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ORIGINAL ARTICLE

Non-surgical periodontal treatment of cyclosporin A-induced gingival overgrowth: immunohistochemical results

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AIM: The present study was planned to analyze the effects of a 12-month non-surgical periodontal treatment on histologic and immunohistochemical features of cyclosporin A (CsA)-induced gingival overgrowth (GO).
MATERIALS AND METHODS: Gingival samples were collected from 21 liver transplant subjects exhibiting CsA-induced GO prior to, and 12 months after non-surgical periodontal therapy including oral hygiene instructions, scaling and 2-month recall appointments, and also from 18 healthy control subjects. Gingival biopsy specimens were stained with hematoxylin–eosin and monoclonal antibodies for vimentin, CD3 (T-lymphocytes), CD20 (B-lymphocytes), CD34 (endothelium) and Ki-67 (fibroblasts proliferation rate), using a streptavidin-biotin-peroxidase complex method.
RESULTS: Total inflammatory cells, gingival vessels and fibroblast proliferation rate demonstrated significant reduction after non-surgical periodontal treatment ($P < 0.0001$) in overgrown gingiva, while B- and T-lymphocytes remained nearly unchanged ($P = 0.61$ and 0.33, respectively). At the 12-month evaluation no significant differences were found when comparing the gingival biopsies from CsA-treated patients and those from healthy controls ($P > 0.05$).
CONCLUSIONS: Control of clinical inflammation by means of non-surgical periodontal treatment results both in lowering of inflammatory infiltrate and in changes in connective tissue composition. Thus, plaque-induced inflammation would seem to modulate the drug-gingival tissue interaction.
CLINICAL RELEVANCE: A strict plaque control program play a pivotal role in the management of transplant patients exhibiting cyclosporin A-GO.
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Introduction

Gingival overgrowth (GO) is a well-documented side effect associated with the systemic use of cyclosporin A (CsA), an immunosuppressive drug extensively used for the prevention of organ transplant rejection, as well as in the treatment of immuno-related disorders (Boltchi *et al*, 1999). It would appear from both human and animal models that plaque-induced inflammatory changes have a significant part in the pathogenesis of such a disease (Seymour *et al*, 2000; Romito *et al*, 2004; Akiyama *et al*, 2006). However, many questions regarding the interaction between the drug and the plaque-induced inflammation still remain unanswered.

The drug may influence directly the inflammatory response by either affecting the nature of the cellular infiltrate or the release of cytokines, prostaglandins or growth factors (Nurmenniemi *et al*, 1999, 2002). Alternatively, the local inflammatory changes secondary to bacterial plaque may orchestrate the drug–fibroblast interaction (Seymour *et al*, 2000; Nurmenniemi *et al*, 2001). Finally, an additive action between the drug and the microbial plaque may be suggested. Drug sequestration in gingival crevicular fluid is greater in the inflamed compared with non-inflamed sites (Ellis *et al*, 1995). Furthermore, CsA may stimulate both cell proliferation in the presence of bacterial lipopolysaccharide, and the release of cytokines in inflamed gingival sites (Barber *et al*, 1992; Sugano *et al*, 1998).

No immunohistochemical data are available in the literature to evaluate the extent to which the drug and the plaque-induced inflammation separately contributes to the induction of GO.

Therefore, the aim of the present study was to determine whether non-surgical periodontal treatment affects

histologic and immunohistochemical gingival features of transplant patients on CsA therapy while complete maturation of soft tissue has been accomplished.

Patients and methods

Patients and clinical procedures

The study was approved by the Ethical Committee of the Medical Faculty, University of Torino, and written informed consent was obtained from each subject before their enrolment in the study. Twenty-one liver transplant patients (7 females, 14 males), aged 25–57 years (mean 46.05 ± 9.15), exhibiting GO were included in the study. They had been on a CsA immunosuppression regimen for an average of 7.95 years (range 4–12) with dosages between 125 and 300 mg day^{-1} (mean 190.48 ± 53.31). None of these patients had a history of any other drug known to affect periodontal tissues. The control group consisted of 18 systemically healthy adults (6 females, 12 males), aged 26–46 years (mean 40.11 ± 6.18) with clinically healthy periodontium and not taking any medication likely to influence periodontal health within the last 6 months.

