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Activity of inflammatory bowel disease influences the expression of cytokines in gingival tissue

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ABSTRACT

This study assessed the cytokine expression in gingival and intestinal tissues from periodontitis patients with inflammatory bowel disease (IBD) and evaluated if IBD activity is a covariate to the amount of gingival cytokines. Paired gingival and intestinal tissues were collected from 21 patients and homogenised using a cell disruptor. Cytokine expression (IL-1β, IL-4, IL-6, IL-10, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IL-17A, IL-17F, IFN-γ, sCD40L, and TNF-α) was evaluated using bead-based multiplex technology. An inflammation score was developed using the intestinal cytokines that showed good accuracy to discriminate IBD active patients from those in remission and then a similar score was applied to gingival tissue. IL-4, IL-10 and IL-21 expressions were significantly increased in gingival tissue from patients with an active disease as compared to those with a disease in remission. The inflammation score (mean value of IL-1β, IL-6, IL-21, and sCD40L) was significantly higher in gingival tissue from patients with IBD activity. There was a significant correlation between gingival and intestinal inflammation scores (rho = 0.548; $P = 0.01$). Significantly higher IL-23 and IFN-γ levels and lower IL-31 and TNF-α levels were observed in gingival tissues than in intestinal ones. Activity of inflammatory bowel disease influenced the cytokine expression in gingival tissue.

1. Introduction

Periodontal disease is a biofilm-induced chronic inflammatory disease that affects tooth-supporting tissues. Its severe form, which can lead to tooth loss, affects over 740 million people worldwide [1]. Similar to the pathogenesis of inflammatory bowel disease (IBD), periodontal disease results from a complex interplay between the microbiota and the host immune-inflammatory response, and is influenced by genetic and environmental factors. Although the microbiota is a requisite, the persistence and dysregulation of the host immune and inflammatory responses are mainly responsible for the destruction of the periodontal tissues [2,3].

Studies have shown that IBD patients present with an increased prevalence of periodontal disease [4,5], as well as a higher severity and a greater extent of periodontitis [5], but the mechanisms responsible for this elevation are not well understood. As the development of both diseases is related to an aberrant immune response, the inflammatory response might be a link between them. Indeed, our group has previously found higher IL-18 levels in serum from patients with IBD and periodontitis [6]. Nevertheless, an important point that has not yet been addressed is whether or not the activity of IBD could play a role in the inflammatory response in periodontal tissues.

In fact, the activity of IBD influences the inflammatory response not only in the intestinal tissue [7], but also in the blood [8,9]. Globig et al. have shown an enrichment of Th17 and Treg cells in active lesions in patients with both Crohn's disease (CD) and ulcerative colitis (UC) compared with quiescent/mildly inflamed lesions [10]. Also, positive correlations have been found between disease activity scores and mRNA levels of IL-17A, IL-23, and IL-6 in patients with CD and UC [11]. A similar pattern is observed in sera, where increased levels of TNF-α have been recorded in patients with active UC in comparison to patients with inactive disease [8]. With that in mind, we hypothesise that the intestinal activity of IBD may influence the cytokine expression in gingival tissue. As a consequence of the high inflammatory activity, bone degradation, a hallmark of periodontitis, could be induced [12].

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IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, INF-γ, sCD40L, and TNF-α are all potentially relevant cytokines in the Th17 response, which plays a significant role in both periodontitis [13] and IBD [10]. Therefore, this study aimed to assess cytokine expression in gingival and intestinal tissue from periodontitis patients with IBD and evaluate whether or not gingival cytokines covariate according to IBD activity.

2. Material and methods

2.1. Patients and clinical assessment

This cross-sectional study selected 21 patients (17 women and 4 men; mean age 40.52 ± 14.77 years) with IBD and chronic periodontitis from the outpatient clinic of the Department of Gastroenterology at the Pedro Ernesto University Hospital – Rio de Janeiro State University. Ten patients were diagnosed with CD and 11 patients with UC. The diagnoses were made according to well-established clinical, endoscopic, radiologic, and histological parameters. Chronic periodontitis was defined using the definition established by the American Academy of Periodontology [14]. Patients diagnosed with CD or UC were included in the study if they had at least 10 teeth with probing depth (PD) $\geqslant$5 mm and clinical attachment loss (CAL) $\geqslant$4 mm in at least 4 sites, in different teeth. Patients were excluded if they had previously undergone non-surgical periodontal treatment or used antibiotics in the 6 months preceding their enrolment in the study. Pregnant and breastfeeding women were also excluded. This study was approved by the Research Ethics Committee of the Pedro Ernesto University Hospital (protocol number 857.118), and patients gave their written informed consent.

