J Clin Periodontol 2008; 35: 371–378 doi: 10.1111/j.1600-051X.2008.01216.x

Journal of Clinical Periodontology

Transforming growth factor-β1 gene expression and cyclosporine A-induced gingival overgrowth: a pilot study

Małgorzata Radwan-Oczko[1], Maria Boratyńska[2], Marek Ziętek[1] and Tadeusz Dobosz[3]

[1]Department of Periodontology; [2]Department of Nephrology and Transplant Medicine and [3]Department of Forensic Medicine, Molecular Techniques Unit, Silesian Piasts University of Medicine, Wrocław, Poland

Radwan-Oczko M, Boratyńska M, Ziętek M, Dobosz T. Transforming growth factor-β1 gene expression and cyclosporine A-induced gingival overgrowth: a pilot study. J Clin Periodontol 2008; 35: 371–378. doi: 10.1111/j.1600-051X.2008.01216.x.

Abstract

Aims: The relationship between gingival overgrowth (GO) induced by cyclosporine A (CsA) and transforming growth factor-β1 (TGF-β1) remains unclear. The aims of the present study were to evaluate TGF-β1 gene expression under different immunosuppressive treatments and its association with TGF-β1 gene functional polymorphism and GO in renal transplant recipients.

Material and Methods: The study included 98 CsA-treated renal transplant recipients (with and without GO) and 44 tacrolimus-treated transplant patients (without GO). TGF-β1 mRNA expression was measured using a real-time quantitative polymerase chain reaction assay. The levels were correlated with TGF-β1 gene polymorphisms at codons 10 and 25, with different immunosuppressive treatment and GO.

Results: The level of TGF-β1 gene expression was insignificantly lower in the CsA-treated group compared with the tacrolimus group, and significantly lower in the group with GO compared with patients without GO. In tacrolimus- and CsA-treated patients, but not in patients with GO, the level of TGF-β1 gene expression was associated with functional phenotypes of TGF-β1. The incidence, degree and extent of GO were higher in recipients with lower TGF-β1 gene expression.

Conclusions: Lower level TGF-β1 gene expression, not functional polymorphism, in patients treated with CsA may be considered to be a risk factor for GO.

Key words: gingival overgrowth; immunosuppression; renal transplant; TGF-β1 gene expression

Accepted for publication 18 February 2008

Gingival overgrowth (GO), an unwanted effect related to cyclosporine A (CsA) and calcium channel blockers' administration in renal transplant recipients, is well recognized (O'Valle et al. 1995, James et al. 2000, Routray et al. 2003). Although the alternative immunosuppressive drug, tacrolimus, became more widely used, cyclosporine remains an important drug for prevention of organ transplant rejection (Berloco et al. 2001). Numerous studies have investigated local and systemic factors that contribute to development of GO, but the exact mechanism for this pathology is still unknown (Afonso et al. 2003, Radwan-Oczko et al. 2003, Bartoli et al. 2004). A significant correlation between oral hygiene and gingival or periodontal status and the extent and severity of GO was proved. Nevertheless, the elimination of the local factors during the initial phase of periodontal therapy resulted in a more ameliorating inflammation than in a reduction in GO (Kantarci et al. 1999, Leung et al. 2003, Radwan-Oczko et al. 2004, Aimetti et al. 2005a, b). Some studies suggested that the total dose and trough blood concentration of cyclosporine and the human leucocyte antigen HLA-DR2 are related to the development of GO (Thomas et al. 2000). The level of CsA evaluated in whole saliva, and the level of cytokines in gingival crevicular fluid (GCF) were also considered to be important determinants of this pathology (Modeer et al.

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
The study was self-funded by the authors and their institution.

1992, Atilla & Kütükcüler 1998). But today various factors related to GO remain an area of controversy.

Attention was also drawn to immunopathological and molecular mechanisms and genetic predispositions concerning the multifactorial pathogenesis of cyclosporine-induced gingival changes. Transforming growth factor-β1 (TGF-β1) has been widely recognized as a key fibrogenic cytokine. Recent studies considered the role of plasma and GCF levels of TGF-β1 and TGF-β1 gene polymorphisms. There are studies pointing out that the CsA therapy increases the level of this cytokine (Buduneli et al. 2001, Wright et al. 2004). However, the results are equivocal. Earlier studies described the association between increased levels of TGF-β1 and both hereditary GO (Wright et al. 2000) and CsA-induced GO (James et al. 1998). However, the latter type of overgrowth is not predominantly fibrotic and the pathology can result from deficiency of this cytokine. In the latest investigation, in which the influence of periodontal status and TGF-β1 plasma concentration on GO was analysed, the low plasma level of this cytokine was found to be a risk factor of GO in patients receiving CsA and concomitantly calcium channel blockers (Ellis et al. 2004).

In our previous study, we did not find any relationship between TGF-β1 plasma levels and GO. We identified the high producer phenotype of TGF-β1 to be overrepresented in patients suffering from GO. The lowest incidence of GO was observed in the 10 C/C genotypes (Radwan-Oczko et al. 2006).

In the last decade, tacrolimus (FK506) has been introduced as an alternative immunosuppressant to CsA (Berloco et al. 2001, Chand et al. 2001). This calcineurin inhibitor has also been investigated for its impact on GO. Renal transplant patients treated with tacrolimus do not suffer from GO even if calcium channel blockers are used concomitantly (James et al. 2001, Radwan-Oczko et al. 2004). Moreover, significant or complete regression of GO and improvement of gingival conditions were noticed after conversion from CsA to tacrolimus both in the objective and in the subjective evaluation (Busque et al. 1999, Kohnle et al. 1999, Thorp et al. 2000, Hernandez et al. 2003, Radwan-Oczko & Boratyńska 2006). Tacrolimus, like CsA, increases TGF-β1 production.

