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Inflammatory lesions in the gingiva following resective/non-resective periodontal therapy

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Abstract

Background: Findings from previous experiments have revealed that inflammatory cell infiltrates may remain in the gingiva following clinically successful non-surgical periodontal therapy.

Purpose: To investigate the presence of inflammatory lesions in the gingiva following a periodontal treatment procedure that included either soft-tissue resection [gingivectomy (GV)] or non-resective open-flap debridement (OFD).

Material and Methods: Fifteen patients with advanced generalized chronic periodontitis were recruited. Following oral hygiene instruction and supragingival debridement, one tooth site in each quadrant (non-molar, probing pocket depth >5 mm, bleeding on probing$^+$ and $>50\%$ bone loss) was selected and a soft-tissue biopsy was obtained and prepared for immunohistochemical analysis. Using a split-mouth design, two quadrants were randomly selected for periodontal therapy including GV, while the two remaining quadrants were exposed to non-resective OFD procedure. Six months after completion of surgical treatment, a new set of biopsies was obtained from GV and OFD sites.

Results: The inflammatory lesions residing in the gingival biopsies obtained prior to surgical therapy were 1.33–1.41 mm^2 large and contained similar proportions of CD19$^+$- (B-cells, 15%), CD3$^+$- (T-cells, 7%) and elastase$^+$- (polymorphonuclear cells, 2%) cells in the two treatment groups. The corresponding lesions identified in the soft-tissue specimens obtained after 6 months of healing were twice as large at OFD as at GV sites (0.19 *versus* 0.08 mm^2, $p = 0.002$). The densities of CD19$^+$- and elastase$^+$-cells in these lesions were significantly greater at OFD than at GV sites.

Conclusion: The findings of the present study indicate that surgical therapy including soft-tissue resection results in regenerated gingival units that contain smaller lesions with lower densities of immunocompetent cells when compared with the lesions remaining in sites treated by non-resective means.

Key words: cell adhesion molecule; B-lymphocytes; immunohistochemistry; immunology; periodontal therapy; periodontitis

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The inflammatory lesion present at sites with advanced periodontal tissue breakdown is dominated by lymphocytes and plasma cells while the densities of macrophages, polymorphonuclear (PMN) cells and fibroblasts are small (e.g. Page & Schroeder 1976, Berglundh et al. 1998). Lindhe et al. (1980) suggested that the composition of the cell infiltrate varied with the size and extension of the lesion. Thus, comparing samples from gingivitis and periodontitis sites, it was demonstrated that (i) the number of B-cells in relation to T-cells increased with greater size of the infiltrate (Gemmell et al. 1994), (ii) T-cells dominated in lesions from gingivitis sites while periodontitis lesions harboured a larger proportion of B- than T-cells (Yamazaki et al. 1993), and that (iii) cytotoxic T-cells were more numerous in periodontitis than in gingivitis lesions (Jully et al. 1986, Gemmell et al. 1994). In the studies referred to (Jully et al. 1986, Yamazaki et al. 1993, Gemmell et al. 1994), the periodontitis sites selected for examination had been exposed to non-surgical periodontal treatment prior to biopsy, but had maintained features of pathology such as bleeding on probing (BoP) and deepened pockets. In other words, the treatment provided had failed to eliminate clinical signs of inflammation.

A group of patients with severe chronic periodontitis (Berglundh et al.

1999) were placed in a careful plaque-control programme that included daily self-performed oral hygiene measures as well as regularly repeated professional tooth cleaning (Axelsson & Lindhe 1974). In addition, all subjects received several sessions of comprehensive non-surgical periodontal therapy. Post-treatment examinations revealed that clinical signs of gingivitis hereby were resolved, that probing pocket depths (PPDs) were reduced and probing attachment levels (PALs) were improved. Histological examinations of gingival biopsies obtained prior to and 12–24 months after treatment disclosed that there was (i) a marked decrease of the size of the inflammatory lesion, (ii) an apparent reduction in the proportion of B-cells, but (iii) an almost unaltered proportion of T-cells in the infiltrate (Berglundh et al. 1999). In other words, the non-resective treatment delivered, markedly altered but failed to entirely resolve the inflammatory lesion in the gingiva in this group of patients.

The aim of the present report was to describe some histological features of clinically healthy gingiva in subjects with advanced chronic periodontitis that were treated with either resective or non-resective means.

Material and Methods

Fifteen patients (five females and 10 males with a mean age of 56.1 ± 8.4 years) with advanced chronic periodontitis were recruited for the study. The regional ethics committee approved the study protocol and the patients gave their informed consent. Patients were on an individual basis given detailed case presentation and oral hygiene instruction. They also received a series of professional supragingival tooth cleaning. Teeth that could not be maintained were removed and, if indicated, provisional partial dentures (fixed or removable) were produced and inserted (Fig. 1a–d).

