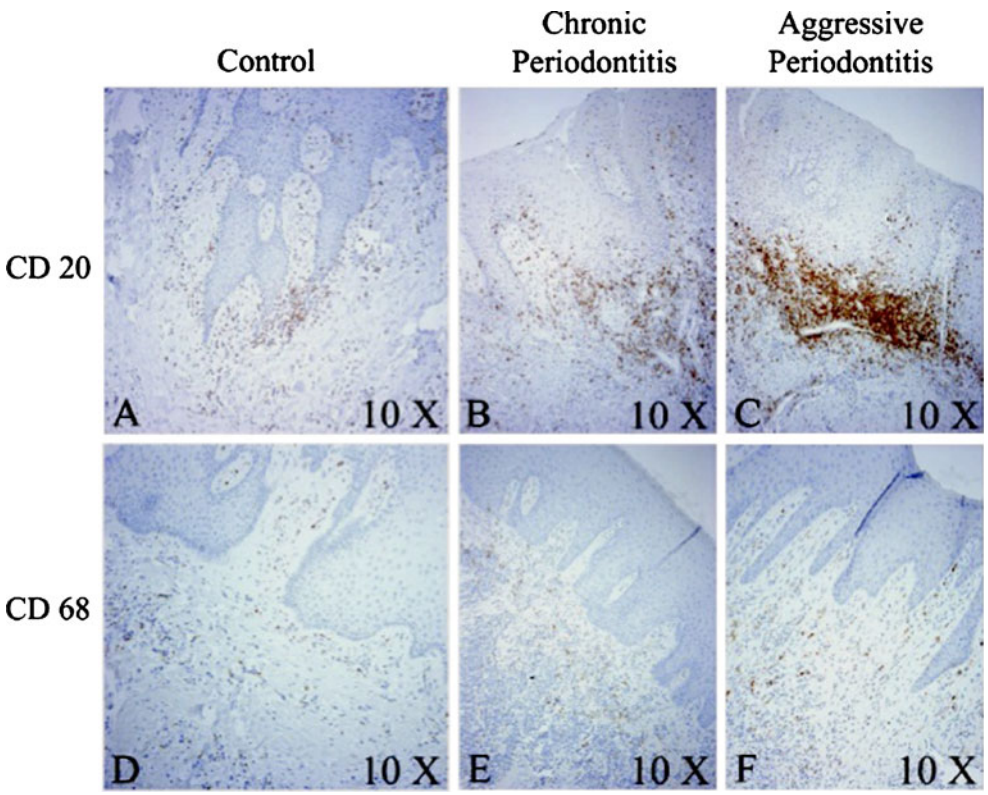

Fig. 3 Immunohistochemical images showing CD 20 and CD 68 expression pattern in control (**a**, **d**), chronic (**b**, **e**), and aggressive (**c**, **f**) periodontitis. CD20 and CD68 staining (PAP) ×10

for GAgP and ChP to provide the best possible diagnosis of GAgP.

Complementary, we registered higher inflammatory infiltrate for aggressive periodontitis when compared to chronic periodontitis. Similarly to other evaluations [2, 29], in the present study the same inflammatory cell pattern of CD 4, CD 8, CD 20, and CD 68+ cells has been observed in both forms of the disease, with differences in cell types distribution.

However, GAgP was described as presenting rapid attachment loss and bone destruction, usually in persons under 35 years of age. CP was described as most prevalent in adults with a slow to moderate rate of progression. All of such features continue to be, to a certain extent, age dependant and require knowledge of the rate of disease progression. Because in cross-sectional studies there are no reliable means of determining the actual time of disease initiation, rate of progression or even disease activity, subject classification is primarily based on the clinical measurements observed at a given point in time. Thus, while it is highly unlikely that GAgP will be misdiagnosed when only subjects under the age of 35 years exhibiting severe and extensive periodontal destructions are included in such groups, it is impossible to ascertain what proportion of the individuals included in GCP groups are actually GAgP subjects that were evaluated after the age of 30. While a certain amount of such overlap cannot entirely be ruled out in the present study, a conscious effort was made to minimize it, e.g., we established an age gap between GAgP and GCP subjects. Individuals of up to 30 years of age were included in the GAgP group and only subjects that were 35 years of age or more were selected for the GCP group.

Lappin et al. [3] examined the distribution of lymphocyte subpopulations in biopsies from periodontal lesions with reference to the probing depth, in patients with chronic and aggressive periodontitis. Similar to the present results, higher T and B cell counts in patients with aggressive periodontitis than in patients with chronic periodontitis were observed, suggesting a different pathogenesis for chronic and aggressive periodontitis. Complementary, Sigusch et al. [5] also sustained the hypothesis of a possible variance in the immune pathology of both forms of periodontitis, particularly considering the high CD 20 cell count in aggressive periodontitis. Similarly, greater CD 20+ cell representation in aggressive versus chronic periodontitis has been observed in the current evaluation.