Thus, the aim of the present study was to investigate and compare the level of TGF-β1 gene expression in renal transplant recipients under CsA therapy with and without GO and in patients under tacrolimus therapy. Furthermore, the association of the TGF-β1 gene expression with functional polymorphisms and development, degree and extent of GO was investigated.

Material and Methods

In this study, 142 outpatients after renal transplantation from the Department of Nephrology and Transplant Medicine, Silesian Piasts University of Medicine, Wrocław, were enrolled. All patients provided informed consent to participate in the study. The research was launched following local committee approval and conducted in accordance with the Helsinki Declaration of 2002. There were 85 males and 57 females with a mean age of 39 years (range 15–70) and mean 48 months (range 4–312) after transplantation. In the immunosuppressant treatment, CsA (98 individuals) and tacrolimus (44 individuals) were given. Both CsA and tacrolimus were used in combination with azathioprine or mycophenolate mofetil and prednisolone. Besides, 75% of the patients received calcium channel blockers concomitantly. The dose of immunosuppressive drugs depended on the through level and the time lapse after transplantation. The patients had stable renal graft function.

The CsA-treated group consisted of 54 patients with (GO+) and 44 recipients without (GO −) GO. Patients in the tacrolimus-treated group were free of GO. The data collected included the following clinical and gingival variables: immunosuppressive drug total dose, time after transplantation, gender, plaque and bleeding indices.

GO was observed in degrees from 1 to 3 as assessed according to a 4-degree scale. The scale described in earlier investigations (Radwan-Oczko et al. 2006) ranges from 0 – indicating no overgrowth, to 3, indicating enlargement covering more than half of the tooth crown. The extent of GO present was from 33% to 100% dental units. The average number of teeth in the investigated groups was from 22 to 23. For clinical evaluation of bleeding, the SBI% index and for plaque the API% index were used (Mühlemann & Son 1971, Lange et al. 1977). All dental examinations were performed by the same dentist.

TGF-β1 quantitative gene expression (E) was estimated in 96 renal transplant recipients (35 CsA group with GO, 31 CsA group without GO and 30 treated with tacrolimus) by the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) method. Forty-eight patients were not assayed for mRNA TGF-β1, because of systemic factors, which might influence the expression of this cytokine (ischaemic heart disease, atherosclerosis, HCV infection, administration of inhibitor of HMG-CoA reductase and/or angiotensin-converting enzyme inhibitor). Genetic polymorphism, on the other hand, is invariable under different systemic influences.

TGF-β1 gene expression was measured in peripheral mononuclear cells using real-time RT-PCR. Total RNA was extracted using the PAXgene Blood RNA Kit (PreAnalytix, Hombrechticon, Switzerland). Purity and yield were determined spectrophotometrically and 2 μg of total RNA was reverse transcribed by the ThermoScript™ RT-PCT system (Invitrogen, Carlsbad, California). All quantitative real-time PCR (TaqMan™, Branchbuvg, New Jersey) primers, probes and Universal Master Mix were obtained from Applied Biosystems (Foster City, California, USA). All PCRs were performed using 2.5 μl cDNA per reaction in triplicates of 25 μl volume on an ABI Prism 7900HT Sequence Detection system (TaqMan) using a two-step PCR protocol after the initial denaturing of the cDNA (10 min. at 95°C) with 40 cycles of 95°C for 15 s and 60°C for 1 min.

cDNA aliquots were quantified for target genes using the threshold cycle (C_t) method normalized for the housekeeping gene GAPDH. The TGF-β1 target in unknown samples was quantified by measuring ΔC_t($\Delta C_t = Ct_{\text{target gene}} - Ct_{\text{endogeneouscontrol}}$). The parameter C_t is defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passes a fixed threshold above the baseline. The C_t value is inversely proportional to the number of target copies and thus better suited for quantification than maximum fluorescence. The larger the starting quantity of the target molecule, the earlier a significant increase in fluorescence is observed. The precise amount of total RNA added to each reaction (based on absorbance) is difficult to assess. We therefore use the GAPDH

gene as the endogenous control for normalization of different amounts of RNA across samples. On the basis of ΔCt values, the levels of mRNA TGF-β1 expression (E) were determined.

In all patients, the two biallelic polymorphisms of the TGF-β1 gene were studied at codon 10 (at position +869) and at codon 25 (position +915) according to the methodology presented in our previous work (Radwan-Oczko et al. 2006). The genotypic combinations T/T at codon 10, G/G at codon 25 (10T/T 25G/G) and 10T/C · 25G/G were described as high producer phenotypes; combinations 10T/C · 25G/C, 10C/C · 25G/G and 10T/T · 25G/C as intermediate producer phenotypes and 10C/C · 25G/C, 10T/T · 25C/C, 10T/C · 25C/C and 10C/C · 25C/C as low producer phenotypes (Hoffmann et al. 2001).

The association between the functional polymorphisms of TGF-β1 and the genotypes with the level of TGF-β1 gene expression was analysed.

Statistical analysis

Data were presented as mean values ± standard deviations (SD). Student's t-test and Mann–Whitney U tests with a value $p<0.05$ were accepted as statistically significant.