After 8 weeks of plaque control, a clinical baseline examination (Day 0) was performed. This included assessment of plaque (plaque index; Silness & Löe 1964), gingivitis [modified gingival index (MGI); Lobene et al. 1986], PPD, PAL and the amount of soft-tissue recession [Rec, from cemento-enamel junction (CEJ)] at four surfaces of all available teeth.

Two different means of access treatment were employed in each patient to facilitate scaling and root planing, i.e. resective therapy: internal bevel gingivectomy (GV), and non-resective therapy: open-flap debridement (OFD) (Wennström et al. 2003). A split-mouth design was utilized. Thus, in two quadrants (one in the maxilla and one in the mandible) the pockets were scheduled to be eliminated by soft-tissue resection (GV), while in the contra-lateral quadrants, the OFD procedure was to be used. Biopsy sites were selected among approximal surfaces of non-molar teeth and had, at this baseline examination, a

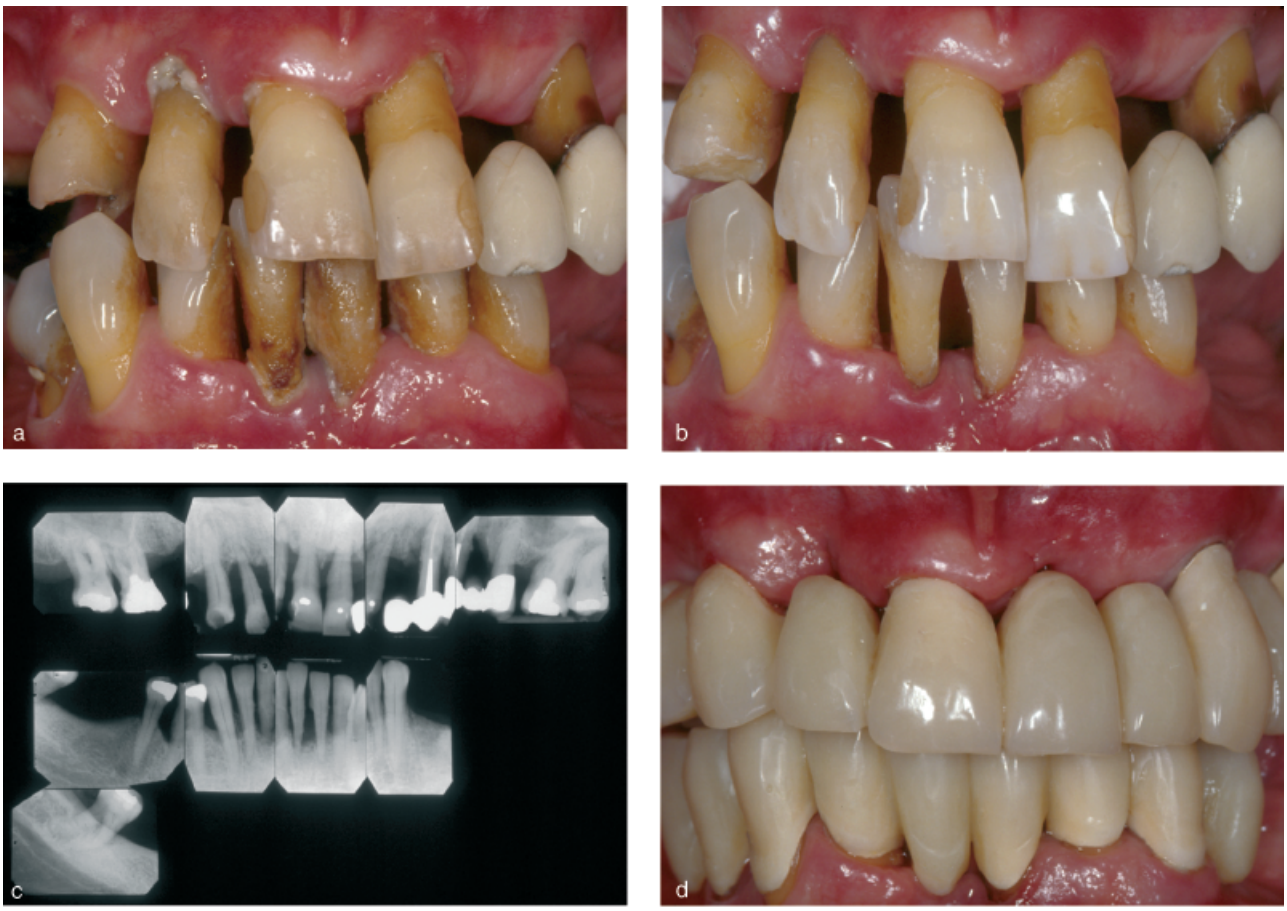

Fig. 1. Clinical photographs of subject no. 4. (a) Before and (b) after 8 weeks of plaque control during initial therapy. The amount of alveolar bone loss is revealed in the full-mouth radiographs (c). The provisional restorations that were placed are illustrated in (d).

