Immunohistochemical Evaluation of CD25+ Cell Expression in the Progression of Periodontal Disease

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It was assessed the immunohistochemical profile of CD25+ cells in cases of chronic gingivitis (CG) and chronic periodontitis (CP). Immunohistochemistry was carried out using streptoavidin-biotin complex and anti-CD25 antibody in 17 cases of CG and 25 cases of CP. Sixteen cases (94.1%) of CG were immunopositive. CD25 was focally expressed in 50% of the sample and diffusely expressed in 25%. The stained cells were localized not only beneath the epithelium, but also far from it. In relation to the cellular density quantification of CD25+ cells, score ++ was the most common. Concerning CP, all cases were immunopositive. CD25+ cells were expressed in focal or diffuse pattern either close or far from the epithelium. Diffuse distribution of positive cells throughout the connective tissue was seen in 60% of the cases and 32% showed focal or diffuse cellular pattern. Sixteen cases (64%) received score +++. It was identified that CD25+ cells are present in either a focal or a diffuse pattern in connective tissue. Significant differences in the density of cellular immunostaining between CG and CP were found. The greatest density was observed in CP cases, which suggests that the infiltrate of lymphocytes show a higher degree of cellular activation in periodontitis compared with gingivitis.

Key Words: periodontal disease, gingivitis, periodontitis, immunohistochemistry, CD25.

INTRODUCTION

Periodontal disease represents a chronic inflammatory pathology of infectious origin that affects periodontal tissues. The interactions between host and bacteria determine the nature of the resulting disease such as other infectious processes (1,2).

Several studies using immunohistochemical markers of cellular activation have indicated that T and B lymphocytes in crevicular fluid and gingival tissues are active in periodontal disease. These cells are greatly implicated in the host local immune response. It is also well known its functional potential to release cytokines and antibodies (via plasmacyte differentiation) when activated, such as the anti-inflammatory cytokine IL-10, which induces the expression of tissue inhibitors of metalloproteinases (TIMPs) and osteoprotegerin (OPG),

the respective inhibitors of MMPs and RANKL systems. It is therefore thought to attenuate disease severity (3).

Active B lymphocytes express surface molecules, such as CD21, CD22, CD23, CD 25 and CD 69, in periodontal lesions. The expression of these cellular activation markers is variable according to the bacterial species that stimulated B cell activation in gingivitis and periodontitis (4).

Previous studies regarding the cellular phenotype in periodontal disease found that B lymphocytes are intensely activated in periodontitis (5,6). In an immunohistochemical experiment, Yamazaki et al. (6) demonstrated a higher number of active B lymphocytes (CD25+) in periodontitis than gingivitis. The anti-CD25 monoclonal antibody recognizes the a α subunit of the IL-2 receptor (IL-2Rα), which is expressed not only on the surface of active B lymphocytes, but also in NK cells

and T lymphocytes under activation (3).

T-helper lymphocytes in periodontitis active sites have a higher expression of CD25 activation marker than T cells in stable sites (7). The present study was undertaken to evaluate the cellular activation of the lymphocytic infiltrate in distinct stages of periodontal disease. Thus, CD25$^+$ cell expression profile in gingivitis and periodontitis tissues was assessed immunohistochemically in the present study.

MATERIAL AND METHODS

Tissue Samples

In this study, were used 42 paraffin-embedded tissue specimens from the files of the Department of Oral Pathology of the Federal University of Rio Grande do Norte, Brazil, which were diagnosed clinically as chronic gingivitis (CG) (17 cases) or chronic periodontitis (CP) (25 cases). The sample was obtained from patients who have undergone oral surgery due to esthetic and functional reasons at the Periodontics and Oral Surgery Clinics of the referred Department.

Immunohistochemistry

Each specimen was fixed in formalin and embedded in paraffin. Tissue sections, 3-µm thick, were cut and mounted on glass silanesed microscope slides (3-aminopropyltriethoxysilane; Sigma Chemical Co., St Louis, MO, USA). Immunohistochemistry was carried out using the streptoavidin-biotin complex method. The sections were treated with primary antibody against CD25 (Ab-1/ II2R.1) specific for IL-Rα. Antigen retrieval was performed with *Steamer* during 15 min. The dilution used was 1:40 and sections were incubated for 60 min.