Results

The clinical characteristics of the groups investigated are presented in Table 1. When we compared CsA- and tacrolimus-treated groups, there was a significant difference only in time lapse after transplantation. Comparison of clinical data between the group with (GO+) and without GO (GO−) showed insignificant differences except for plaque and bleeding indices, which were higher in patients with overgrowth. Also, calcium channel blocker use was similar in the CsA-treated groups; thus, it has not influenced the results of the investigations. Slightly fewer calcium channel blockers were used in the tacrolimus-treated patients, which were not susceptible to overgrowth.

In the group of patients suffering from GO, 79.6% displayed high producer phenotype, 16.7% intermediate and only 3.7% low producer phenotype. In the group without GO, high producer phenotype was present in 72.7% patients, intermediate in 20.5% and low producer phenotype in 6.8%, almost twice more frequently than in the group with GO.

In the whole CsA-treated group of patients, the level of TGF-β1 gene expression was lower by 8% (not significantly) in comparison with the tacrolimus-treated group (Fig. 1).

In tacrolimus-treated patients, the level of TGF-β1 gene expression was associated with functional phenotypes of TGF-β1 and was the lowest for low and the highest for high producer phenotypes. In the whole group treated with CsA (regardless of GO), excluding low producer phenotype, the relationship between the level of TGF-β1 gene expression and functional phenotypes was similar. When CsA-treated patients were compared with tacrolimus-treated patients, the level of TGF-β1 expression in the CsA group was significantly lower for the high producer phenotype and significantly higher for the intermediate producer phenotype and more than four times higher for the low producer phenotype (only three patients) (Table 2).

Patients treated with CsA, who had T/C alleles at codon 10 and G/C alleles at codon 25, displayed significantly higher levels of TGF-β1 expression when compared with the tacrolimus-treated group; conversely, the carriers of T/T alleles at codon 10 had significantly lower levels of expression than when treated with tacrolimus. Figure 2 shows a significantly lower TGF-β1 expression for the T/T allele when compared with the carrier C/C allele in the CsA-treated group of patients. In codon 25, the level of expression was comparable with G/G and G/C allele carriers (Fig. 3).

In the group with GO, TGF-β1 gene expression was lower by 25% and conversely in the group without GO, expression was higher in reference to the tacrolimus-treated group (Fig. 1). When groups with and without GO

Table 1. Baseline clinical characteristics of investigated groups

	Whole CsA-treated group	Tacrolimus-treated group	CsA-treated groups	
			with GO	without GO
Number of patients	98	44	54	44
Gender distribution (F:M)	40:58	17:27	18:36	22:22
Age (years)	40.1 ± 11.4	37.7 ± 12.5	39.9 ± 10.1	40.3 ± 13.0
Time lapse after transplantation (months)	61.3 ± 45.6	17.5 ± 8.2*	55.8 ± 35.6	68.0 ± 55.2
CsA/tacrolimus total dose (g)	298 ± 189.2	6.6 ± 12.2	306 ± 190.4	288 ± 189.4
Calcium channel blockers use (%)	78.5	68.1	77.7	79.5
API%	52 ± 30.9	45 ± 27.8	57 ± 27.9	47 ± 33.8†
SBI%	27 ± 27.7	17 ± 18.2	34 ± 31	19 ± 20.3‡

Significant differences between investigated groups.
*$p<0.00$; Students' t test; CsA *versus* tacrolimus group.
†$p<0.05$; Mann–Whitney U test; GO+ *versus* GO− group.
‡$p<0.00$; Mann–Whitney U test; GO+ *versus* GO− group.
CsA, cyclosporine A; GO, gingival overgrowth.

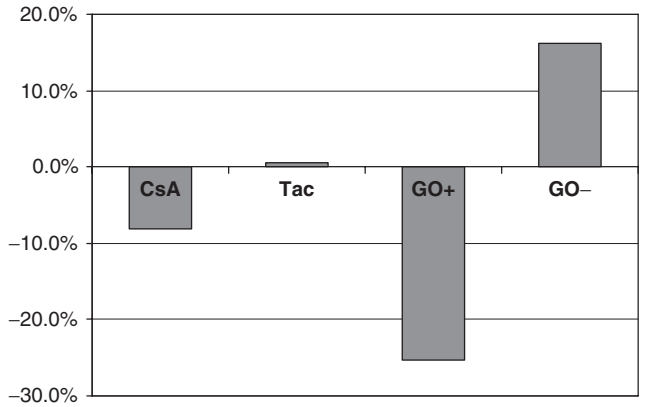

Fig. 1. Level of transforming growth factor-β1 gene expression in cyclosporine A (CsA)-treated and GO+ and GO− groups in reference to the level of the tacrolimus-treated group (0%). Significant difference between GO+ *versus* GO− group; $p<0.05$. GO, gingival overgrowth.

Table 2. Transforming growth factor-β1 gene expression (E) in CsA- and tacrolimus-treated groups

	n	CsA-treated group	n	Tacrolimus-treated group
Whole group	66	1.42 ± 2.88	30	2.873 ± 9.67
Low-producer phenotype	3	2.2955 ± 2.75	2	0.533 ± 0.09
Intermediate-producer phenotype	9	0.9961 ± 0.36	7	0.7184 ± 0.19*
High-producer phenotype	54	1.4441 ± 3.13	21	3.814 ± 11.51*
Alleles C/C in codon 10	6	1.5983 ± 1.9	6	0.7044 ± 0.21
Alleles T/C in codon 10	39	1.7906 ± 3.64	9	0.6542 ± 0.16*
Alleles T/T in codon 10	21	0.6863 ± 0.21	15	5.0719 ± 13.54*
Alleles G/G in codon 25	57	1.4156 ± 3.4	25	3.3302 ± 10.57
Alleles G/C in codon 25	9	1.4609 ± 1.55	5	0.5876 ± 0.12*

Significant difference between CsA- *versus* tacrolimus-treated groups.
*$p < 0.05$.
CsA, cyclosporine A.

