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Cytokine expression in gingival and intestinal tissues of patients with periodontitis and inflammatory bowel disease: An exploratory study

Juliana Santos Bittencourt Menegat[a], Ronaldo Lira-Junior[a], Mariana Alves Siqueira[a,b], Fernanda Brito[a], Ana Teresa Carvalho[c], Ricardo Guimarães Fischer[a], Carlos Marcelo Figueredo[a,*]

[a] Department of Periodontology, Faculty of Odontology, Rio de Janeiro State University, Brazil
[b] Department of Periodontology, Faculty of Odontology, Veiga de Almeida University, Brazil
[c] Department of Internal Medicine, Faculty of Medicine, Rio de Janeiro State University, Brazil

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ABSTRACT

Objective: To evaluate the expression of the cytokines IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IL-17A, IL-17F, sCD40L, and TNF-α in gingival tissue and intestinal mucosa of patients having both periodontitis and inflammatory bowel disease (IBD) and assess how they cluster in both tissues.

Methods: This cross-sectional study selected 28 patients with periodontitis (18 with Crohn's disease and 10 with ulcerative colitis) from the IBD gastroenterology outpatient clinic at the Pedro Ernesto University Hospital. Patients were assessed using questionnaire, medical chart check and periodontal examination. Gingival and intestinal biopsies were collected and homogenized using a cell disruptor. Cytokines expression was evaluated through multiplex technology. Cluster analysis was performed based on cytokine's correlation strength and presented in dendrograms.

Results: Crohn's disease and ulcerative colitis patients exhibited no significant difference between them in cytokine levels ($p > 0.05$), so they were analysed together. Significantly higher levels of IL-17A, IL-17F, IL-22, IL-25, IL-33, IL-10, and INF-γ were found in gingival tissues in comparison with intestinal mucosa ($p < 0.05$). In gingival tissue, cytokines formed the following clusters: IL-25/IL-10/IL-33 ($r = 0.775$), IL-22/IL-23/IL-6 ($r = 0.681$) and IL-6/IL-25/IL-33/IL-10 ($r = 0.660$). In intestinal mucosa, the following clusters were formed: IL-6/IL-21/IL-10 ($r = 0.880$), IL-17A/IL-6/IL-21/IL-10 ($r = 0.826$), IL-17F/IL-33/IL-25 ($r = 0.813$) and IL-23/IL-2/IL-17A/IL-6/IL-21/IL-10 ($r = 0.785$).

Conclusion: Expression of IL-17A, IL-17F, IL-22, IL-25, IL-33, IL-10, and INF-γ was significantly increased in gingival tissue in comparison with intestinal mucosa of patients with periodontitis and IBD. The cytokine clustering pattern was different in gingival and intestinal tissues.

1. Introduction

Periodontitis is a chronic inflammatory disease resulting in destruction of the tooth insertion apparatus, and in severe cases, may result in tooth loss. Despite the necessity of bacterial insult for its occurrence, the central role in the periodontitis pathogenesis is exerted by the host response (Kinane & Bartold, 2007). The immunoinflammatory profile of periodontitis has been studied in different groups of chronic inflammatory diseases, such as cardiovascular disease (Lira-Junior, Figueredo, Bouskela, & Fischer, 2014), rheumatoid arthritis (Koziel, Mydel, & Potempa, 2014), chronic kidney disease (Brito et al., 2012), diabetes mellitus (Preshaw & Bissett, 2013), and inflammatory bowel disease (IBD) (Brito et al., 2008; Figueredo et al., 2011).

Increased prevalence of periodontitis has been described in IBD patients (Brito et al., 2008; Flemming, 1991) and the immunoinflammatory response seems to be the key factor in the relationship between the two diseases (Kinane & Bartold, 2007; Brito et al., 2008; Bouma & Strober, 2003). Our group has demonstrated that interleukin (IL)-4 levels were significantly lower in gingival fluid of periodontitis sites in patients with IBD. Individuals with ulcerative colitis (UC) had significantly higher levels of IL-6 in periodontitis sites when compared to a control group. However, our study did

* Corresponding author at: Rio de Janeiro State University, Faculdade de Odontologia, Boulevard 28 de Setembro 157, 2o Andar, Vila Isabel, Rio de Janeiro, RJ 20551-030, Brazil.
E-mail address: cmfigueredo@hotmail.com (C.M. Figueredo).

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not show a Th1 or Th2 cytokine pattern in gingival fluid of these patients (Figueredo et al., 2011).

Due to the role that IL-17 seems to play in regulating the immune system and its possible contribution to the clinical disorder, the identification and characterization of related molecules have been of particular interest. Several studies have indicated a potential role for IL-17 in mediating inflammation at the beginning and progression of periodontal destruction (Beklen et al., 2007; Mahanonda et al., 2008). This cytokine may affect the bone loss process, both locally and systemically (Behfarnia, Birang, Pishva, Hakemi, & Khorasani, 2013). In fact, a shift from a Th1 response to a response mixing Th1 and Th17 has been frequently observed during the course of Crohn's disease in recent studies (Verdier, Begue, Cerf-Bensussan, & Ruemmele, 2012; Zorzi et al., 2013).

