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ABSTRACT

The aim of the present study was to examine differences in cellular characteristics of human peri-implantitis and periodontitis lesions. Two groups of patients were included: 40 patients with generalized severe chronic periodontitis and 40 patients presenting with severe peri-implantitis. Soft tissue biopsies were obtained from diseased sites (probing pocket depth ≥ 7 mm with bleeding on probing) and prepared for histologic and immunohistochemical analysis. In contrast to periodontitis samples, peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers, and densities of CD138-, CD68-, and MPO-positive cells than periodontitis lesions. Peri-implantitis lesions also extended to a position that was apical of the pocket epithelium and not surrounded by noninfiltrated connective tissue. They further presented with significantly larger densities of vascular structures in the connective tissue area lateral to the infiltrated connective tissue than within the infiltrate. This study suggests that peri-implantitis and periodontitis lesions exhibit critical histopathologic differences, which contribute to the understanding of dissimilarities in onset and progression between the 2 diseases.

KEY WORDS: biopsy, dental implant, immunohistochemistry, inflammation, inflammatory cell, peri-implant disease.

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Composition of Human Peri-implantitis and Periodontitis Lesions

INTRODUCTION

Peri-implantitis is an increasing problem in implant dentistry (Mombelli *et al.*, 2012). It is recognized by bleeding on probing with loss of supporting tissues (Lindhe *et al.*, 2008; Lang *et al.*, 2011). Although clinical and radiologic signs of periodontitis and peri-implantitis have many features in common, results from experimental studies indicate that significant histopathologic characteristics exist that may explain differences in disease onset and progression (Lindhe *et al.*, 1992; Schou *et al.*, 1993; Berglundh *et al.*, 2011; Carcuac *et al.*, 2013). In a review on periodontitis and peri-implantitis lesions, Berglundh *et al.* (2011) reported that there is comprehensive information on human periodontitis lesions, while few studies have examined peri-implantitis lesions prepared from human samples. Furthermore, analysis of human peri-implantitis was made on a small number of samples and patients, and comparisons to periodontitis were exceptional.

Animal models in this field provide access to the entire disease process, including soft and hard tissues. In an experimental study of dogs, Carcuac *et al.* (2013) reported that peri-implantitis lesions were considerably larger, extended closer to the crestal bone, and contained larger number of osteoclasts than periodontitis lesions. As the findings in experimental studies need to be validated in human protocols and a more comprehensive analysis of cellular and functional characteristics of the lesions is required, evaluations of human disease samples obtained from patient groups of sufficient size and with well-described clinical characteristics of diseased sites are needed. The aim of the present study was to perform the requested assessments of human peri-implantitis and periodontitis lesions.

MATERIAL & METHODS

Two groups of patients from the Clinic of Periodontics, Mölndal, Public Dental Health Services, Västra Götaland, Sweden, were included. One group consisted of 40 patients with generalized severe chronic periodontitis (24 women and 16 men; age range, 40-89 yr; mean, 64 ± 11.45 yr). The patients exhibited bone loss $\geq 50\%$ and probing pocket depth ≥ 7 mm with bleeding on probing at ≥ 4 teeth. A second group of 40 patients presenting with severe peri-implantitis was also recruited (23 women and 16 men; age range, 46-93 yr; mean, 70 ± 10.41 yr; function time for implants, 2-10 yr). The subjects in this group demonstrated ≥ 1 implant with peri-implant bone loss ≥ 3 mm and a peri-implant probing pocket depth ≥ 7 mm, with bleeding on probing and/or suppuration.

The study protocol was approved by the local human review board, and before enrollment, the patients of the 2 groups received information regarding the purpose of the study and signed an informed consent. None of the subjects had a known systemic disorder that could have affected the periodontal and peri-implant tissue conditions. Smoking habits were recorded in both groups.

No patients had received any treatment regarding periodontal or peri-implant diseases during the last 6 mo. On an individual basis, the patients were given a detailed case presentation and oral hygiene instruction. They also received professional supragingival tooth/implant cleaning.