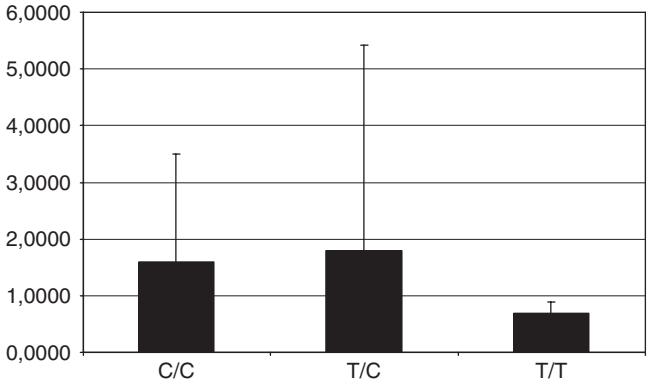

Fig. 2. Level of transforming growth factor-β1 gene expression in the cyclosporine A (CsA)-treated group for C/C, C/T and T/T alleles in codon 10. Significant difference in the CsA-treated group between C/C and T/T alleles; $p = 0.02$. Error bars represent SD.

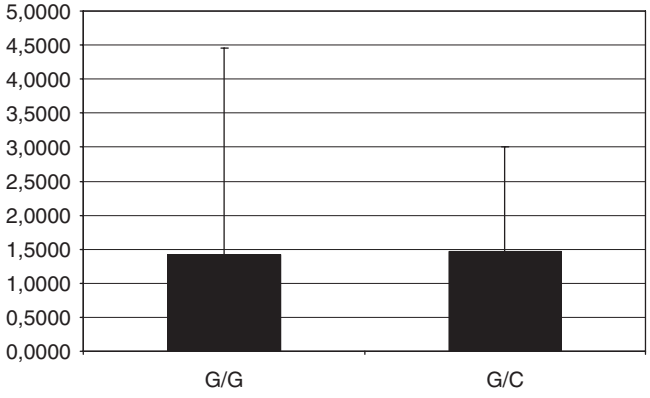

Fig. 3. Level of transforming growth factor-β1 gene expression in the cyclosporine A-treated group for G/G and G/C alleles in codon 25. Error bars represent SD.

Table 3. Transforming growth factor-β1 gene expression (E) in group with (GO+) and without (GO−) gingival overgrowth

	n	GO+	n	GO−
Whole group	35	0.949 ± 1.28	31	1.955 ± 3.94*
Low- + intermediate-producer phenotype	3	0.914 ± 0.194	9	1.457 ± 1.551
High-producer phenotype	32	0.953 ± 1.341	22	2.159 ± 4.595*

*$p < 0.05$.
GO, gingival overgrowth.

(without distinction for polymorphism) were compared, there was a significantly lower level of TGF-β1 expression in the group with GO (Table 3 and Fig. 1). A similar observation was found for patients bearing a high producer phenotype (Table 3 and Fig. 4). In case of patients bearing low and intermediate phenotypes, the level of TGF-β1 expression in the group with GO was also lower by 48% (lack of statistical significance).

The relationship between the TGF-β1 gene expression level and the incidence, degree and extent of GO was also investigated. Also, when gene expression amounted to 1 or >1 ($E \geqslant$), the level of expression amounted to <1 ($E <$), all investigated parameters that described GO were higher (not significantly) in relation to lower TGF-β1 gene expression levels (Figs 5–7).

Discussion

Many earlier studies have evidenced the influence of CsA therapy on TGF-β1 synthesis both in in vitro and in vivo studies. It was shown that cyclosporine increases the circulating TGF-β1 level, its expression in kidney tissue (Shin et al. 1998, Khanna et al. 1999) and GCF level (Buduneli et al. 2001, Wright et al. 2004). Our previous data (Radwan-Oczko et al. 2006) showed an insignificantly higher TGF-β1 plasma level in patients treated with cyclosporine compared with healthy controls and tacrolimus-treated patients. These findings were not confirmed in other investigations (Hughes et al. 1999, Hetzel et al. 2001).

TGF-β1 gene expression was also investigated in association with TGF-β1 gene polymorphisms in renal transplant recipients under different immunosuppressive therapies. A lower TGF-β1 gene expression was found in renal recipients treated with tacrolimus, especially for the low producer phenotype (Ochsner et al. 2002). The data also pointed out the relationship between mRNA TGF-β1 expression and TGF-β1 genotypes (Guo et al. 2002). Our study confirmed these results for patients treated with tacrolimus and a subgroup treated with CsA but without GO. Comparison of cyclosporine- and tacrolimus-treated patients revealed similar levels of TGF-β1 gene expression. Our studies evaluated, for the first time, the TGF-β1 gene expression level in