Full mouth radiographic examination and clinical assessments were carried out by the same investigator. Full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were used to assess the overall degree of clinical inflammation and the home plaque control in both groups. The clinical periodontal parameters including gingival index (GI, Löe and Silness, 1963), and plaque index (PI, Silness and Löe, 1964) were recorded on the upper and lower anterior teeth (canine to canine) in the CsA-treated group and at the sampling sites of both groups at baseline. The degree of GO (hyperplastic index, HI) was graded numerically on plaster study models by the method previously described by Seymour *et al* (1985). A score of 30% was considered the critical value to distinguish CsA responders from non-responders (Seymour and Smith, 1991).

Based on the results, CsA-treated patients who had severe overgrowth (> 30%) and did not have alveolar bone loss were selected for the study. The whole-blood concentration of cyclosporin by radioimmunoassay was determined at the beginning and at the end of the observation period.

In order to reduce or eliminate gingival inflammation, transplant patients underwent instruction on daily plaque control and supra- and sub-gingival scaling procedures with ultrasonic and hand instruments. After completion of the etiologic periodontal therapy, patients were placed on a strict recall maintenance program which included reinforcement of oral hygiene instructions and full mouth scaling every 2 months. They were re-evaluated at 12 months and periodontal variables were recorded. Only the anterior maxillary and mandibular teeth were re-evaluated; the posterior segments of the dental arches were not analyzed.

Gingival samples

In CsA-treated patients, inter-dental sites in single rooted teeth which exhibited a HI score of 4 or 5 and clinically detectable inflammation were selected as sampling sites. None of the patients had any restorations, crowns or bridge construction around the GO. Gingival specimens were collected as described by Vardar *et al* (2004) before the etiologic phase of periodontal treatment and repeated on the same sampling sites 12 months thereafter. In the control group, clinically healthy gingival tissue specimens were obtained during crown lengthening procedures.

Histologic and immunohistochemical procedures

The processing as well as the histologic and immunohistochemical analysis were performed by a blinded investigator. Upon excision, tissue samples were immediately embedded in 10% formalin before immunohistochemical and histologic examination. They were then fixed in 4% formalin overnight, dehydrated in graded alcohols (80%, 90%, 100%) and xylol, and embedded in paraffin at 55–60°C. Serial 3–5 μm sections were made for each tissue specimen on a Leica® ultramicrotome (Leica Microsystems, Wetzlar, Germany). Sections were collected on glass slides for histologic procedures and on 0.1% poly-L-lysine-coated slides for immunohistochemical observations. The biopsy samples were treated with Bio Clear® (Bio Optica, Milan, Italy) and rehydrated through graded decreasing series of alcohols and water.

Light microscopy studies on the sections of healthy and overgrown gingiva were carried out by hematoxylin and eosin staining to determine the epithelial hyperplasia, the acanthosis and the degree of inflammation.

Immunohistochemistry examination was carried out using the antigen retrieval system, i.e. two passages in a microwave oven at 700–750 W for 5 min. The specimens were immersed in a solution of citrate buffer (2.1 g of citric acid monohydrated in 1 l of distilled water) during the exposure and maintained at pH 6 with the addition of NaOH 0.1 N. Immunohistochemistry was performed using an automatized immunostainer (Dako®, Milan, Italy). Endogenic oxidative enzymes were blocked by washing in phosphate-buffered saline (PBS) containing 2% hydrogen peroxide (H_2O_2). The sections were then incubated for 30 min in 2% bovine serum albumin (BSA) followed by 1 h at 37° C with the monoclonal primary antibody diluted in BSA, washed in PBS for 5 min and incubated for 30 min at room temperature in biotinylated secondary antibody (LsAb2; Dako®). A streptavidin-biotin-peroxidase system was used for the detection of primary antibody bindings. Light microscopy (Leitz Laborlux D) was used to examine immunoreactions using a chromogenic substratum dimethyl amino benzidine (DAB/H_2O_2), the sections were washed in PBS, and mounted before examination.