Biopsy and Histologic Processing

Diseased interproximal tooth/implant sites were identified that exhibited probing pocket depth ≥ 7 mm with bleeding on probing. Following local anesthesia (Xylocain Dental Adrenalin, 20 mg/mL + 12.5 μ g/mL; Dentsply Pharmaceutical, York, PA, USA), 2 parallel incisions, 4 mm apart, were made with a 12D scalpel blade (Hu-Friedy, Chicago, IL, USA) through the soft tissue until bone contact was achieved. The 2 incisions were connected with a perpendicular incision placed at a distance of 4 mm from the tooth/implant. The biopsies, including the entire supracrestal soft tissue portion of the diseased site, were carefully retrieved and prepared for histologic and immunohistochemical analysis.

The tissue samples were rinsed in saline, mounted in mesh basquets (Tissue-Tek Paraform Sectionable Cassette System; Sakura Finetek Europe, Netherlands), and placed in 4% buffered formalin for 48 hr. The samples were stored in 70% ethanol, kept at 4°C, and subsequently dehydrated and embedded in paraffin. Microtome serial sections (5 μ m thick) were cut and mounted on glass poly-D-lysine-coated slides and stained with hematoxylin and eosin.

Immunohistochemistry

Immunohistochemical preparation was performed with an EnVision kit (EnVision System-HRP; DAB, DakoCytomation, Glostrup, Denmark). The primary mouse monoclonal antibody to CD3 (1:50 dilution) was used to identify T cells, while B cells, plasma cells, macrophages, and endothelial cells were detected through mouse monoclonal antibodies to CD20 (1:400), CD138 (1:50), CD68 (1:200), and CD34 (1:100), respectively. Polyclonal rabbit anti-human myeloperoxidase was used to detect polymorphonuclear leukocytes (1:1,500). The sections were dewaxed and incubated in antigen retrieval solution (DIVA; Biocare Medical, Concord, CA, USA) at 60°C over night and subsequently incubated with primary antibodies for 30 min and with Dako's Peroxidase Block for 10 min. The specimens were then incubated with a characterized and diluted mouse or rabbit primary antibody, followed by a labeled polymer for 30 min and a substrate/chromogen for 10 min. Counterstaining was performed with hematoxylin. Finally, the sections were mounted and coverslipped. Human oral mucosa tissue sections were used as positive controls, while negative controls were produced by substituting the primary antibody with nonimmune serum.

Histologic Analysis

The histologic examinations were performed in a Leica DM-RBE microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500 MC; Leica, Wetzlar, Germany).

The surface area of the infiltrated connective tissue (ICT) in the connective tissue (area ICT) was evaluated by outlining its circumference with a mouse cursor.

The histologic quantitative assessments of cell markers were performed with a microscope equipped with an image system (Leitz DM-RBE Q-500 MC; Leica, Wetzlar, Germany). For the identification of positive cell markers, an interference contrast setting at a magnification of $\times 400$ was applied as previously described (Liljenberg *et al.*, 1994; Zitzmann *et al.*, 2001). A point-counting procedure was used to determine the percentage of positive cell markers within the ICT. A lattice comprising 400 points was superimposed over the tissue area. Cross points that indicated the positive cell in the compartment to be examined were counted and related to the total counts for the entire ICT (%) and expressed as area proportions (%) of ICT. In addition, the mean size of positive cells was assessed by using a mouse cursor in 10 randomly selected sections of each category of markers in both patient groups. Based on the data on cell density and size of ICT with the cell size, the number of total positive cells for each marker in the ICT was estimated. The density of vascular structures of the ICT was determined *via* the point-counting procedure with the reference of endothelial structures expressing CD34. The density of vascular units was also performed in a 200- μ m-wide zone of the connective tissue immediately lateral to the ICT. To assess the intra-individual variation of the immunohistochemical analysis, double assessments were performed within 2-mo intervals on 10 sections representing each marker used.

Data Analysis

Mean values and standard deviations were calculated for each variable and patient. Differences between patient groups were analyzed with the Student's *t* test for unpaired observations ($n = 80$). The null hypothesis was rejected at $p < .05$. For superiority of peri-implantitis lesions in relation to periodontitis lesions, with an α of 0.05, a given standard deviation of 1.1% to 2.5%, and a power of 80%, a difference in area proportions of cells of 3% required a sample size of 30 subjects in each group. Analysis of covariance was performed to analyze possible effects of sex, age, and smoking on the results.