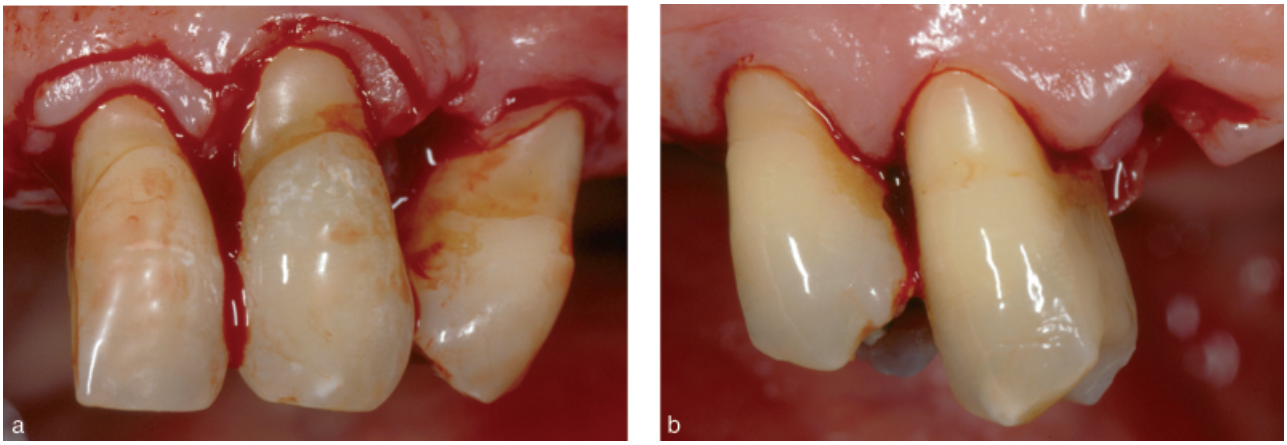

Fig. 2. Clinical photograph that illustrate the gingival incision made at gingivectomy sites (a). Intra-crevicular incisions in open-flap debridement sites are outlined in (b).

PPD of >5 mm and were BoP^{+}. Soft-tissue biopsies were obtained from one diseased site in each quadrant (Fig. 2a, b). Following local anaesthesia (Articain HCl/Ultracain D-S®, Hoechst, Frankfurt, Germany), two parallel incisions, 4 mm apart, were made with a scalpel blade type 12D, an international description available through several manufacturers (e.g. Hu-Friedy, Chicago, IL, USA) through the soft-tissue until bone contact was achieved. The two incisions were connected with a perpendicular incision placed about 4 mm apical of the gingival margin. The biopsies were carefully removed and prepared for immunohistochemical analysis.

Quadrants assigned to GV treatment: buccal and lingual scalloped incisions were placed 2–3 mm apical of the gingival margin (Fig. 2a) and directed towards the bone crest. The pocket epithelium and the adjacent inflamed connective tissue were separated from the non-inflamed gingiva and excised. Mucoperiosteal flaps were elevated and granulation tissue was removed. Following careful debridement of the teeth, buccal and lingual flaps were replaced to the bone crest and sutured.

Quadrants assigned to OFD treatment: an intra-crevicular incision was made (Fig. 2b) and mucoperiosteal flaps were carefully elevated. No instrumentation was applied to the internal aspect of the mucosa, but the exposed root surfaces of the teeth were meticulously debrided. The flaps were replaced and sutured.

All sutures were removed after 10 days. Following surgical therapy, patients were recalled every 2–3 weeks for supportive oral hygiene control and professional tooth cleaning. Six months after the completion of the surgical treatment (Day 180), a clinical and radiological re-examination was performed (Fig. 3a, b). In each subject, soft-tissue biopsies were obtained at approximal surfaces of non-molar teeth from one GV-treated and one OFD-treated site.

Immunohistochemical preparation

The tissue samples were rinsed in saline, embedded (Tissue-Tek®, Sakura Finetek, Zoeterwoude, the Netherlands), and subsequently snap frozen in liquid nitrogen and stored in a freezer at -70°C. From each tissue portion, 5 µm thick sections were prepared in a cryostat and exposed to immunohistochemical staining. A panel of antibodies was used and the avidin–biotin method (ABC) was applied for the staining (Hsu et al. 1981). Sections of human tonsils were used as positive controls, and to