CD20 (L26 clone), CD3 (PS1 clone), CD34 (Q bend 10 clone), vimentin (V 9 clone) and Ki-67 (Mib-1 clone) were used as primary antibodies. CD20, L26 clone (Dako®) is a molecule expressed by the B-lymphocytes which is then lost during the maturation in plasma cells.

CD3, PS1 clone (Novocastra®, Borjea (RO), Italy) is a molecule expressed by mature T- lymphocytes and CD34, Q bend 10 clone (Lab Vision Corporation, Neomarkers®, Newmarket, UK) is a molecule expressed in multipotent

stem cells and in the endothelium. Vimentin, V9 clone (Dako®) is expressed in all cells of mesenchymal origin and it was employed to detect fibroblasts in the stroma. Ki-67, clone Mib-1 (Immunotech®, Beckman Coulter Inc., Fullerton, CA, USA) is an antigen expressed in the nuclei of proliferating cells located in G1, S, G2 and M phase of the cell cycle, and it was used to calculate the cellular proliferation index of fibroblasts.

Positive and negative controls were used as appropriate in each staining run. Serial sections were routinely stained without primary antibodies or non-immune serum and served as negative controls. Reactive human lymph node tissue was used as the positive control.

Histologic and immunohistochemical evaluation

Three fields were selected at random in each tissue specimen for histologic and immunohistochemical evaluation. Both hyperplasia and acanthosis of the oral epithelium were quantified at a magnification of 50× in hematoxylin-stained sections. They were measured in μm using a micrometric ocular from the outer epithelial surface to the epithelial–connective tissue border and from the deepest point in the epithelial–connective tissue interface to the more coronal area in the dermal papillae inserting into the epithelium. The number of inflammatory cells in the connective tissue beneath the oral gingival epithelium was also determined from hematoxylin-stained sections to assess the relative degree of inflammation. The inflammatory cell infiltrate in gingival specimens was expressed as the count of lymphocytes, macrophages and plasma cells mm^{-2} using a manual counter at a magnification of 200×. Data analysis was based on the average of the counts.

The CD3, CD34, CD20, Ki-67 and vimentin-labeled cell distributions were expressed as percentages of cells that were positive for the antigens, compared to the total number of nucleated cells in a selected area, at a magnification of 50×. Data analysis was based on the average of the percentages. Corresponding non-immune-stained slides from serial sections were used as controls to determine background staining which was low or negligible.

Statistical analysis

When data were shown to be normally distributed (acanthosis, hyperplasia, inflammatory cells, CD34, CD3 and Ki-67) baseline and post-therapy values in the CsA-induced GO group were compared by the Student's *t*-test for paired samples. For the nonparametric parameters the Wilcoxon paired test was used. The differences between CsA-GO sites and healthy control samples were tested by unpaired Student's *t*-test (acanthosis, hyperplasia, inflammatory cells, CD34, CD3, and Ki-67) or the Mann–Whitney rank test (vimentin and CD20). Significance level was set at $P < 0.05$.

Results

Clinical aspects

There was no statistically significant difference between the two study groups in terms of demographic characteristics ($P > 0.05$) (Table 1). When the scoring method described by Seymour *et al* (1985) was applied, the mean overgrowth score for the CsA-treated group was 43.52 ± 13.81%. All patients were responders to immunosuppressive therapy and had clinically significant overgrowth (HI >30%). Three hundred and sixty-four gingival units were scored in the anterior segments, of which 287 (78.85%) were overgrown. The initial mean CsA whole blood level was 134.29 ± 44.90 ng ml^{-1}. At the end of the observation period it increased to 138.57 ± 41.90 ng ml^{-1}, but the difference was not statistically significant ($P > 0.05$).