RESULTS

There were no statistically significant differences regarding distribution of age and sex between the 2 patient groups. The proportion of smokers was 27.5% in both groups. Micrographs illustrating periodontitis and peri-implantitis lesions are presented in Figure 1. In the sections representing the periodontitis group, the lesion resided in a well-defined compartment of the connective tissue that was walled off by a pocket epithelium toward the pocket and a non-ICT portion on its lateral and apical aspects. In the peri-implantitis specimens, however, the ICT occupied a considerably larger portion of the connective tissue adjacent to an ulcerated pocket epithelium. In addition, the ICT in this group of specimens extended to a position that was apical of the pocket epithelium and not surrounded by a zone of non infiltrated connective tissue.

Periodontitis

Peri-implantitis

Figure 1. Sections prepared from periodontitis and peri-implantitis sites. Pocket area located to the left. Haematoxylin and eosin, CD3, CD20, CD138, CD68, and MPO markers. Magnification x25 and x400.

The results from the analysis of the size of ICT and area proportions of cell markers are reported in Table 1. The ICT in the peri-implantitis sites was more than 2 times larger than the lesions in the periodontitis sections ($3.48 \pm 2.54 \text{ mm}^2$ vs. $1.49 \pm 1.05 \text{ mm}^2$). This difference was statistically significant. The area proportions of the ICT that was occupied by CD138-, CD68-, and MPO-positive cells were significantly larger in peri-implantitis than in periodontitis specimens, while a reverse relationship was found for CD20-positive cells. The density of vessels within the ICT was significantly larger in periodontitis than in peri-implantitis. In the connective tissue portion lateral to the ICT, however, the proportion of vascular structures was significantly larger in peri-implantitis than in periodontitis. In addition, the differences in vascular density between the 2 tissue compartments

were statistically significant for both periodontitis and peri-implantitis specimens.

The percentage distribution of total number of cells in ICT of periodontitis and peri-implantitis lesions with the relative overall size of the ICT is depicted in Figure 2. This figure also illustrates the large discrepancy on the overall size of the ICT between the 2 types of specimens.

The results from the assessments of cell size, the calculated total number positive cells, and number of cells/ mm^2 within the ICT are reported in Table 2. The estimated total number of inflammatory cells within ICT was significantly larger in peri-implantitis than in periodontitis sections. The numbers of CD3-, CD138-, CD68-, and MPO-positive cells were significantly larger in peri-implantitis than in periodontitis lesions. The

Table 1. Size and Area Proportions of ICT for Positive Cells and Vascular Units of Periodontitis and Peri-implantitis Sites

	Periodontitis (n = 40)	Peri-implantitis (n = 40)
Size of ICT (mm ²)	1.49 ± 1.05	3.48 ± 2.54*
% area proportions of ICT		
CD3	7.82 ± 5.36	6.87 ± 4.42
CD20	4.97 ± 5.23*	3.10 ± 2.79
CD138	8.96 ± 6.71	13.24 ± 9.22*
CD68	2.13 ± 3.17	3.68 ± 3.53*
MPO	4.28 ± 2.52	10.90 ± 7.53*
Vascular units within the ICT	7.81 ± 5.09*	2.75 ± 2.60
Vascular units lateral to the ICT	2.31 ± 2.34	8.58 ± 8.93*

Values in mean ± SD.

ICT, infiltrated connective tissue.

**p* < .05.

Figure 2. Percentage distribution of total number of cells in periodontitis and peri-implantitis lesions. CD3 (blue), CD20 (purple), CD138 (red), CD68 (brown), and MPO (green). Note the difference in size of infiltrated connective tissue (ICT). *n* = 80.

overall density of inflammatory cells within the ICT (*i.e.*, the number of cells/mm²) was significantly higher in peri-implantitis than in periodontitis specimens. Specifically, the densities of CD138-, CD68-, and MPO-positive cells were significantly higher in peri-implantitis than in periodontitis lesions, whereas an opposite association was observed for CD20-positive cells. The largest total number of cells or cells/mm² among the different phenotypes was found for MPO- and CD138-positive cells in peri-implantitis lesions. These 2 cell categories in peri-implantitis not only occurred in 3- to 6-times larger numbers than their counterparts in periodontitis lesions but also outnumbered other cell groups in both types of lesions.