Guo et al., 2009 demonstrated that the 3 Th populations respond to an IL-1 family member, IL-33, which is the newest IL-1 family member, being first reported in 2005 (Schmitz et al., 2005). It is mainly expressed by tissue barrier cells, suggesting a central role in the barrier defense. Through mechanisms still poorly understood, IL-33 seems to be released as an alarm, due to its ability to activate cells from both innate and adaptive immune system. Although its role is not clear, it may be an important cytokine in the initiation and perpetuation of inflammatory diseases (Liew, Pitman, & McInnes, 2010). An increased production of IL-33 has been described in patients with UC, as well as its relation with the Th1-cytokines suppression (Groβ, Doser, Falk, Obermeier, & Hofmann, 2012). On the other hand, IL-33 seems to influence Th2 response, inducing the production of IL-4, IL-10 and IL-31 (Maier, Werner, Duschl, Bohle, & Horejs-Hoeck, 2014).

A comprehensive analysis of cytokine expression has not been performed in gingival and intestinal tissues of patients with IBD yet. Studies have shown similarities in inflammatory mechanisms in intestinal and periodontal tissues, with both diseases resulting in an imbalance between pro-inflammatory and anti-inflammatory mediators (Maier et al., 2014; Oz, Chen, & Ebersole, 2010). Therefore, an evaluation of similar cytokines expression in both tissues might demonstrate some similarities in pathogenic pathways. Thus, the aim of this study was to evaluate the expression of IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IL-17A, IL-17F, sCD40L, and TNF-α in gingival tissue and intestinal mucosa of patients with periodontitis and IBD and assess how they cluster in both tissues.

2. Material and methods

2.1. Subjects

This cross-sectional study selected 28 patients (19 women and 9 men; mean age 43.36 ± 14.32 years) with IBD and chronic periodontitis from Gastroenterology outpatient clinic at the Pedro Ernesto University Hospital-Rio de Janeiro State University (HUPE-UERJ). Eighteen patients were diagnosed with CD and 10 patients with UC. The diagnosis of CD and UC had been established according to clinical, endoscopic, radiologic, and histological parameters. Chronic periodontitis was defined according to American Academy of Periodontology (Armitage, 1999). Patients diagnosed with CD or UC were included in the study if they had at least 8 teeth with probing depth (PD) ≥ 5 mm and clinical attachment level (CAL) ≥ 4 mm in at least 4 sites, in different teeth. Patients were excluded if they had gone through previous non-surgical periodontal treatment and used antibiotics in the previous 6-months from the beginning of the study. Pregnant and breastfeeding women were also excluded. This study was approved by the Research Ethics Committee of the HUPE-UERJ. Patients signed a written informed consent.

IBD activity was evaluated clinically and laboratory employing the Harvey-Bradshaw index (Harvey & Bradshaw, 1980) for CD and Truelove and Witts score (Truelove & Witts, 1955) for UC. Periodontal examination was performed by a single calibrated examiner, 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, excluding third molars and BOP and VPI in four sites per tooth.

Patients with CD used as medication immunomodulators (n = 13), 5-aminosalicylate derivatives (n = 11), corticosteroids (n = 1), immunomodulatory derivatives plus 5-aminosalicylate (n = 10). Four DC patients were not taking any medication. UC patients used 5-aminosalicylate derivatives (n = 8), immunomodulators (n = 5), corticosteroids (n = 1) and immunomodulatory derivatives plus 5-aminosalicylate acid derivatives (n = 4). One UC patient was not taking any medication.

2.2. Sample collection and preparation

Sample involved in this study was composed by 36 tissue specimens. Due to difficulties in collecting both tissue biopsies from the same patient, it was not possible to match gingival and intestinal tissues. Therefore, we ended up with 24 samples from gingiva and 12 samples from intestine. Only 3 biopsy pairs were matched. Gingival tissue was collected from inflamed sites previously selected at the time of the periodontal examination, after anesthesia, with a punch of 1.5 mm positioned toward the periodontal pocket bottom. Sites for collection should present PD ≥ 4 mm and CAL ≥ 3 mm. Intestinal biopsy, from the most inflamed area, was performed at the time of colonoscopy by the Gastroenterology staff at the HUPE. After collecting, gingival and intestinal tissues were stored in 10% formalin.

Tissue was weighted on an analytical balance (Ohaus, Parisppany, USA) and then transferred to a microtube containing ultrapure 3.0 mm zirconia beads, 200 μl of phosphate buffered saline (PBS, Sigma-Aldrich St-Louis, USA) and 100 μl of protease inhibitor (Sigma-Aldrich, St. Louis, USA). Tissue homogenization was performed using a cell disruptor (L-3 Beader, Loccus biotechnology Cotia, Brazil) at a speed of 4000 rpm (6 cycles of 30 s with interval of 20 s on ice between cycles). After homogenization, homogenate was collected and centrifuged at 10,000 rpm for 10 min. The supernatant was then stored at −70 °C until analysis.

2.3. Luminex assay

The levels of IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IL-17A, IL-17F, sCD40L, and TNF-α were determined using a multiplex bead immunoassay. Fifty microliters of the homogenized samples were analyzed using a commercially available kit (Bio-Rad Laboratories, Hercules, CA) in a multiplex analyzer (Bio-plex 200®, Bio-Rad Laboratories, Hercules, CA) according to the instructions of manufacturer. The concentrations of the unknown samples were calculated from the standard curve using the Bio-Plex Manager Software (Bio-Rad Laboratories, Hercules, CA). Cytokines levels were adjusted for biopsy weight and were presented in picogram/milligram of tissue (pg/mg).

2.4. Statistical analysis

Data analysis was performed using SPSS 19.0 (IBM, Chicago, USA). The normality of data was checked with Kolmogorov-Smirnov test. Continuous variables are presented as median (interquartile range) and categorical variables as percentages. Age