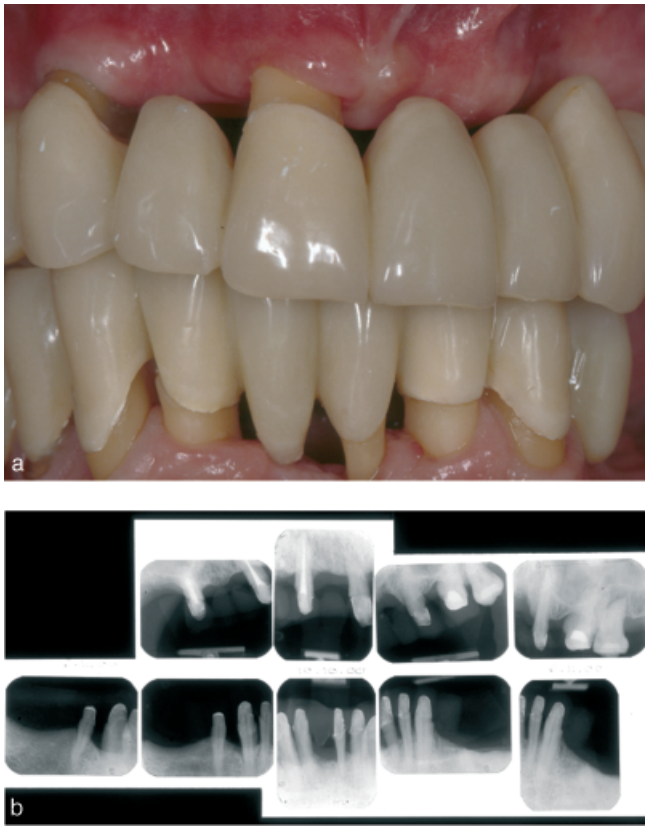

Fig. 3. Clinical photograph that illustrate gingival healing in subject no. 4 at day 180 (a). The amount of remaining bone is revealed in the radiographs (b).

determine the appropriate antibody dilution. Since the mucosal vascular addressins (MAdCAM-1) protein is selectively expressed on high endothelial vessels (HEVs) of mucosal lymphoid organs and on lamina propria venuels of intestinal mesentery (Lehnert et al. 1998), human small intestinal tissue was used as positive control for the BMS170 labelling. All sections were counter-stained with methyl green, dehydrated and mounted (for details see Zitzmann et al. 2001). The CD3 monoclonal antibody was used to identify T-cells, the CD4 marker detected T-helper cells and the CD8 marker cytotoxic T-cells (Table 1). B-cells were identified by CD19 monoclonal antibodies, while PMN leucocytes were detected with anti-PMN elastase monoclonal antibodies. CD54, CD62E and CD106 markers were used to detect cells that expressed inter-cellular (ICAM), endothelial (ELAM) and vascular (VCAM) leucocyte adhesion molecules. The BMS170 monoclonal antibody was applied to identify MAdCAM-1.

Histological analysis

Morphometric measurements were performed using a Leitz DM-RBE microscope (Leica, Wetzlar, Germany) equipped with a Leica Q-500 MC® image system. The keratinized gingiva and the junctional (pocket) epithelium were depicted. The infiltrated connective tissue (ICT) was identified and its size was measured using a mouse cursor (objective × 10). For the enumeration of labelled cells, a point counting procedure with a double lattice (25/400 points) was applied (objective × 40) as previously described (Liljenberg et al. 1994, Zitzmann et al. 2001).

Statistical analysis

Mean values ± standard deviations (SD) were calculated for each variable using the subject as the experimental unit. Differences between the two treatment procedures (GV *versus* OFD) and between baseline and healing at 6 months were analysed using Student's *t*-test for paired observations. *p*-values <0.05 (adjusted for multiple testing; Miller 1981) were considered as significant.

Results

Clinical observations

The results from the clinical examinations performed at baseline and after 6 months of healing are reported in Table 2. At baseline, the biopsy sites of the two prospective treatment groups had a mean PPD value that varied between 6.9 mm (GV) and 6.6 mm (OFD). The corresponding PAL values varied between 7.4 and 7.3 mm and the soft-tissue margin was located on the average between 0.5 and 0.7 mm apical of the CEJ.

During healing following resective therapy (GV), de novo gingival units formed that, at the 6-month interval, were clinically healthy (MGI score = 0.13; only 1 site was BoP^+) and had a mean PPD value of 1.9 ± 0.5 mm. The margin of the newly formed units was located 4.2 mm apical of the CEJ. Thus, the resective treatment performed had resulted in an additional soft-tissue recession that amounted to 3.7 mm.

Also the non-resective therapy (OFD) resulted in the establishment of clinically healthy gingival units (MGI score = 0.25; only two sites were BoP^+) that had a mean PPD value of 2.9 mm. Thus, during healing in OFD-treated quadrants, the gingival units contracted on the average 2.5 mm. As a result the gingival margin of the biopsy sites in this group became located on the average 3.2 mm apical of CEJ (Table 2).

Histological findings

Baseline (day 0)

The different sites selected for biopsy at baseline and after 6 months of healing are presented in Table 3. In all sections, an ulcerated pocket epithelium was present and the connective tissue compartment was dominated by a large inflammatory cell infiltrate (ICT). The size of this ICT was similar in sites assigned to GV (1.33 mm^2) and OFD (1.41 mm^2) treatment (Table 4).