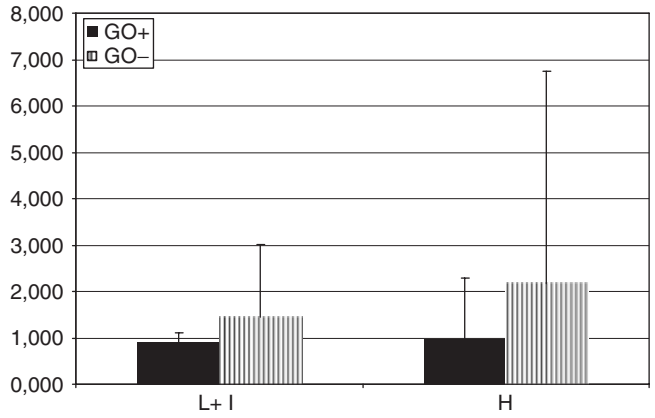

Fig. 4. Level of transforming growth factor-β1 gene expression for both low and intermediate phenotypes and high producer phenotype in GO+and GO− groups. Significant difference between the GO+ and GO− groups investigated for the H phenotype; $p<0.05$. Error bars represent SD. GO, gingival overgrowth.

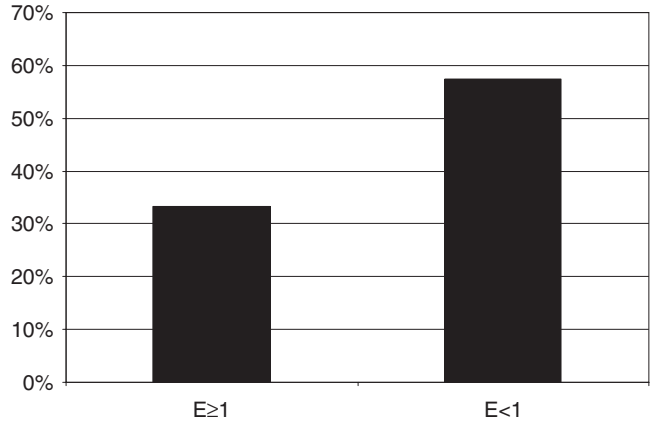

Fig. 5. Incidence of gingival overgrowth in the cyclosporine A-treated group in patients with higher than and equal to and lower than 1 transforming growth factor-β1 gene expression.

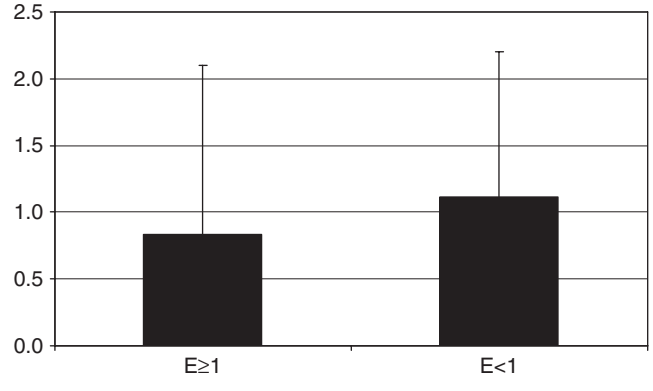

Fig. 6. Degree of gingival overgrowth level in the cyclosporine A-treated group in patients with higher than and equal to and lower than 1 transforming growth factor-β1 gene expression.

CsA-treated renal transplant patients with and without GO. The results showed that the level of expression in patients suffering from GO was significantly lower in comparison with subjects without this pathology and also lower in comparison with tacrolimus-treated patients. Greater frequency, higher degree and larger extent of GO were observed in patients with a lower ($E<1$) level of TGF-β1 gene expression (Figs 4–6).

Surprisingly, no relationship between functional polymorphisms and level of expression of TGF-β1 was identified in the patients with GO. Such a relationship was observed for all the other patients, i.e. patients without GO and patients treated with tacrolimus. The patients with GO had an equally low level of TGF-β1 expression in the low, the intermediate and the high producer phenotypes. Low TGF-β1 gene expression is responsible for diminished TGF-β1 protein production. In our previous paper, we described a lower plasma level of TGF-β1 protein in patients with GO compared with patients without GO. In the high producer phenotype, the plasma level of TGF-β1 was lower, which corresponds to the finding of low TGF-β1 gene expression in this phenotype concerning patients with GO. Currently, it is impossible to establish as to whether low TGF-β1 expression is the primary reason or result of GO. In the patients with GO, CsA failed to increase TGF-β1 expression. It is not clear which additional factors play a role in the pathomechanism in susceptible patients. On the other hand, many factors are able to change mRNA expression and act in changing latent to active form of TGF-β1 protein in vivo. Hence, evaluations of mRNA expression in comparison with the plasma or GCF level should be the subject of further investigations. The best and most reliable source for assessment of mRNA expression is gingival tissue (with and without overgrowth). In this study, patients refused to take gingival samples.

Ellis et al. (2004) reported a lower plasma TGF-β1 concentration in patients with GO. They found a significantly inverse correlation between plasma levels of TGF-β1 and GO, suggesting that patients are at a greater risk of more severe overgrowth with a lower, rather than a higher, TGF-β1 concentration. The plasma level of TGF-β1 may not fully reflect its local tissue level; however, the expression of mRNA for TGF-β1 in peripheral blood mononuclear cells, which migrate to impaired tissues, where they are responsible for local production of this cytokine, provides more accurate information about tissue expression.

