

CD26 immuno-expression and periodontal disease progression

P. M. L. Manna, J. E. Costa, and R. S. Gomez*

Department of Oral Surgery and Pathology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Immunological mechanisms participate in the pathogenesis of human chronic inflammatory periodontal disease (CIPD). Human CD4⁺ lymphocytes express functionally heterogeneous profiles of cytokine production. CD26 is an integral membrane glycoprotein, that is, a marker of Th1-like cytokine development. The purpose of the present study was to compare the immuno-expression of CD26 receptor in periodontal sites with and without clinical attachment loss (CAL). Five patients with rapidly progressing periodontitis and one with juvenile periodontitis were investigated. Each patient presented at least one site with and without CAL. Ten sites with CAL and nine without any CAL were biopsied, followed by the immunohistochemical identification of the CD26 receptor using the MIB-DS2/7 antibody. The results demonstrated that the percentage of positive cells for this antigen in the periodontal sites with CAL was not significantly different from those without attachment loss. Therefore, Th1 cell impairment may not be directly involved with periodontal attachment loss.

INTRODUCTION

Immunological mechanisms have been implicated in the pathogenesis of human chronic inflammatory periodontal disease (CIPD) for over 30 years. Defects in polymorphonuclear leukocytes [1], depressed cellular immune response [2, 3], polyclonal β -cell activation [4], and imbalance in the cytokine network [5–10] are some of the alterations reported. However, few studies have compared immunological features of active *versus* nonactive periodontal lesions [11].

Human CD4⁺ lymphocytes express functionally heterogeneous profiles of cytokine production [12–14]. Th1 CD4⁺ produce interleukin-2 (IL-2) and interferon- γ (IFN- γ), whereas Th2 cells produce mainly interleukins-4 (IL-4) and 5 (IL-5). The same pattern of cytokine profile has also been described in CD8⁺ lymphocytes. The presence of IL-4 or IL-12 contributes to these highly polarized phenotypes [13–16]. Some signaling molecules, like Stat4 and Stat6, appear essential for Th1 and Th2 development, respectively. The Th1 subset induces cell-mediated immune responses, while the Th2 subset is associated with humoral-type responses.

The study of Th1 and Th2 subsets in inflammatory diseases is hampered by the lack of reliable surface markers for these cell phenotypes. Additionally, human T-cells clones form a continuous spectrum in which Th1 and Th2 cells may be only two of the possible extreme phenotypes [17]. CD30 was reported to be a marker of the Th2 profile [18], but this receptor is not strictly limited to Th2 cells [19].

CD26 is an integral type II membrane glycoprotein of 110 kDa with a dipeptidyl peptidase IV activity [20, 21]. This receptor is expressed in 10%–60% of peripheral blood T cells, and T-cell activation is accompanied by its enhanced expression [20–22]. CD26 immunostaining correlates with the pro-

duction of IFN- γ in granulomatous diseases [23] and additional studies implicated the CD26 receptor as a marker of Th1-like cytokines development [17, 24].

To examine the cellular immune response and Th1 subsets in human chronic inflammatory periodontal disease pathogenesis, in the present study we investigated the immuno-expression of CD26 receptor in periodontal sites with and without clinical attachment loss (CAL).

MATERIALS AND METHODS

Subjects

Six patients with early onset periodontitis (five affected with rapidly progressing periodontitis and one with juvenile periodontitis) were included in this study. They were initially treated with oral hygiene instructions, scaling and root planning, as well as plaque index assessment. After six weeks, monthly evaluations were done over a nine-month period. The evaluations consisted of probing depth, clinical attachment loss and bleeding on probing measures, using an electronic controlled-force probe (Florida Probe, Florida Probe Corporation, Gainesville, and Florida).

Small gingival biopsies were done according to the following criteria: (a) ≥ 1 mm of CAL since the baseline therapy and ≥ 5 mm of pocket depth; (b) no CAL after the baseline measurement but associated with teeth designated for extraction. The biopsies were performed by incision with approximately 1.5 mm thickness extending from the sulcus outward through the oral epithelium and apical to the depth of the periodontal sulcus. Each patient presented at least one site with and without CAL. Ten sites with CAL and nine without CAL were biopsied. Biopsies from both groups (with and

without CAL) were matched as closest as possible to the probing depth and clinical attachment level at the initial examination, and supragingival plaque. No patient had a history of disease or medications which might affect the microbial flora, immune system or inflammatory response. Informed consent was received from each subject and the research project was approved by the University's Ethics Committee.

Immunohistochemistry

Although many antibodies specific for CD26 receptor are available, not all of them have been found useful in identifying a Th1-like immune reaction in human tissues. Different antibodies against CD26 receptor were tested for discrimination between Th1-like and Th2-like reactions in leprosy [24]. According to these authors, although all seven antibodies used were specific for this antigen, only the MIB-DS2/7 and 2A6 were capable to identify a Th1-like immune reaction in human disease. Therefore, in the present study we used the clone MIB-DS2/7, kindly provided by Dr. Ulrike Seitzer, to identify the CD26 receptor.