Table 1. Specificity of monoclonal mouse anti-human antibodies used for the immunohistochemical analysis

Antibodies (clone)	Specificity	Dilutions	Isotype
CD3 (T3-4B5)	pan T cells	1:50	IgG1
CD4 (MT310)	T-helper cells	1:20	IgG1
CD8 (DK25)	cytotoxic T-cells	1:50	IgG1
CD19 (HD37)	B-cells	1:25	IgG1
Elastase (NP57)	PMN	1:400	IgG1
CD 54 (6.5B5)	ICAM-1	1:50	IgG1
CD 62E (1.2B6)	ELAM-1	1:50	IgG1
CD 106 (1.4C3)	VCAM-1	1:25	IgG1
BMS 170* (CA102.2C1)	MAdCAM-1	1:100	IgG1

Source: DAKO, A/S, Glostrup, Denmark.
**Source*: AH-Diagnostics, Stockholm, Sweden.
PMN, polymorphonuclear cells; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; ELAM, endothelial leucocyte adhesion molecule; MAdCAM, mucosal vascular addressins.

Table 2. Results of clinical measurements made at baseline and after 6 months of healing. The measurements were made at the different biopsy sites that are identified in Table 3

	Baseline (Day 0)		Healing (Day 180)	
	GV	OFD	GV	OFD
PLI	0.25 (0.32)	0.22 (0.26)	0.09 (0.20)	0.13 (0.22)
MGI	3.16 (0.47)	3.09 (0.49)	0.13 (0.22)	0.25 (0.26)
PPD (mm)	6.9 (1.3)	6.6 (0.8)	1.9 (0.5)*	2.9 (0.7)
PAL (mm)	7.4 (1.4)	7.3 (1.3)	6.0 (1.5)	6.1 (1.1)
Rec (mm)	0.5 (1.0)	0.7 (1.3)	4.2 (1.5)*	3.2 (1.3)

Plaque index scores 0–3 (PLI, Silness & Löe 1964).
Modified gingival index scores 0–4 (MGI, Lobene et al. 1986).
Mean values and standard deviations (SD).
*Indicates statistically significant difference between GV and OFD treatment.
PPD, probing pocket depth; PAL, probing attachment level; Rec, recession; GV, gingivectomy; OFD, open-flap debridement.

$CD19^+$-cells dominated in the lesions at both GV and OFD sites (Table 4; Fig. 4a,b) and occupied a proportion that was twice as large as that of $CD3^+$-cells (14.8–14.9% *versus* 7.4–7.2%). PMN-cells (elastase$^+$-cells) were frequently observed within the pocket epithelium, but were also present in the ICT and occupied 1.9% (GV) and 2.2% (OFD) of the lesion. The density of the different antibodies that disclosed the presence of cell adhesion molecules varied between 2.8% and 5.4%, while the proportion of $BMS170^+$-cells (MAd-CAM-1) was 0.7–0.8% (Fig. 5a–d). Endothelial MAd-CAM-1 expression was detected in all 15 subjects.

Healing (day 180)

In nine out of 15 GV sites, a small inflammatory lesion (range 0.07–0.15 mm^2) was identified in the marginal portion of the gingival connective tissue, while in the other six GV sites only few scattered inflammatory cells were observed to form an ICT (range 0.02–0.04 mm^2). In 13 OFD sites, the size of the inflammatory lesion varied between 0.12 and 0.40 mm^2, while two sites harbored smaller lesions (0.05–0.06 mm^2). On the average, the size of the residual lesion in OFD was significantly larger (0.19 mm^2) than the size of the newly formed lesion in GV (0.08 mm^2) units (Table 5).

The $CD3^+$-cells occupied 4.5% (GV) and 5.4% (OFD) of the ICT (Table 5). The density of $CD19^+$-cells was almost twice as large in OFD than in GV sites (10.1% *versus* 5.4%; Fig. 6a, b). A larger density of elastase$^+$-cells was observed in the ICT of OFD (0.9%) compared with GV (0.5%). The differences regarding $CD19^+$- and elastase$^+$-cells were statistically significant. The proportion of endothelial cell markers ($CD54^+$-, $CD62^+$-, $CD106^+$-cells) varied between 1.8% and 5.3%. The density of MAd-CAM-1 ($BMS170^+$-cells) was larger in OFD sites (0.3%) than in GV sites (0.1%). The MAd-CAM-1 expression was found in eight out of 15 GV sites and 11 out of 15 OFD sites.