By other hand, Suarez et al. [30] reported a low T cell (CD 3) count in aggressive periodontitis, which could be considered as an indication that other lymphocytic cells, like B lymphocytes, might play a more important role in the immune response to aggressive periodontitis. Additionally, the authors pointed out that T cells were not the most relevant at the advanced stage of the disease, when the biopsies were taken. Their analysis of T cell subpopulations revealed a decrease in total CD 3+ cells in aggressive

periodontitis, which was mainly caused by a reduction in CD 4+ cells. Meanwhile, the CD 8+ cell subpopulation remained stable in aggressive periodontitis and in the control group. Therefore, CD 8 + cells might not have important role in aggressive periodontal disease pathogenesis.

Furthermore, Amer et al. [31] found an increased proliferation of B cells in the peripheral blood from patients with aggressive periodontitis, whereas Shi et al. [29] reported that systemic markers (i.e.: white blood cell and neutrophils counts) were generally increased in patients with aggressive periodontitis, with the exception of lymphocyte numbers that were found decreased. Yamazaki et al. [32] demonstrated a distinct amplification in the CD 19/CD 3 (B cell/T cell) ratio within the periodontal lesion. This further suggested that B cells in the tissue also dominate the periodontal lesion. Indeed, Afar et al. [16] demonstrated a marked increase in a B-cell subpopulation—namely the autoantibody-producing CD20+ CD5+ cells—of patients with aggressive periodontitis.

Earlier studies in chronic periodontitis showed that T lymphocytes are dominant in the cellular infiltrate of healthy/gingivitis lesions, while periodontitis lesions are associated with high numbers of B cells in the infiltrate [10, 23, 32]. However, other studies [2, 29, 31] do not support these data, where CD 20+ cells seemed to be identified more in aggressive than in the chronic periodontitis. We can speculate that the inclusion criteria utilized by the aforementioned studies were not the same used in the present study and consequently, the periodontal status of those patients should be different. In addition, this feature may indicate that both forms of periodontitis could be characterized by a new lymphocyte pattern. Indeed, it might be that the previous assumption was only based on the results from the analysis of the sulcular epithelium; in the current study a high CD 20 count was also found in the sulcular epithelium of chronic periodontitis patients, but not in the lamina propria and oral epithelium.

Complementary, it we also investigate CD 68+ cells in order to get a more detailed aspect of the inflammatory process. The present results showed an increase in CD 68+ cells in aggressive periodontitis specimens, when compared with chronic periodontitis and the control group. This raise in the CD 68+ cells is a particular characteristic of the aggressive form of periodontal disease. Firstly, it is the key mediator of this inflammatory process and secondly its increased number supports the current significance of this study. Herewith, the report shows the need to further investigate the molecular interactions of CD 68+ cells in this inflammatory process. On the other hand, there were no differences in chronic periodontitis versus healthy patients. Bodineau et al. [9] analyzed the inflammatory infiltrate in chronic periodontitis patients, and also they did not find a substantial increase in CD 68 cell/CD 45 RB (leukocytes) cell ratio when judged against the control group. Lappin et al. [3] compared CD 68+ cells in early onset periodontitis and adult periodontitis and they did not notice significant differences. Pernu and Knuuttila [33] reported low CD 68+ cell count in healthy specimens; interestingly, they identified an increase of CD 68+ cells in patients treated with nifedipine and or cyclosporine A for cardiac issues and in renal transplant patients.

In conclusion, the present study demonstrated a higher prevalence for CD 20+ cells in the aggressive periodontitis lesions. However, further studies should be conducted to confirm and identify a clear trend and pattern of inflammatory cells and the mechanisms sustaining the disease in order to have a more specific treatment.

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Disclosure The authors declare that they have no conflict of interest.

References

1. International Workshop for a Classification of Periodontal Diseases and Conditions (1999) Ann Periodontol 4:1–112
2. Kinane, DF & Lindhe J (1996) Pathogenesis of periodontal disease. In: Lindhe J (ed) Textbook of Periodontology. ch. 5. Munksgaard, Copenhagen, Denmark
3. Lappin DF, Koulouri O, Radvar M, Hodge P, Kinane DF (1999) Relative proportions of mononuclear cell types in periodontal lesions analyzed by immunohistochemistry. J Clin Periodontol 26:183–189
4. Liu RK, Cao CF, Meng HX, Gao Y (2001) Polymorphonuclear Neutrophils and their mediators in gingival tissues from generalized aggressive periodontitis. J Periodontol 72:1545–1553
5. Sigusch BW, Wutzler A, Nietzsch T, Glockmann E (2006) Evidence for a specific crevicular lymphocyte profile in aggressive periodontitis. J Periodontal Res 41:391–396
6. Baer PN (1971) Case for periodontosis as a clinical entity. J Periodontol 42:516–520
7. Schenkein HA, Van Dyke TE (1994) Early-onset periodontitis: systemic aspects of etiology and pathogenesis. Periodontol 2000 (6):7–25
8. Berglund T, Liljenberg B, Lindhe J (2002) Some cytokine profiles of T-helper cells in lesions of advanced periodontitis. J Clin Periodontol 29:705–709
9. Bodineau A, Coulomb B, Tedesco AC, Seguier S (2009) Increase of gingival matured dendritic cells number in elderly patients with chronic periodontitis. Arch Oral Biol 54:12–16
10. Murakami S, Okada H (1997) Lymphocyte—fibroblast interactions. Crit Rev Oral Biol Med 8:40–50
11. Seguier S, Godeau G, Brousse N (2000) Immunohistological and morphometric analysis of intraepithelial lymphocytes and Langerhans cells in healthy and diseased human gingival tissues. Arch Oral Biol 45:441–452
12. Seymour GJ, Greenspan JS (1979) The phenotypic characterization of lymphocyte subpopulations in established human periodontal disease. J Periodontal Res 14:39–46