A low level of TGF-β1 expression in the microenvironment of CsA toxicity may initiate the pathomechanism leading to GO. TGF-β1 exhibits many possible mechanisms by which it protects

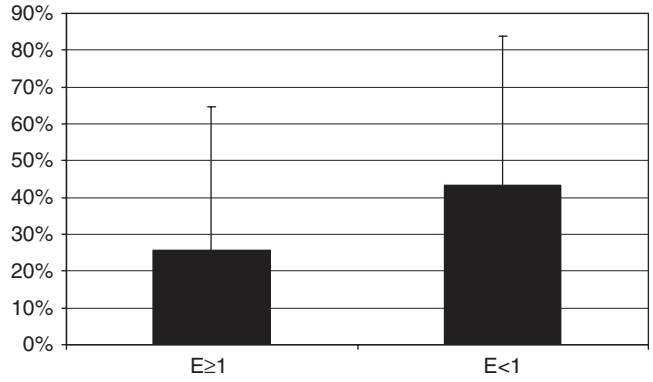

Fig. 7. Extent of gingival overgrowth in the cyclosporine A-treated group in patients with higher than and equal to and lower than 1 transforming growth factor-β1 gene expression.

gingiva from pathological overgrowth, e.g. it counteracts many pro-inflammatory effects, inhibits proliferation of endothelial and epithelial cells, stimulates apoptosis and exerts immunomodulatory effects. Deficiency of this molecule in the environment impaired by CsA toxicity may enhance inflammation, epithelial cell proliferation and, most of all, disturb gingivae repair (Buduneli et al. 2001). To date, GO in patients treated with CsA was associated with a higher level of TGF-β1 and its fibrogenic effect (James et al. 1998, Wright et al. 2000, Buduneli et al. 2001). Recent experimental studies suggest that the overgrowth of the gingivae is the result of the increase of the epithelial thickness, stemming from cellular hypertrophy in the keratinized epithelia and from cellular hyperplasia in the junctional epithelium, increased angiogenesis, vasodilatation, inflammation and a lesser grade of fibrosis (Mariani et al. 1996, Ayanoglou & Lesty 1999, Nurmenniemi et al. 2001). In the light of these data, lower TGF-β1 expression associated with the lack of control and coordination of repair processes of gingiva injured by CsA toxicity may be a pathogenic factor of GO. It is not known what level of mRNA TGF-β1 expression allows the proper function of this cytokine. TGF-β1 knockout mice died due to multiorgan inflammations, while transfection of the TGF-β1 gene to the transplanted heart inhibited immunological and inflammatory responses (Kulkarni et al. 1993, Qin et al. 1994).

Deficiency of TGF-β1 may cause disequilibrium between numerous growth factors (e.g. PDGF, FGF, TNF-α, IL-1, IL-6) in the surrounding gingival tissue, resulting in GO (Werner & Grose 2003). We believe that higher TGF-β1 gene expression in CsA-treated patients exerts a protective influence in the development of GO, in contrast to low expression, which does not keep the cell proliferation and inflammatory response in gingival tissue under control.

It is unknown as to why patients with GO reveal low systemic expression of mRNA TGF-β1. We hypothesized that these patients had primary (before transplantation) lower mRNA TGF-β1 expression, caused by environmental or genetic factors, apart from the functional TGF-β1 gene polymorphism. The survey of patients followed from the pre-transplantation period up to the incident of gingival hyperplasia should explain this issue. Secondly, TGF-β1 positively regulates its own production, and a relationship exists between the level of expression and stimulation (low expression causes low stimulation; high expression causes high stimulation) (Chand et al. 2001, Guo et al. 2002). Furthermore, expression of TGF-β1 depends on many growth factors and inflammatory cytokines, because they act in a network of relationships. Production of TGF-β1 is blocked, e.g. by IL-1, IL-15, epithelial growth factor, inhibition of vascular endothelial growth factor and platelet-derived growth factor. Blood mononuclear cells, the main source of TGF-β1 and other cytokines, are affected by immunosuppressive treatment and consequently, the production of these factors is decreased. Interindividual variations of pharmakogenetics, pharmacodynamics and pharmacokinetics of immunosuppressive drugs (CsA, azathioprine/mycophenolate mofetil and prednisone) may result in systemic underexpression of mRNA TGF-β1 in some patients.

In conclusion, a low level of TGF-β1 gene expression, not the functional polymorphism in patients treated with CsA, may be considered to be a risk factor in the multifactorial aetiopathogenesis of CsA-induced GO.

References

Afonso, M., de Oliveira, V., Bello, V., Shibli, J. A. & Sposto, M. R. (2003) Cyclosporin A-induced gingival overgrowth in renal transplant recipients. *Journal of Periodontology* **74**, 51–56.

Aimetti, M., Romano, F. & Debernardi, C. (2005a) Effectiveness of periodontal therapy on the severity of cyclosporin A-induced gingival overgrowth. *Journal of Clinical Periodontology* **32**, 846–850.

Aimetti, M., Romano, F., Priotto, P. & Debernardi, C. (2005b) Non-surgical periodontal therapy of cyclosporin A gingival overgrowth in organ transplant patients. Clinical results at 12 months. *Minerva Stomatologica* **54**, 311–319.

Atilla, G. & Kütükcüler, N. (1998) Crevicular fluid interleukin-1beta, tumor necrosis factor-alpha and interleukin-6 levels in renal transplant patients receiving cyclosporine A. *Journal of Periodontology* **69**, 784–790.

Ayanoglou, C. M. & Lesty, C. (1999) Cyclosporin A-induced gingival overgrowth in the rat: a histological, ultrastructural and histomorphometric evaluation. *Journal of Periodontal Research* **34**, 7–15.