IBD activity was evaluated clinically and laboratory using the Harvey-Bradshaw index [15] for CD and the Truelove and Witts score [16] for UC. Patients were taking the following medications: mesalazine (n = 7), mesalazine plus azathioprine (n = 8), mesalazine, azathioprine plus TNF-α inhibitor (n = 4) and mesalazine plus steroid (n = 2).

A calibrated examiner performed a comprehensive periodontal examination using a manual probe (UNC-15, Hu-Friedy Manufacturing Company, Inc., Chicago, IL, USA). The examination included assessment of PD, CAL, bleeding on probing (BoP) and visible plaque index (VPI). PD and CAL measurements were determined at six sites per tooth. BOP and VPI were determined at four sites per tooth, excluding third molars. Intra-examiner concordance was 95% for both PD and CAL within 1 mm.

2.2. Tissue collection

This study involved 42 tissue specimens from 21 patients. Gingival and intestinal tissues were always paired and collected no longer than seven days apart. Gingival tissue was collected from inflamed sites, after anesthesia, with a punch of 1.5 mm positioned towards the periodontal pocket bottom. Sites for collection had PD $\geqslant$ 4 mm and CAL $\geqslant$ 3 mm. Intestinal biopsy was collected from the most inflamed area and performed at the time of colonoscopy. After collection, tissues were washed with phosphate buffered saline (PBS, Sigma-Aldrich St-Louis, USA) and stored at −70 °C until homogenisation.

Tissue was weighed on an analytical balance (Ohaus, Parisppany, USA) and then transferred to a microtube containing ultrapure 3.0 mm zirconia beads, PBS and protease inhibitor (Sigma-Aldrich, St. Louis, USA). Tissue homogenisation was performed using a cell disruptor (Loccus Biotechnology, Brazil) at a speed of 4000 rpm. After homogenisation, homogenate was collected and centrifuged at 10,000 rpm for 10 min. The supernatant was then stored at −70 °C until analysis.

2.3. Multiplex assay

The expression of fifteen cytokines (IL-1β, IL-4, IL-6, IL-10, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IL-17A, IL-17F, IFN-γ, sCD40L, and TNF-α) was evaluated using a bead-based multiplex immunoassay. Fifty microliters of the homogenised samples were analysed using a commercially available kit (Bio-Rad Laboratories, Hercules, CA, USA) in a multiplex analyser (Bio-plex 200, Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The concentrations of the unknown samples were calculated from the standard curve using the Bio-Plex Manager Software (Bio-Rad Laboratories, Hercules, CA, USA). Cytokine levels were adjusted for biopsy weight and are presented as pg/mg of tissue.

2.4. Inflammation score

Firstly, we selected the intestinal cytokines that showed a significant difference between disease activity and remission. Then, the area under the curve was calculated for these cytokines and, in order to develop an inflammation score, we selected those with good accuracy (>0.80) in discriminating between activity and remission in intestinal tissue. Four cytokines showed good accuracy (IL-1β, IL-6, IL-21, and sCD40L) and were chosen to constitute the score. The score was determined as the mean value of the four cytokines. After the score was established, it was also calculated as the mean value of the four gingival cytokines.

2.5. Statistical analysis

Data analysis was performed using SPSS 20.0 (IBM, Chicago, USA). Cytokine data are presented as mean and standard error. Wilcoxon test was used to compare the cytokine levels in gingival and intestinal tissues. Mann-Whitney and Fisher exact tests were used, when indicated, to compare cytokine expression between IBD activity and remission. Spearman's rank correlation coefficient (rho) was used for the correlation analysis. Differences were considered to be statistically significant at $P < 0.05$. A cytokine network was built based on the significant correlation coefficients (rho > 0.5; $P < 0.05$) between cytokine pairs for gingival and intestinal tissues, using the nwcommands (http://nnwcommands.org).

Sample size calculation, based on a pilot study (n = 8), showed that with a sample size of 20 patients, there is 80% power to detect, at a 0.05 level, a mean difference of 50% in IL-17A levels between gingival and intestinal samples.

3. Results

This study enrolled 21 patients with IBD and periodontitis, 8 in IBD activity and 13 in remission. All patients were diagnosed with moderate to severe chronic periodontitis. There were no significant differences in demographic and periodontal parameters between patients with active IBD and those in remission (Table 1).

3.1. Gingival versus intestinal tissue

Cytokine expression in gingival and intestinal tissues is depicted in Fig. 1. Cytokine levels were similar in intestinal tissue between CD and UC patients (data not shown), so that CD and UC were analysed as a unique IBD group. Regarding gingival tissue, IL-23 showed increased levels in patients with CD in comparison to patients with UC ($P = 0.002$).