Table 1 Demographic characteristics of the study groups

	CsA-GO (n = 21)	*H (n = 18)*
Age (mean ± SD)	46.05 ± 9.15	40.11 ± 6.18
Female/male	7/14	6/12
Smoker	0	0

CsA-GO, cyclosporin A-induced gingival overgrowth; H, healthy.

The home plaque control and the number of bleeding sites improved over the 1-year experimental period compared with the baseline values. At baseline, transplant patients showed FMPS and FMBS scores of 52.64 ± 16.53% and 46.82 ± 21.43% respectively; the same parameters amounted to 15.74 ± 4.87% and 16.46 ± 3.90% at the 1-year evaluation. In the anterior sextants PI scores decreased by 1.17 from a baseline value of 1.62 ± 0.72 and GI scores by 1.33 from a baseline score of 1.74 ± 0.63. The lowering of PI and GI values was accompanied by a reduction in HI mean scores to 10.05 ± 6.00%. At the end of the observation period 255 gingival units (70%) were not still affected. Sites with the presence of bacterial biofilm and persisting inflammation exhibited residual GO. Seventy-seven gingival units presented HI score of grade 1, 21 sites displayed score of grade 2, and 11 a score of grade 3. No differences between test and control groups were detected at the 12-month examination in FMPS or FMBS (13.82 ± 3.41% and 12.19 ± 2.89%, respectively).

A similar clinical pattern was observed at sampling sites (Table 2). Baseline PI and GI values of the transplant group were significantly higher than those of the healthy control group. At 1 year examination all

Table 2 Clinical periodontal parameters at sampling sites of the study groups (mean ± SD)

	CsA-GO (n = 21)		*H (n = 18)*
	Baseline	*12 months*	*Baseline*
PI	1.90 ± 0.56*	0.33 ± 0.37**	0.17 ± 0.14
GI	2.57 ± 0.42*	0.23 ± 0.26§	0.10 ± 0.1
HI	4.24 ± 0.24*	0.48 ± 0.68*·**	0

CsA-GO, cyclosporin A-induced gingival overgrowth; H, healthy; PI, plaque index; GI, gingival index; HI, hyperplastic index.
*Significant difference from the healthy control group ($P < 0.0001$).
**Significant lower than the baseline values within the same group ($P < 0.0001$).

clinical measurements displayed a statistically significant reduction and were comparable to the control group.

Histologic aspects

The histologic data are presented in Table 3. No significant difference was found in the epithelial layer in terms of hyperplasia and acanthosis between CsA-GO and healthy control specimens at baseline ($P > 0.05$). At the end of the observation period the degree of acanthosis and the epithelial thickness remained nearly unchanged ($P = 0.50$ and 0.09, respectively).

The total number of inflammatory cells in the transplant group was significantly higher than the healthy control group at baseline ($P < 0.0001$). All CsA-GO gingival specimens revealed a moderate to severe inflammatory cell infiltrate made up of plasma cells, lymphocytes, and cells with a macrophage-like aspect and multiple vessels in the lamina propria. This chronic inflammatory infiltrate was drastically reduced after non-surgical periodontal treatment (particularly plasma cells and macrophages) from 345.2 ± 221.9 cells mm^{-2} to 92.9 ± 46.3 cells mm^{-2} ($P < 0.0001$) (Figure 1a–c).

When 12-month gingival samples from transplant subjects were compared with those of periodontally healthy controls no significant differences were found ($P = 0.24$).

Immunohistochemical aspects

The immunohistochemical data are summarized in Table 3. Analyzing the total T-lymphocytes (CD3) and the B-lymphocyte population (L26) behavior in the immunosuppression group after etiologic periodontal treatment, the CD3-labeled cells showed a moderate, nonstatistically significant generalized decrease ($P > 0.05$), whereas the number of B-lymphocytes remained almost unchanged ($P = 0.33$). Similarly, the number of fibroblasts and mesenchymal cells was not significantly changed by the periodontal therapy ($P > 0.05$).