Bartoli, F., Castronovo, G. & Stabile, A. (2004) Risk factors conditioning the incidence and severity of cyclosporine A-induced gingival overgrowth and methods of prevention. *Minerva Stomatologica* **53**, 165–170.

Berloco, P., Rossi, M., Pretagostini, R., Sociu-Foca, Cortesini, N. & Cortesini, R. (2001) Tacrolimus as cornerstone immunosuppressant in kidney transplantation. *Transplantation Proceedings* **33**, 994–996.

Buduneli, N., Kütükcüler, N., Aksu, G. & Atilla, G. (2001) Evaluation of transforming growth factor β_1 level in crevicular fluid of cyclosporin A-treated patients. *Journal of Periodontology* **72**, 526–531.

Busque, S., Demers, P., Saint-Louis, G., Boily, J. G., Tousignant, J., Lemieux, F., Martin, G., Smeesters, C., Corman, J. & Daloze, P. (1999) Hypertrichosis and gingival hypertrophy regression in renal transplants following the substitution of cyclosporine by tacrolimus. *Annales de Chirurgie* **53**, 687–689.

Chand, D. H., Southerland, S. M. & Cunningham, R. J. (2001) Tacrolimus: the good, the bad and the ugly. *Pediatric Transplant* **5**, 35–36.

Ellis, J. S., Morgan, C. L., Kirby, J. A., Taylor, J. J. & Thomason, J. M. (2004) Plasma TGF-β_1 as a risk factor for gingival overgrowth. *Journal of Clinical Periodontology* **31**, 863–868.

Guo, Z., Binswanger, U. & Knoflach, A. (2002) Role of codon 10 and codon 25 polymorphisms on TGF-β1 gene expression and protein

synthesis in stable renal allograft recipients. *Transplantation Proceedings* **34**, 2904–2906.

Hernandez, G., Arriba, L., Cruz Frias, M., Macorra, J. C., Vicente, J. C., Jimenez, C., Andres, A. & Moreno, E. (2003) Conversion from cyclosporin A to tacrolimus as non-surgical alternative to reduce gingival enlargement: a preliminary case series. *Journal of Periodontology* **74**, 1816–1823.

Hetzel, G. R., Plum, J., Özcan, F. & Heering, P. (2001) Transforming growth factor-β_1 plasma levels in stable renal allograft recipients under different immunosuppression. *Transplantation* **4**, 586–587.

Hoffmann, S. C., Stanley, E. M., Cox, E. D., Craighead, N., Di Mercurio, B. S., Koziol, D. E., Harlan, D. M., Kirk, A. D. & Blair, P. J. (2001) Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. *Transplantation* **72**, 1444–1450.

Hughes, J. R., Hughes, V. F., Trull, A. & Metcalfe, S. M. (1999) Blood levels of TGF-β1 in liver transplants recipients receiving either tacrolimus or micro-emulsified cyclosporine. *Transplantation* **4**, 583–586.

James, J. A., Irwin, C. R. & Linden, G. J. (1998) Gingival fibroblast response to cyclosporin A and transforming growth factor β_1. *Journal of Periodontal Research* **33**, 40–48.

James, J. A., Jamal, S., Hull, P. S., Macfarlane, T. V., Campbell, B. A., Johnson, R. W. G. & Short, C. G. (2001) Tacrolimus is not associated with gingival overgrowth in renal transplant patients. *Journal of Clinical Periodontology* **28**, 848–852.

James, J. A., Marley, J. J., Jamal, S., Campbell, B. A., Short, C. D., Johnson, R. W. G., Hull, P. S., Spratt, H., Irwin, C. R., Boomer, S., Maxwell, A. P. & Linden, G. J. (2000) The calcium channel blocker used with cyclosporin has an effect on gingival overgrowth. *Journal of Clinical Periodontology* **27**, 109–115.

Kantarci, A., Cebeci, I., Tuncer, Ö., Carin, M. & Firatli, E. (1999) Clinical effects of periodontal therapy on the severity of cyclosporin A-induced gingival hyperplasia. *Journal of Periodontology* **70**, 587–593.

Khanna, A. K., Cairns, V. R., Becker, C. G. & Hosenpud, J. D. (1999) Transforming growth factor (TGF)-β mimics and anti TGF-β antibody abrogates the in vivo effects of cyclosporine. *Transplantation* **67**, 882–889.

Kohnle, M., Lülkes, P., Zimmermann, U., Philipp, T. & Heemann, U. (1999) Conversion from cyclosporine to tacrolimus in renal transplant recipients with gum hyperplasia. *Transplantation Proceedings* **31**, 44–45.

Kulkarni, A. B., Huh, C. G., Becker, D., Geiser, A., Lyght, M., Flanders, K. C., Roberts, A. B., Sporn, M. B., Ward, J. M. & Karlson, S. (1993) Transforming growth factor beta 1null mutation in mice causes excessive inflammatory response and early death. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 770–777.

Lange, D. E., Plagmann, H. Ch., Eenboom, A. & Prommesberger, A. (1977) Klinische bewertungsverfahren zur objektivierung der mundhygiene. *Deutsche Zahnärztliche Zeitschrift* **32**, 44–46.

Leung, W. K., Yau, J. Y. Y., Jin, L. J., Chan, A. W. K., Chu, F. C. S., Tsang, C. S. P & Chan, T. M. (2003) Subgingival microbiota of renal transplant recipients. *Oral Microbiology and Immunology* **18**, 37–44.