The analysis of covariance of patient characteristics revealed that distributions of sex, age, and smokers between the periodontitis and the peri-implantitis groups did not influence the results from the histologic assessment.

DISCUSSION

This study evaluated histopathologic characteristics in human periodontitis and peri-implantitis lesions. It demonstrated that peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers, and densities of CD138-, CD68-, and MPO-positive cells than periodontitis lesions. Peri-implantitis specimens, in contrast to periodontitis samples, also presented with significantly larger densities of vascular structures in the connective tissue area lateral to the ICT than within the infiltrate. The study suggests that peri-implantitis and periodontitis lesions exhibit critical histopathologic differences, which contribute to the understanding of dissimilarities in onset and progression between the 2 diseases.

As previous reports on evaluations of differences between human peri-implantitis and periodontitis lesions are few and included small numbers patients, the present study aimed at performing a comprehensive examination of histopathologic differences between the 2 diseases. Thus, the number of patients in each group (*n* = 40) and the severity of the conditions in the selected sites and cases suffice necessary requirements of statistical power and distinctions of clinical signs of disease. In addition, sampling of biopsies in both diseases was, in most cases, carried out in conjunction with surgical therapy. From an ethical point of view, sampling of biopsies under such conditions is restricted to the soft tissue component, as the supporting bone is not accessible. Although the biopsy-sampling procedure is aimed at including the entire supracrestal soft tissue portion of the diseased site, small parts of the apical portions of the lesion may occasionally, for technical reasons, not be retrievable in narrow osseous defects. Yet, biopsies obtained from animal experiments include the entire peri-implant and periodontal hard and soft tissue components and may, from such a perspective, be superior to the human protocol. Indeed, in an experimental study from our laboratory, Carcuac *et al.* (2013) reported that experimentally induced peri-implantitis lesions were larger and extended closer to the bone crest than periodontitis lesions. The finding on differences in size of the lesions between the 2 conditions reported by Carcuac *et al.* corroborates data presented in the present study.

Table 2. Cell Size, Total Estimated Number, and Density of Positive Cells in the ICT of Periodontitis ($n = 40$) and Peri-implantitis Sites ($n = 40$)

	CD3	CD20	CD138	CD68	MPO
Cell size (μm^2)	58 $\pm$ 4.08	63 $\pm$ 0.62	61 $\pm$ 0.43	95 $\pm$ 7.8	44 $\pm$ 1.02
Total no. of cells in ICT					
Periodontitis	2,138 $\pm$ 2,015	1,235 $\pm$ 1,683	2,624 $\pm$ 2,898	280 $\pm$ 375	1,492 $\pm$ 1,310
Peri-implantitis	4,672 $\pm$ 5,340*	1,817 $\pm$ 2,129	9,140 $\pm$ 10,850*	1,364 $\pm$ 2,016*	10,035 $\pm$ 12,366*
No. of cells per mm^2					
Periodontitis	1,348 $\pm$ 924	788 $\pm$ 829*	1,464 $\pm$ 1,096	206 $\pm$ 324	983 $\pm$ 579
Peri-implantitis	1,185 $\pm$ 762	464 $\pm$ 437	2,164 $\pm$ 1,506*	388 $\pm$ 372*	2,505 $\pm$ 1,730*

Values in mean $\pm$ SD.

ICT, infiltrated connective tissue.

* $p < .05$.