13. Van Dyke TE, Serhan CN (2003) Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. J Dent Res 82:82–90
14. Wassenaar A, Reinhardus C, Abraham-Inpijn L, Kievits F (1996) Type-1 and type-2 CD8+ T-cell subsets isolated from chronic adult periodontitis tissue differ in surface phenotype and biological functions. Immunology 87:113–118
15. Takeichi O, Haber J, Kawai T, Smith DJ, Moro I, Taubman MA (2000) Cytokine profiles of T-lymphocytes from gingival tissues with pathological pocketing. J Dent Res 79:1548–1555
16. Afar B, Engel D, Clark EA (1992) Activated lymphocyte subsets in adult periodontitis. J Periodontal Res 27:126–133
17. Gemmell E, Woodford V, Seymour GJ (1996) Characterization of T lymphocyte clones derived form porphyromonas gingivalis infected subjects. J Periodontal Res 31:47–56
18. Celenligil H, Kansu E, Ruacan S, Evatalay K, Caglayan G (1990) Immunohistological analysis of gingival lymphocytes in adult periodontitis. J Clin Periodontol 17:542–548
19. Celenligil H, Kansu E, Ruacan S, Evatalay K, Caglayan G (1993) In situ characterization of gingival mononuclear cells in rapidly progressive periodontitis. J Periodontol 64:120–127
20. Cobb CM, Singla O, Feil PH, Theisen FC, Schultz RE (1989) Comparison of NK-Cell (Leu-7+ and Leu-11b+) populations in clinically healthy gingiva, chronic gingivitis and chronic adult periodontitis. J Periodontal Res 24:1–7
21. Fujita S, Takahashi H, Okabe H, Ozaki Y, Hava Y, Kato I (1992) Distribution of natural killer cells in periodontal diseases: an immunohistochemical study. J Periodontol 63:686–689
22. Ishii T, Mahanonda R, Seymor GJ (1992) The establishment of human T cell lines reactive with specific periodontal bacteria. Oral Microbiol Immunol 7:225–229
23. Seymour GJ (1991) Importance of the host response in the periodontium. J Clin Periodontol 18:421–426
24. Pietruska MD, Zimnoch L, Waszkiel D, Stokowska W, Duraj E (2002) Evaluation of lymphocyte populations and subpopulations extracted from inflamed periodontal tissues. Rocz Akad Med Bialymst 47:218–225
25. Armitage GC (1999) Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4:1–6
26. Faveri M, Figueiredo LC, Duarte PM, Mestnik MJ, Mayer MP, Feres M (2009) Microbiological profile of untreated subjects with localized aggressive periodontitis. J Clin Periodontol 36: 739–749
27. Duarte PM, Neto JB, Casati MZ, Sallum EA, Nociti FH Jr (2007) Diabetes modulates gene expression in the gingival tissues of patients with chronic periodontitis. Oral Dis 13:594–599
28. Ximenez-Fyvie LA, Almaguer-Flores A, Jacobo-Soto V, Lara-Cordoba M, Moreno-Borjas JY, Alcantara-Maruri E (2006) Subgingival microbiota of periodontally untreated Mexican subjects with generalized aggressive periodontitis. J Clin Periodontol 33:869–877
29. Shi D, Meng H, Xu L, Zhang L, Chen Z, Feng X, Lu R, Sun X, Ren X (2008) Systemic inflammation markers in patients with aggressive periodontitis: a pilot study. J Periodontol 79:2340–2346
30. Suarez LJ, Ocampo AM, Duenas RE, Rodriguez A (2004) Relative proportions of T cell subpopulations and cytokines that mediate and regulate the adaptive immune response in patients with aggressive periodontitis. J Periodontol 75:1209–1215
31. Amer A, Singh G, Darke C, Dolby AE (1990) Spontaneous lymphocyte proliferation in severe periodontal disease: role of T and B cells. J Oral Pathol Med 19:49–52
32. Yamazaki K, Nakajima T, Aoyagi T, Hara K (1993) Immunohistological analysis of memory T lymphocytes and activated B lymphocytes in tissues with periodontal disease. J Periodontal Res 28:324–334
33. Pernu HE, Knuuttila MLE (2001) Macrophages and lymphocyte subpopulations in nifedipine- and cyclosporine A-associated human gingival overgrowth. J Periodontol 72:160–166