Analysis of the Immunostained Cells

Scores from 0 to "+++" were established to evaluate the immunostaining cellular density in the lamina propria of the specimens in accordance with the methodology described by Lins et al. (5), modified for this study. The following parameters were considered: Score 0 = negative expression, Score + = discrete number of immunopositive cells; Score ++ = moderate number of positive cells; Score +++ = large number of immunopositive cells. A descriptive analysis of the

microscopic findings regarding localization (beneath and/or far from the epithelium) and distribution pattern of CD25$^+$ cells in gingival connective tissue was performed.

Statistical Analysis

Differences between CG and CP were assessed using Kolmogorov-Smirnov test and the non-parametric Mann-Whitney U-test. Differences were considered to be statistically significant if the probability value was less than 0.05.

RESULTS

Chronic Gingivitis

Sixteen cases (94.1%) showed immunopositivity for CD25. In 4 cases (25%), CD25$^+$ cells were seen exclusively in a diffuse pattern. In other 4 cases (25%), positive cells were either focally or diffusely arranged (beneath or far from the epithelium). Eight cases (50%) demonstrated only focal immunostaining with 5 cases showing clusters of positive cells beneath or far from the epithelium whereas 3 cases showed immunostaining only distant from the epithelium (Fig. 1). Score ++ was the most commonly attributed to the immunostaining cellular density of CD25 in the sample (7 cases). Five cases received score + and 4 cases were score +++ (Table 1).

Chronic Periodontitis
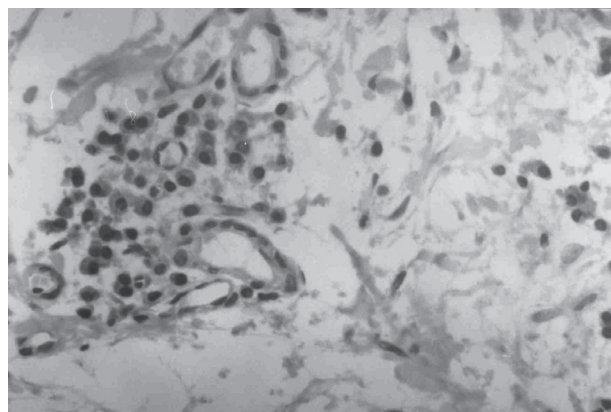
All specimens were CD25 immunopositive.

Figure 1. Clusters of CD25+ cells distant from epithelium in chronic gingivitis.

Reactive cells were arranged in a focal and/or diffuse pattern (beneath or far from the epithelium). Fifteen cases (60%) showed CD25^{+} cells diffusely distributed throughout connective tissue (Fig. 2), and 8 cases (32%) demonstrated either diffuse or focal cellular distribution. Finally, only 2 cases (8%) showed focal immunostaining. Concerning the immunostaining density of CD25, 16 cases were score +++, 7 cases score ++ and only 2 cases were score +. Thus, score +++ was the most common score in this group.

CD25 immunostaining in the epithelium, endothelium and fibroblasts was a frequent finding either in gingivitis or periodontitis. Both lymphocytes and plasmacytes in the inflammatory infiltrate were CD25^{+}. In some cases, clusters of CD25^{+} cells were also seen close to blood vessels.

Statistical Results

The mean value score was ++ in both groups. Differences in CD25+ density between CP and CG were statistically significant (p=0.0092).

DISCUSSION

Recent studies indicate that T-helper lymphocytes can be classified in two distinct subsets when they are activated and undergo clonal expansion. These groups are Th1 and Th2 depending on cytokine profile and metabolic activities. Data from several studies show that Th1 lymphocytes are involved with active secretion of cytokines such as interleukin 2 (IL-2), interferon gamma (IFN-γ), tumor necrosis factor beta (TNF-β) and
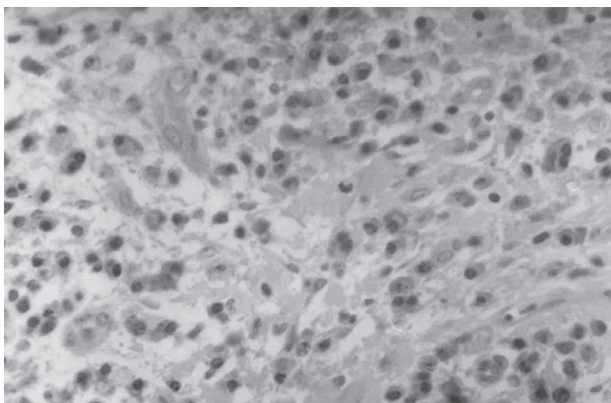

Figure 2. CD25^{+} cells diffusely distributed throughout connective tissue.

interleukin 12 (IL-12), which are called Th1 cytokines. On the other hand, Th2 lymphocytes secrete cytokines such as IL-4, IL-5, IL-6, IL-10 and IL-13, which are designated as Th2 cytokines (8,9). According to the Garlet et al. (10) Th1 cytokines are important in cell-mediated immunity whereas Th2 cytokines participate in T-dependent response.