A different trend was observed in the cell population immunostained with CD34 (endothelial cells). An intense expression was observed in overgrown gingiva which decreased significantly at the completion of the periodontal program ($P < 0.0001$) (Figure 2a–c). Concerning the Ki-67 results, the fibroblast proliferation rate was significantly influenced by the reduction in plaque-induced inflammation ($P < 0.0001$).

When comparing the 12-month gingival biopsies from CsA-treated patients and those from the healthy group a similar immunohistochemical pattern was observed. The percentages of CD3, L26, CD34, Ki-67 and vimentin-labeled cells were still greater in CsA-GO sites than in healthy controls, but no significant differences were found between these groups ($P > 0.05$).

Discussion

In the present study we evaluated the effects of a 12-month non-surgical periodontal treatment on histologic and immunohistochemical features of CsA-induced overgrown gingiva. The elimination of plaque-related inflammation may better clarify the sole effects of CsA on gingival tissues. An association between CsA-GO and inflammation has been recorded

Table 3 Histologic and immunohistochemical parameters at sampling sites of the study groups (mean ± SD)

	CsA-GO (n = 21)				*P** CsA-GO/H	
	Baseline	*12 months*	*P**	*H (n = 18)*	*Baseline*	*12 months*
Hyperplasia (μ)	480.9 ± 73.3	469.0 ± 96.8	0.62	444.5 ± 76.5	0.14	0.39
Acanthosis (μ)	240.5 ± 81.6	216.7 ± 69.5	0.09	200.0 ± 82.2	0.13	0.50
No. inflammatory cells mm^{-2}	345.2 ± 221.9	92..9 ± 46.3	< 0.0001	78.9 ± 18.2	< 0.0001	0.24
Ki-67 (%)	15.7 ± 8.5	3.81 ± 3.5	< 0.0001	2.4 ± 2.03	< 0.0001	0.13
Vimentin (%)	16.7 ± 6.6	13.6 ± 8.8	> 0.06	14.4 ± 9.2	0.37	0.73
CD3 (%)	29.5 ± 15.0	27.6 ± 9.4	0.61	29.7 ± 13.8	0.97	0.58
CD20 (%)	4.3 ± 3.3	3.8 ± 3.1	0.33	3.9 ± 3.7	0.7	1.000
CD34 (%)	31.9 ± 10.3	19.5 ± 8.0	< 0.0001	14.4 ± 9.2	< 0.0001	0.09

CsA-GO, cyclosporin A-induced gingival overgrowth; H, healthy.
*$P < 0.05$ (statistically significant).

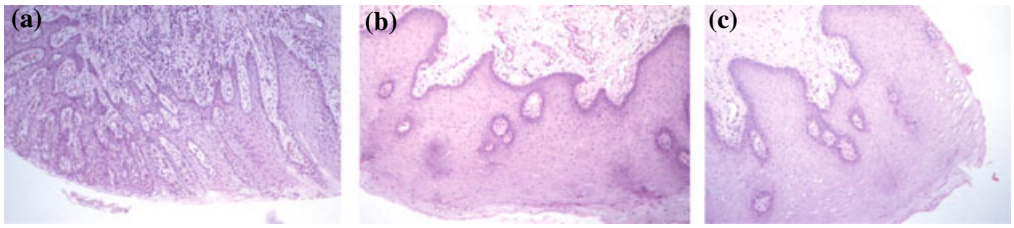

Figure 1 Histologic sections of cyclosporin A (CsA) overgrown and normal gingiva stained with hematoxylin–eosin (original magnification ×200). (**a**) High number of chronic inflammatory cells prior to treatment in the CsA specimen. (**b**) Marked decrease in degree of inflammatory infiltrate 12 months post-treatment. (**c**) Small number of inflammatory cells in the control gingiva