Discussion

The present study that included subjects with advanced chronic periodontitis, evaluated some aspects of periodontal tissue healing that occurred during a 6 months interval of plaque control after

Table 3. The biopsy sites (tooth sites) that were examined and/or sampled at baseline and after 6 months of healing following resective (GV) and non-resective (OFD) therapy

Patient	Age	Base line		Healing (6 months)	
		GV	OFD	GV	OFD
1. C. B.	50	13, 31	24, 45	14	23
2. L. A.	56	23, 43	13, 32	44	34
3. S. Z.	55	14, 42	25, 35	45	34
4. W. A.	57	25, 42	11, 33	23	13
5. W. Y.	56	21, 32	13, 43	24	15
6. P. W.	57	12, 33	24, 45	14	23
7. M. C.	67	25, 32	14, 45	24	15
8. H. W.	66	12, 45	23, 34	13	24
9. P. F.	56	23, 42	14, 34	43	33
10. K. C.	69	25, 34	11, 41	22	13
11. M. E.	43	24, 45	14, 35	44	34
12. T. H.	52	24, 43	11, 33	45	34
13. J. S.	56	23, 42	13, 33	45	35
14. M. B.	38	24, 35	14, 45	34	44
15. P. D.	63	15, 45	23, 35	44	33
Mean	56.07				
SD	8.40				

Location of the tooth site in the first–fourth quadrants is described using the FDI system.
GV, gingivectomy; OFD, open-flap debridement, SD, standard deviation.
All biopsies were harvested from interproximal sites.

Table 4. Baseline biopsies; results of the morphometric measurements (Day 0)

Intended treatment	GV	*p*-value (n = 15)	OFD
Size ICT (mm^2)	1.33 (0.66)	0.741	1.41 (0.98)
% of ICT			
CD3	7.38 (2.67)	0.732	7.14 (2.43)
CD4	5.04 (2.30)	0.659	4.72 (2.26)
CD8	2.23 (1.17)	0.469	2.07 (0.91)
CD19	14.90 (5.84)	0.919	14.76 (4.64)
Elastase	1.94 (1.04)	0.427	2.16 (0.93)
CD54	4.65 (1.33)	0.087	5.40 (1.39)
CD62	2.84 (1.39)	0.646	2.95 (1.83)
CD106	5.02 (1.60)	0.238	5.39 (1.76)
BMS170	0.73 (0.66)	0.875	0.76 (0.59)

Mean values and standard deviations (SD).
GV, gingivectomy; OFD, open-flap debridement; ICT, infiltrated connective tissue.

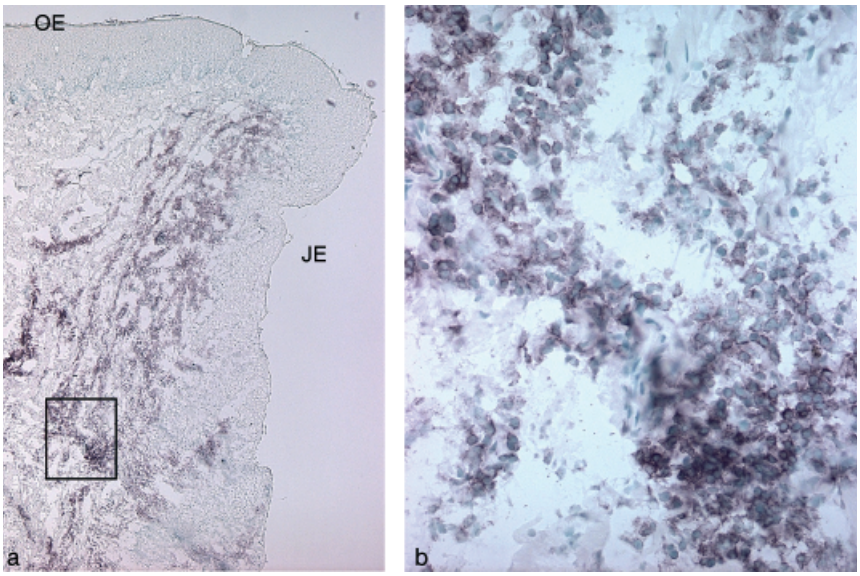

Fig. 4. Bucco-lingual section of a biopsy before treatment (subject no. 4). CD19 labelling, original magnification × 50, OE, oral epithelium; JE, junctional epithelium (a). (b) Higher magnification (× 400) of the area outlined in (a).

the completion of a treatment procedure including either resective (GV) or non-resective (OFD) means. It was demonstrated that after both therapies, clinically healthy gingival units occurred. Thus, only three out of 30 sites examined, were BoP^+ at 6 months. The regenerated gingiva in GV-treated quadrants had at the re-examination a smaller PPD value than the contracted unit in OFD-treated quadrants. These findings are in agreement with data previously published (e.g. Westfelt et al. 1985, Becker et al. 1988, Kaldahl et al. 1988) and illustrate that resective therapy is more effective in reducing PPD (at sites with initially deep pockets) than a non-resective approach.

During healing, the lesion in OFD sites of the current subject sample was reduced from 1.41 to 0.19 mm^2. This degree of resolution of the inflammatory lesion is in agreement with data recently presented by Berglundh et al. (1999).