In periodontal disease, macrophages and Langerhans cells are able to stimulate Th1 lymphocytes, which release great concentrations of IL-2, originally described as T growth cell factor (TGCF). IL-2 autocrine

Table 1. Density scores of CD25+ cells in cases of chronic gingivitis (CG) and chronic periodontitis (CP).

Case	CG-CD25^{+}	CP-CD25^{+}
1	+++	++
2	++	+++
3	+	+++
4	+++	++
5	+++	++
6	+++	++
7	++	+++
8	+	++
9	+	+++
10	++	+++
11	++	+++
12	++	+++
13	0	+++
14	+	+
15	+	+
16	++	+++
17	++	+++
18		+++
19		++
20		+++
21		+++
22		+++
23		++
24		+++
25		+++

pathway plays a key role in the stimulation of Th1 lymphocytes that secrete not only IL-2, but also TNF-β and IFN-γ (11). These cytokines play essential roles in the pathogenesis of periodontal diseases, having a positive correlation between existence and activity of periodontal diseases and tissue cytokine levels (12).

The secretion of maximum levels of IL-2 is regarded as stimuli for Th2 clones of T-helper lymphocytes local secretion of great deal of IL-6 is induced by the clonal expansion of Th2 lymphocytes (13). According to Longhi et al. (14), the IL-6 is a multifunctional cytokine expressed by both lymphoid and non-lymphoid cells that has been implicated in increasing the severity of various diseases. In adition to being a potent bone-resorptive cytokine together IL-1 and TNF-α, IL-6 is an important mitogenic factor for clonal expansion of B and Th2 lymphocytes (15). According to Guzeldemir et al. (12) IL-1 and TNF-α are considered major mediators of periodontal diseases.

IL-6 does play a pivotal role in regulating the immune system, resolving the innate immune response and directing the transition from innate to acquired immunity, a process that can, at least in part, be attributed to its effect on recruitment, activation and survival of different leukocyte subsets (15). Giving support to these findings, Shao et al. (16) have stated that one of IL-6 major functions is the final maturation induction of B cell to plasmocyte. It has been speculated that the high number of B cells and plasmocytes in periodontitis may be a result of the increased IL-6 production in local sites of the disease since IL-6 has a crucial role in T-dependent humoral immune response (17).

Il-1 and TNF-α have been implicated in the pathogenesis and clinical course of periodontal diseases because of its multiple pro-inflammatory properties. IL-1 modulates extracellular matrix components, enhances bone resorption in the periodontal tissues, stimulates fibroblasts and other nucleated cells to produce matrix metalloproteinase, activates plasminogen, and triggers prostaglandin synthesis. TNF-α induces the secretion of collagenase by fibroblasts, stimulates the resorption of cartilage and bone, and has been implicated in the destruction of periodontal tissue in periodontitis (12).

Evidence from different studies has demonstrated higher levels of IL-6 and IL-1 and TNF-α polymorphisms in inflammatory sites of periodontitis tissues. According to Shao et al. (16), this finding is consistent with IL-6 proliferative activity on B lymphocytes and plasmacytes and to the predominance of these cells in advanced

periodontal lesions. Furthermore, IL-6 and cytokines, such as IL-2, IL-4 and IL-5, are also involved in the control of activation, proliferation, growth and B cell differentiation (4,15-16). Guzeldemir et al (12) observed that IL-1 gene polymorphisms appear to have a role in susceptibility to localized aggressive periodontitis, considering these results as risk factors rather than diagnostic criteria.

Based on the aforementioned studies, it can be inferred that IL-2 stimulates initially the clonal expansion of Th1 lymphocytes and Th2 lymphocytes later. Furthermore, it acts directly or indirectly (Th2 cytokines via, especially IL-6) in the proliferation of B lymphocytes. According to different authors (5,6,13), the IL-2 direct stimulation on B and T lymphocytes is possible inasmuch as these cells express cell receptors for IL-2 when they are activated.

Corroborating the study of Dendrou and Wicker (17), anti-CD25 monoclonal antibody recognizes the α subunit of the IL-2 receptor (IL-2Rα), which is expressed not only on the surface of B lymphocytes surface, but also in NK cells and T lymphocytes in activation.