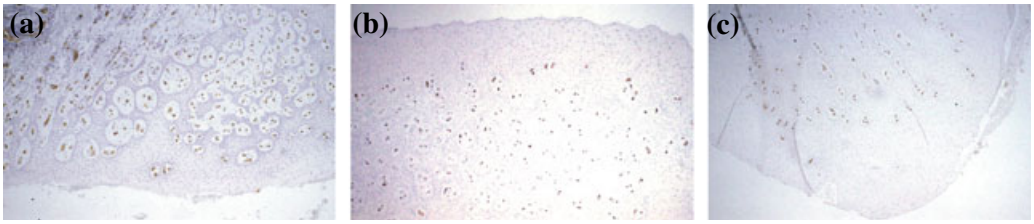

Figure 2 CD34 antigen expression in the cyclosporin-overgrown gingiva and in the control tissues (original magnification ×50). (**a**) Intense immunolocalization of CD34 antigen prior to periodontal treatment in the connective tissue of cyclosporin A (CsA)-treated gingiva. (**b**) Marked decrease in the number of gingival vessels 12 months post-therapy. (**c**) Expression of CD34 antigen in the control gingiva. The immunostaining observed in healthy samples was similar to that detected in CsA-GO specimens after periodontal treatment

in several clinical papers, and the presence of a chronic inflammatory cells infiltrate in lamina propria has been reported in morphological studies (Seymour and Jacobs, 1992; Thomason *et al*, 2005). However, the exact interactions between the drug and the inflammatory process are still unclear. Almost all studies investigating the morphological and immunohistochemical aspects of overgrown gingival tissues have been carried out in inflamed gingival samples obtained prior to periodontal treatment (Seymour *et al*, 2000). To our knowledge, this is the first communication analyzing the immunohistochemical characteristics of CsA-induced overgrown gingiva before and 12 months after etiologic and supportive periodontal therapy. It was previously reported in the literature that 1 year is the time necessary for proper maturation of the soft tissues to occur (Aimetti *et al*, 2005). Montebugnoli *et al* (2000) reported that scaling and oral hygiene program were effective in reducing plaque and gingival indices after 3 months, while changes in gingival volume took longer to show a significant improvement. In the present investigation the removal of dental plaque and inflammation corresponded to a marked decrease in the severity of gingival enlargement. At 1-year examination the reduction in PI and GI values by 80–90% was accompanied by a similar decrease in HI scores. These clinical outcomes could be, in part, attributable to the stringent supra- and subgingival plaque control protocol. The significant modifications in gingival volume occurred without any variation in CsA blood concentration.

In transplant patients who are responders to the immunosuppressive therapy (HI $>30\%$) the drug alone would not seem to modulate the histologic and immunohistochemical features of gingival tissues. No differences were observed between clinically non-inflamed gingiva harvested from CsA-treated patients at 12 months post-therapy and normal, clinically non-inflamed gingival biopsy material from healthy individuals without medication. It is important to point out that we analyzed only some morphological and immunohistological aspects of gingival enlargement and their possible relationship with bacterial biofilm. The CsA-GO is a multifactorial disease in which various factors may play a significant role and where many aspects of connective tissue homeostasis can serve as targets for inducing drug to cause overgrowth.

Histology examination showed that squamous epithelium thickness, acanthosis or numerical density of fibroblasts are not related to the presence of GO or to the degree of gingival inflammation. These findings are in agreement with the previous observations by Uzel *et al* (2001) who described a number of fibroblastic cells and connective tissue fibers in CsA-GO samples similar to non-inflamed healthy gingiva after professional supra- and sub-gingival debridement and plaque control.

By contrast, previous studies described enhanced fibroblast cellularity and thickening of the oral epithelium in CsA gingival enlargements (Wondimu *et al*, 1995; Ashrafi *et al*, 1996; Spolidorio *et al*, 2001).