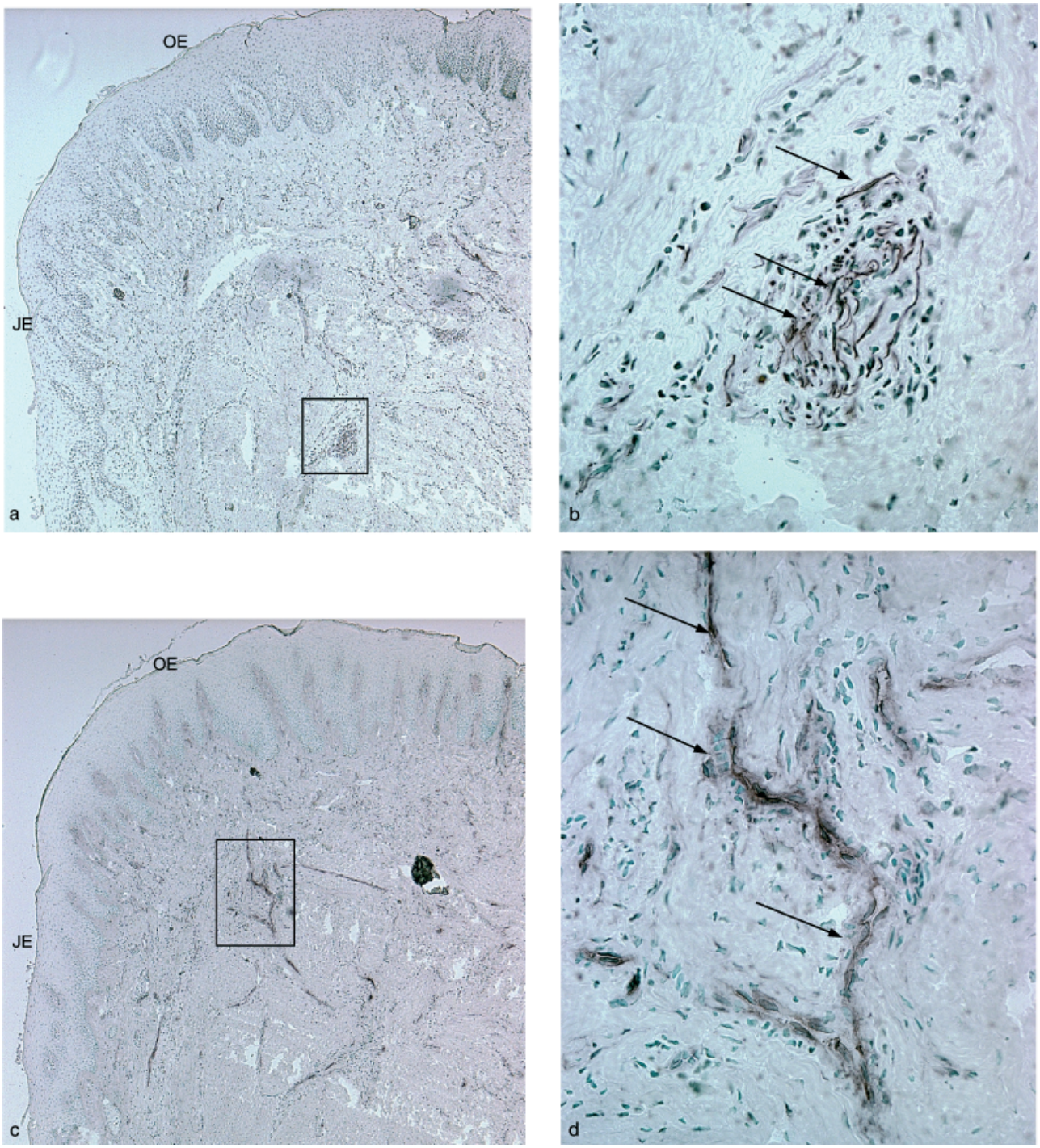

Fig. 5. Bucco-lingual section of a biopsy before treatment (subject no 11). Mucosal vascular addressins-1 labeling (a) original magnification × 50, OE, oral epithelium; JE, junctional epithelium. (b) Higher magnification (× 400) of the area outlined in (a). Arrows indicate ''activated'' endothelial cells. (c) Same biopsy with vascular cell adhesion molecule (VCAM) labelling, original magnification × 50. (d) VCAM labelling × 400.

Table 5. Biopsies obtained after 6 months of healing; results of the morphometric measurements

Treatment	GV	*p*-value ($n = 15$)	OFD
Size ICT (mm^2)	0.08 (0.04)	0.002*	0.19 (0.10)
% of ICT			
CD3	4.52 (2.00)	0.152	5.39 (2.14)
CD4	3.21 (1.93)	0.320	3.73 (1.78)
CD8	1.42 (0.59)	0.042	1.82 (0.85)
CD19	5.41 (2.48)	0.0001*	10.06 (3.21)
Elastase	0.51 (0.36)	0.008*	0.88 (0.35)
CD54	3.99 (1.21)	0.149	4.73 (1.87)
CD62	1.76 (0.92)	0.561	1.91 (0.79)
CD106	4.81 (0.71)	0.128	5.31 (1.40)
BMS170	0.13 (0.13)	0.040	0.30 (0.30)

Mean values and standard deviations (SD).
*Indicates statistically significant difference between GV and OFD treatment.
GV, gingivectomy; OFD, open-flap debridement; ICT, infiltrated connective tissue.