Mariani, G., Calastrini, C., Carinci, F., Bergamini, L., Calastrini, F. & Stabellini, G. (1996) Ultrastructural and histochemical features of the ground substance in cyclosporin A-induced gingival overgrowth. *Journal of Periodontology* **67**, 21–27.

Modeer, T., Wondimu, B., Larsson, E. & Jonzon, B. (1992) Levels of cyclosporin A (CsA) in saliva in children after oral administration of the drug in mixture or in capsule form. *Scandinavian Journal of Dental Research* **100**, 366–370.

Mühlemann, H. R. & Son, S. (1971) Gingival sulcus bleeding – a leading symptom in initial gingivitis. *Helvetica Odontologica Acta* **15**, 107–111.

Nurmenniemi, P. K., Pernu, H. E. & Knuuttila, M. L. E. (2001) Mitotic activity of keratinocytes in nifedipine- and immunosuppressive medication-induced gingival overgrowth. *Journal of Periodontology* **72**, 167–173.

Ochsner, S., Guo, Z., Binswanger, U. & Knoflach, A. (2002) TGF-β1 gene expression in stable renal transplant recipients: influence of TGF-β1 gene polymorphism and immunosuppression. *Transplantation Proceedings* **34**, 2901–2903.

O'Valle, F., Mesa, F., Aneiros, J., Gomez-Morales, M., Lucena, M. A., Ramirez, C., Revelles, F., Moreno, E., Navarro, N., Caballero, T., Masseroli, M. & Garcia del Moral, R. (1995) Gingival overgrowth induced by nifedipine and cyclosporin A. Clinical and morphometric study with image analysis. *Journal of Clinical Periodontology* **22**, 591–597.

Qin, L., Chavin, K. D., Ding, Y., Woodward, J. E., Favaro, J. P., Lin, J. & Bromberg, J. S. (1994) Gene transfer for transplantation. Prolongation of allograft survival with transforming growth factor beta 1. *Annals of Surgery* **220**, 508–513.

Radwan-Oczko, M. & Boratyńska, M. (2006) The influence of different treatment modalities on clinical improvement and reduction of cyclosporine A-induced gingival overgrowth. *Case Report and Clinical Practice Review* **7**, 58–63.

Radwan-Oczko, M., Boratyńska, M., Klinger, M. & Ziętek, M. (2003) Risk factors in kidney transplant recipients treated with cyclosporine A. *Annals of Transplantation* **8**, 40–45.

Radwan-Oczko, M., Boratyńska, M. & Ziętek, M. (2004) Clinical evaluation of marginal parodontium condition in patients after kidney graft treated with calcineurine inhibitors and calcium channel blockers. *Bulletin du Groupement Européen Pour la Recherche Scientifique en Stomatologie et Odontologie* **46**, 45–51.

Radwan-Oczko, M., Boratyńska, M., Ziętek, M., Żołędziewska, M. & Jonkisz, A. (2006) The relationship of transforming growth factor-beta 1 gene polymorphism, its plasma level and gingival overgrowth in renal transplant recipients receiving different immunosuppressive regimens. *Journal of Periodontology* **77**, 865–873.

Routray, S. N., Mishra, T. K., Pattnaik, U. K., Satapathy, C., Mishra, C. K. & Behera, M. (2003) Amlodipine-induced gingival hyperplasia. *Journal of The Association of Physicians of India* **51**, 818–819.

Shin, G. T., Khanna, A., Ding, R., Dharma, V. K., Lagman, M., Li, B. & Suthanthiran, M. (1998) In vivo expression of transforming growth factor-β_1 in humans. *Transplantation* **65**, 313–318.

Thomas, D. W., Newcombe, R. G. & Osborne, G. R. (2000) Risk factors in the development of cyclosporine-induced gingival overgrowth. *Transplantation* **69**, 522–526.

Thorp, M., De Mattos, A., Bennett, W., Barry, J. & Norman, D. (2000) The effect of conversion from cyclosporine to tacrolimus on gingival hyperplasia, hirsutyzm and cholesterol. *Transplantation* **69**, 1218–1224.

Werner, S. & Grose, R. (2003) Regulation of wound healing by growth factors and cytokines. *Physiological Reviews* **83**, 835–870.

Wright, H. J., Chapple, I. L., Blairm, F. & Matthews, J. B. (2004) Crevicular fluid levels of TGF beta 1 in drug-induced gingival overgrowth. *Archives of Oral Biology* **49**, 421–425.

Wright, H. J., Chapple, I. L. & Matthews, J. B. (2000) TGF-β isoforms and TGF-β receptors in drug-induced and hereditary gingival overgrowth. *Journal of Oral Pathology and Medicine* **30**, 281–289.

Address:
Małgorzata Radwan-Oczko
Department of Periodontology
Silesian Piasts Medical University
50-425 Wrocław, ul. Krakowska 26
Poland
E-mail: malgorzata.oczko@poczta.onet.pl

Clinical Relevance

Scientific rationale for the study: Some evidence, although ambiguous, has shown an association between TGF-β1 levels and TGF-β1 gene polymorphisms and CsA-induced GO.

Principal findings: TGF-β1 gene expression in CsA- and tacrolimus-treated groups was, in a distinct way, elevated with phenotypes and genotypes of the TGF-β1 gene. The higher incidence, degree and extent of GO were related to the low level of TGF-β1 expression, which was significantly lower in the group with GO in comparison with the group without GO.

Practical implications: The examination of polymorphisms and expression of the TGF-β1 gene can take advantage of evaluation of the level of GO risk factors related to TGF-β1.