It is of interest that in the present study the proliferation rate of fibroblasts was related to the degree of gingival inflammation. We observed a marked expression of Ki-67 antigen in the lamina propria of transplant patients which decreased at the completion of non-surgical treatment to values comparable to those of control specimens. Saito *et al* (1999) reported an intense expression of Ki-antigen in fibroblasts of hyperplastic gingival tissues and an undetectable immunostaining in the controls. However, the percentage of cells expressing the antigen was not calculated. Presumably, CsA alone is not directly influencing the proliferation rate of fibroblasts, but rather acts through indirect networks via locally released inflammatory mediators or bacterial products (James *et al*, 1995; Pernu and Knuuttila, 2001). Alternatively, it is possible that the inflammatory process may orchestrate directly the cellular activity of fibroblasts. It is known that in inflammatory lesions the mitotic activities of gingival fibroblasts are increased (Stabellini *et al*, 2004). The removal of plaque-induced inflammation may have affected the fibroblast proliferation rate and, indirectly on cellular synthetic activities.

Histological studies reported as prominent ultrastructural findings of CsA enlarged gingiva-rich vascularity and chronic inflammatory cell infiltrate (Mariani *et al*, 1996). We observed a great expansion of the vascular component in the connective tissue of inflamed CsA overgrown gingiva. In accordance with morphological observations we recorded a high expression of CD34 in the lamina propria of immunostained biopsy specimens. CD34 staining appeared to be greater in sites where inflammation was more apparent and decreased after

periodontal treatment where similar values to those of control healthy tissue were observed.

In the present study qualitative differences in immune response were studied regarding the T- (CD3) and B-lymphocyte (CD20) populations. No differences in the percentage of CD3- and CD20-labeled cells were found in relation to inflammation status and cyclosporin medication. The only difference we detected was a higher baseline standard deviation in CsA-GO sites indicating heterogeneity among transplant subjects regarding CD3 expression. A similar heterogeneity was also observed in previous investigations (O'Valle *et al*, 1994). Our findings compare favorably to studies by O'Valle *et al* (1994) and Pernu and Knuuttila (2001) who found no significant differences between CsA-overgrown and healthy gingiva regarding numbers of CD20 cells. However, as no periodontal treatment was applied, our comparison was limited to preoperative data.

We hypothesized that the marked decrease in the total inflammatory infiltrate observed in hematoxylin-stained sections was attributable to a reduction in plasma cells and macrophages. However, no immunohistochemical data are available. The histologic finding of higher numbers of plasma cells in CsA-GO specimens at baseline compared to 12-month evaluation appears to support this view. In individuals on CsA treatment the humoral response would seem to replace the cell-related immune response to plaque-induced inflammatory changes (Pernu and Knuuttila, 2001). These increased proportions of plasma cells could be the result either of the continual antigenic stimulation on the immature B-lymphocytes induced by the bacteria of the plaque or of the altered production of IL-6 in the presence of gingival inflammation or both (O'Valle *et al*, 1994; Williamson *et al*, 1994). Likewise, the elimination of plaque may, in part, explain the decrease in such inflammatory cells.

In conclusion, our results indicate that control of clinical inflammation by means of a strict non-surgical periodontal treatment results in gingival tissue histologically and immunohistochemically comparable to that of healthy control patients, irrespective of the assumption of CsA. A previous report suggested that CsA-GO has two components: fibrotic enlargement of CsA origin and inflammatory lesions caused by microbial dental plaque (Kantarci *et al*, 1999). However, in the present investigation the removal of microbial biofilm resulted both in the elimination of the inflammatory component and in changes in the composition of the connective tissue. These findings seem to support the possibility that fibrosis and local inflammatory factors are related. It is known that numerous lymphokines exert a direct fibrogenic action which can increase that induced by the drug (Kovacs, 1991). The complete regression of gingival enlargement in most gingival units may further corroborate this concept. This assumption does not exclude the importance of other factors in the pathogenesis of GO.

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