While some descriptive studies on peri-implantitis lesions were presented previously, reports on comparisons between human peri-implantitis and periodontitis lesions are scarce. Sanz *et al.* (1991) analyzed soft tissue biopsies from 6 patients with peri-implantitis and reported that about two-thirds of the connective tissue portion of the biopsy was occupied by an infiltrate consisting of plasma cells, mononuclear cells, and enlarged blood vessels. Berglundh *et al.* (2004) analyzed soft tissue biopsies obtained from 12 implant sites with severe peri-implantitis in 6 patients. The histologic analysis demonstrated that the lesion occupied almost the entire connective tissue compartment and extended apical of the pocket epithelium. These observations are in agreement with results presented in the current study. In fact, the data on the mean size of 3.61 mm^2 of the ICT presented in the study by Berglundh *et al.* (2004) are consistent with results in the current report. Bullon *et al.* (2004) analyzed soft tissue biopsies from 5 cases with peri-implantitis and 5 patients with aggressive periodontitis. They reported that peri-implantitis and periodontitis lesions both presented with plasma cells, macrophages, and lymphocytes, among which T cells were more common than B cells. Similar findings were also presented by Cornellini *et al.* (2001) in a study on biopsies prepared from 10 patients with peri-implantitis.

The 2 lesions examined in the present study did not only differ in regard to their size, as the numbers and densities of CD138- (plasma cells), CD68- (macrophages), and MPO-positive cells (PMN cells) were larger in peri-implantitis than in periodontitis lesions. These differences indicate that the inflammatory response in peri-implantitis sites is more intense by promoting cells, which are part of both the innate and the adaptive host response. Studies on gene expression of proinflammatory markers in periodontitis and peri-implantitis sites have presented similar findings. Venza *et al.* (2010) analyzed soft tissue biopsies collected from different patient groups and reported that peri-implantitis sites exhibited higher mRNA expression of IL-6, IL-8, and TNF α than periodontitis. In a study on genome-wide transcriptome profiles in gingival specimens obtained from small patient groups with periodontitis and peri-implantitis, Becker *et al.* (2012) concluded that the 2 conditions represent distinct entities with different mRNA signatures.

The examination of the 2 types of lesions in the present study is relevant in regard to similar appraisals of differences between

peri-implant mucositis and peri-implantitis lesions presented by Gualini and Berglundh (2003). They examined immunohistochemical characteristics of soft tissue biopsies obtained from 16 patients and reported that peri-implantitis lesions contained significantly greater proportions of B cells and elastase-positive cells (indicating PMN cells) than mucositis lesions. Thus, the severity of a condition appears to correlate with the size of the lesion and with a cell profile based on enhanced densities and numbers of the B-cell or plasma cell line with neutrophil granulocytes and macrophages. Peri-implantitis lesions carry such characteristics.

In the study on experimental peri-implantitis and periodontitis referred to earlier, Carcuac *et al.* (2013) reported that periodontitis lesions, in contrast to peri-implantitis lesions, were consistently walled off from the alveolar bone by a zone of non infiltrated connective tissue and that the biofilm in the pocket was separated from the connective tissue by a pocket epithelium. These structural differences appear to be the fundament to the dissimilar histopathologic characteristics of the 2 conditions and explain the findings in the present study on larger numbers and densities of plasma cells and neutrophils in peri-implantitis lesions.

Another observation in the current investigation was the difference in vascular density between the 2 types of lesions. As the healthy supracrestal connective tissue portion around teeth contains larger amounts of vascular structures than does the corresponding tissue compartment around implants (Berglundh *et al.*, 2004), it is likely that an inflammatory infiltrate occupying this zone would exhibit a similar difference in vascular units. Yet, data on vascular densities in peri-implant and periodontal tissues are conflicting. Bullon *et al.* (2004) used the endothelial marker CD34 and reported that the connective tissue lateral to the junctional/sulcular epithelium in peri-implantitis sites contained a larger vascular density than that in periodontitis sites. The restriction of analysis to the coronal part of the tissue in the study by Bullon *et al.* may explain the difference in results on vascular density to the present study.

It should also be noted that the connective tissue zone lateral to peri-implantitis lesions in the present material presented with enhanced density of vessels. This finding indicates a longer distance from blood vessels to target sites for transmigrating neutrophil granulocytes in peri-implantitis lesions. Taken together, the increased peripheral vascular density and the lack

of an epithelial lining between the lesion and the biofilm in the pocket may explain the dominance of neutrophil granulocytes in peri-implantitis lesions as a major difference to lesions in periodontitis.

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