Frozen sections obtained by cryostat (Microm-HM 500 OM) were subjected to the biotin-streptavidin amplified system for CD26 immunostaining. Briefly, the sections were fixed in cold acetone for 10 min and immersed in 3% methanol-hydrogen peroxide solution for 10 min, to block endogenous peroxidase activity. After washing in 20 mM tris-HCl buffer containing 0.19 M NaCl (pH 7.4), the sections were incubated with anti-CD26 (prediluted) in tris-HCl buffer for 18 hours at 4°C. Thereafter, the sections were washed once again in tris-HCl buffer (pH 7.4) and incubated at room temperature with biotinylated rabbit anti-mouse immunoglobulin (DAKO) diluted 1:400 in tris-HCl for 30 min; washed with tris-HCl twice for 10 min; incubated for another 30 min with horseradish peroxidase-conjugated streptavidin (DAKO) prepared according to the manufacturer's directions; washed with tris-HCl; incubated for three minutes with 0.01% diaminobenzidine tetrahydrochloride (DAB) and 0.3% H₂O₂ in tris-HCl buffer (pH 7.4); rinsed in distilled water for 10 min and finally counterstained with Mayer's hematoxylin. To avoid false positive results, a series of tissue sections were stained with omission of the primary antibody.

Cell quantification and statistical analysis

The immunostaining of CD26 was quantitatively analyzed at six high-power microscopic fields at the epithelium/connective tissue interface (400x) through a square micrometer. The definitions of the first microscopic field were established by adjusting the square micrometer at the apical interface of the periodontal pocket epithelium/connective tissue. The results are expressed as the percentage of positive cells.

Statistical analysis

Since percentage values do not demonstrate a normal distribution, a nonparametric analysis Wilcoxon signed rank test was used to compare the means of the sites with and without

CAL. The error in cell counts was determined by six double counts in different moments and was below 2.2% of the cell-specific counts.

RESULTS

Some immunostaining was observed in the connective tissue. The cells immunostained by the antibody MIB-DS2/7 showed a lymphoid morphology. The percentage of CD26⁺ cells in the periodontal sites with CAL (mean: 10.3, median: 24.6, minimal: 1.2, maximal: 47.9) was not significantly different from those without it (mean: 6.9, median: 11.0, minimal: 2.1, maximal: 15.1).

DISCUSSION

Cytokines are particularly important for immune system regulation and T-lymphocytes have a central role in it. Th1 and Th2 cells induce cell-mediated and humoral-type immune responses, respectively [12, 24]. Th1 and Th2 lymphocytes are a polarized spectrum of T-cell differentiation and not distinct T-cell clones and their products regulate the differentiation and the effector functions of the reciprocal subset [13–16]. The definition of Th1 or Th2 cells is based primarily on the type of cytokines produced. Recently, the CD26 receptor was suggested as a marker of Th1-like cytokine development [17, 24]. In the present study, we used the antibody MIB-DS2/7 for CD26 immunostaining. This antibody is useful to identify the Th1 cells in inflammatory diseases [24].

The regulation of the immune system in human chronic inflammatory periodontal disease has been extensively studied. Seymour *et al.* [25] and Gemmell *et al.* [26] proposed that the progression of periodontal diseases is characterized by a shift from a predominantly Th1 to a Th2 cell profile. This hypothesis is supported by some studies [2, 7, 11, 27–31] but has not been confirmed by others [32, 33]. Ellis *et al.* [10] suggested that progressively lower gingival concentrations of IL-12 and higher IL-6 concentrations associated with increasing sulcular depth not only indicates a shift from a Th1 to a Th2 immunologic phenotype, but also suggests a regulatory role for IL-12 in that shift. Considering that different methodologies were used in these studies, a definitive conclusion about Th1 and Th2 cells and periodontal disease activity cannot be achieved.

In the current study, we have not observed any differences in CD26 immunostaining between the periodontal sites with and without CAL. These data suggest that Th1 cell impairment is not directly involved with periodontal attachment loss. However, we used a longitudinal assay to discriminate active *versus* nonactive periodontal sites. The samples in the active sites were collected after periodontal attachment loss. Therefore, the possibility that we analyzed a postactive event cannot be completely ruled-out. Furthermore, the extent of active or inactive periods in periodontal disease is not known [34]. If periodontal pockets have prolonged periods of inactivity plus brief episodes of localized exacerbation, the protocol used would not discriminate the lesions susceptible to

periodontal breakdown from those which are quiescent. This could be due to the poor resolution of the available clinical measurements [35].

In conclusion, our study shows that periodontal sites with and without attachment loss do not have different numbers of Th1 cells and further studies are necessary to clarify the importance of Th1 cells in the biological activity of periodontal disease.

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* Corresponding author.

Current address: Departamento de Clínica, Patologia e Cirurgia Faculdade de Odontologia Rua Conde de Linhares, 141 Belo Horizonte, 30380-030, Brasil
E-mail: rsgomez@net.em.com.br



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