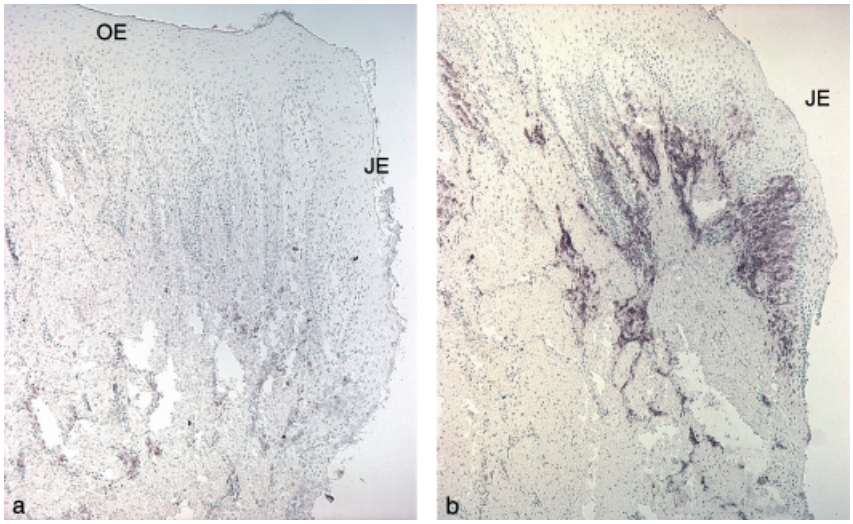

Fig. 6. Bucco-lingual section of a biopsy from a site treated with gingivectomy (a) and sampled following 6 months of healing (subject no. 4). Biopsy from a site treated with open-flap debridement (b) of the same patient at 6 months. CD19 labelling, original magnification × 100, OE, oral epithelium; JE, junctional epithelium.

They studied the cell infiltrate (ICT) in the gingiva of patients with advanced chronic periodontitis prior to and after non-surgical periodontal therapy. Berglundh and co-workers reported that scaling, root planing and subsequent supportive therapy caused a marked decrease of the size of the ICT; from 1.01 mm^2 at baseline to 0.06 and 0.09 mm^2 after 12 and 24 months.

In the baseline biopsies of the present sample, the large ICT was dominated by B-cells. Thus, the proportion of B-cells in the lesion was twice as large as the density of T-cells (15% *versus* 7%). A similar predominance of B-cells in residual periodontitis lesions was reported by e.g. Yamazaki et al. (1993) and Gemmell et al. (1994). They analysed the cellular composition of lesions that persisted following non-surgical therapy and stated that in such ICTs the proportion of B-cells was consistently larger than that of T-cells.

Resective and non-resective therapy resulted, in the present sample, not only in a marked reduction of the size of the ICT, but also in substantial qualitative amendments. A reduction in the proportions was observed for all markers and for $CD19^+$-cells in particular. The qualitative alterations that occurred in the ICT of the current OFD-treated sites, concur with observations reported by Berglundh et al. (1999). They concluded that following non-surgical treatment the density of $CD19^+$-cells was markedly reduced (from 8.3% to 2.0% at 12 months and 1.3% at 24 months), while the proportions of various T-cell populations underwent only modest changes (from 9.9% to 6.9% at 12 months and 6.5% at 24 months).

An important observation made in the current study was that gingival units, which regenerated following resective therapy, at 6 months, harboured a small inflammatory lesion. This ICT, however, engaged an area that was significantly smaller than that occupied by the residual ICT of the OFD units (0.08 *versus* 0.19 mm^2; $p = 0.002$). In the minuscule lesion within the regenerated gingiva (GV sites), the proportions of B-cells and PMN-cells were smaller than in the larger residual lesion of the contracted gingiva of the OFD sites.

In the current study, $BMS170^+$-cells, which indicate expression of the mucosal vascular addressin MAdCAM-1, were present in both GV and OFD sites of all subjects, and in the majority of the samples at 6 months. This cell adhesion molecule selectively supports the binding of lymphoid cells, which are able to recognize and adhere to mucosal HEVs (Lehnert et al. 1998). It was suggested that such specialized vessels play an important role in the immune response in sites of chronic inflammation. Lappin et al. (2003), who sampled mucosal biopsies from clinically healthy and from inflamed gingival tissues in patients with generalized chronic periodontitis, considered the MAdCAM-1 to be one of the addressins responsible for cell trafficking and leucocyte homing in the periodontium. The authors reported that while in a reference material from intestinal mucosa MAdCAM-1 markers were identified, no such expression was detected in the oral tissues (Lappin et al. 2003). In the present investigation, $BMS170^+$-cells (MAdCAM-1 positive) were found both in the infiltrated and in the non-inflamed connective tissue in most of the gingival tissue samples. There are reasons to suggest that this addressin takes part in the recruitment of leucocytes into the periodontitis lesion.

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