This study assessed the cellular activation of lymphocytic infiltrate in different stages of periodontal disease. Thus, an immunohistochemical analysis using anti-CD25 antibody in tissue specimens of gingivitis and periodontitis was performed. The results showed that CD25+ cells were either focally or diffusely distributed throughout lamina propria. Statistical analysis revealed significant differences in CD25+ density between CP and CG. Score ++ predominated in gingivitis whereas score +++ was the most common in periodontitis, thus suggesting a greater degree of cellular activation in lymphocytic infiltrate of periodontitis. This finding could explain the higher tissue damage in advanced periodontal disease due to the enhanced release of catabolic and bone-resorption cytokines by active Th lymphocytes (Th1 and Th2) and the relevant production of antibodies by plasmacytes originated through the differentiation of active B lymphocytes. According to Guzeldemir et al. (12), when humoral immune response (T dependent or independent) is aggravated it may cause harmful effects to periodontal tissues and also contribute to tissue damage.

The current results are supportive those of Yamazaki et al.(6), who reported a predominance of activated B lymphocyte (CD25+) in chronic periodontitis than in chronic gingivitis. They are also in agreement with a another study (18), which indicated that T

lymphocytes express significant levels of CD25 marker in periodontitis active sites. In the referred experiment, the authors observed that the active sites, characterized by higher tissue damage, have a relevant increase in the number of CD25$^+$ cells compared to stable sites. Taking into account this consideration, it can be inferred that acute episodes of periodontal disease are associated with a quantitative increase of CD25$^+$ cells. It is of utmost importance to emphasize that these immunohistochemical findings are only adjuvant in the diagnosis. They are only suggestive of an intense or discrete activity of the disease and the knowledge of the clinical features are extremely necessary for such confirmation.

In a previous study assessing the identification and characterization natural regulatory T cells (Tregs) in the inflammatory infiltrate of human chronic periodontitis (CP), it was found that patients with CP presented an increased frequency of T lymphocytes and CD4+CD25+ T cells in the inflammatory infiltrate of gingival tissues (19). This information suggests that in the present experiment T lymphocytes CD25$^+$ are probably T-helper since T cytotoxic/suppressor does not appear to express CD25 marker.

Also noteworthy is that CD25 immunostaining in the epithelium, endothelium and fibroblasts was a frequent finding either in gingivitis or periodontitis. Regarding the inflammatory infiltrate, both lymphocytes and plasmacytes were CD25$^+$. Since the majority of the cells in the inflammatory infiltrate and other cell types expressed receptor for IL-2, the great immunoreactivity for anti-CD25 antibody confirms the pro-inflammatory nature of this cytokine. Immunostaining for CD25 in gingival epithelium could also be related to the expression of IL-2 in dendritic cells.

Based on the obtained immunohistochemical results, it may be concluded that CD25$^+$cells are focally or diffusely distributed throughout the gingival tissue. Cells expressing CD25 were found in higher density in chronic periodontitis than in chronic gingivitis. The inflammatory infiltrate in periodontitis has an enhanced degree of cellular activation and this could be one explanation to the higher tissue damage observed in advanced periodontal disease.

RESUMO

Foi avaliado o perfil imunohistoquímico das células CD25$^+$ em casos de gengivite (CG) e periodontite crônica (CP). A imunohistoquímica foi realizada utilizando o complexo de streptoavidina-biotina e o anticorpo anti-CD25 em 17 casos de CG e 25 casos de CP. 16 casos (94.1%) de CG foram imunopositivos. O CD25 foi expresso focalmente em 50% da amostra e difusamente em 25% dos casos. As células imunomarcadas estavam localizadas não apenas no epitélio, mas também por todo o tecido conjuntivo. Em relação à quantificação da densidade celular de células CD25$^+$, o escore ++ foi o mais comum. Em relação a CP, todos os casos foram imunopositivos. As células CD25$^+$ foram expressas em padrão ora focal ora difuso, tanto no epitélio como no conjuntivo. A distribuição difusa das células positivas apenas no tecido conjuntivo foi observada em 60% dos casos, e 32% dos casos exibiram padrão celular ora focal ora difuso. 16 casos (64%) foram considerados como escore +++. Identificamos que as células CD25$^+$ estão presentes em padrão ora focal ora difuso no tecido conjuntivo. Diferenças significantes na densidade da imunomarcação celular entre CG and CP foram encontradas. A maior densidade celular foi observada na periodontite, sugerindo que o infiltrado de linfócitos mostrou um maior grau de ativação celular na periodontite comparada à gengivite.

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