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Relationship between *IL-1A* polymorphisms and gingival overgrowth in renal transplant recipients receiving Cyclosporin A

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Abstract

Aim: Levels of interleukin-1α (IL-1α) are elevated in periodontal inflammation. *IL-1A* gene polymorphisms are associated with inflammatory diseases. This study aimed to investigate *IL-1A* gene polymorphism in Cyclosporin A (CsA)-treated renal transplant patients and investigate the association between this polymorphism and gingival crevicular fluid (GCF) levels of several cytokines.

Materials and Methods: Fifty-one renal transplant patients on CsA treatment (25 with and 26 without gingival overgrowth) and 29 healthy controls were recruited for the study. Demographic, pharmacological and periodontal parameters were recorded and gingival overgrowth was assessed.

Results: Multiple regression analysis showed that genotype was significantly associated with gingival overgrowth ($p = 0.02$). Carriage of the *IL-1A* (− 889) T allele was strongly protective [95% confidence interval (CI): 0.046–0.77], although not significantly associated with IL-1α protein levels in GCF. IL-1α, IL-1β and IL-8, but not IL-6, were detected in GCF of CsA-treated patients, but none of them was significantly associated with gingival overgrowth.

Conclusions: This study is the first to associate a gene polymorphism as a risk factor for CsA-induced gingival overgrowth in renal transplant patients, demonstrating that *IL-1A* polymorphism might alter individual susceptibility to CsA. However, there was no association between GCF cytokine levels and the presence of gingival overgrowth or patient *IL-1A* genotype.

Key words: CsA; drug-induced gingival overgrowth; IL-1α; polymorphism; renal transplant

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Cyclosporin A (CsA) is a potent and selective immunosuppressant widely used for the prevention of transplant rejection and for the treatment of various autoimmune diseases (Hassell & Hefti 1991, Faulds et al. 1993). Among the most common side effects is gingival overgrowth, most studies reporting a prevalence of about 30% but some as high as 81% (King et al. 1993, Hefti et al. 1994, Boltchi et al. 1999). A range of risk factors for CsA-induced gingival overgrowth have been suggested including age, sex, genetic pre-disposition, duration of therapy, gingival inflammation and concomitant medication, such as calcium channel blockers (Seymour et al. 1996, Thomason et al. 1996, 2005, Morisaki et al. 2000, de Oliveira Costa et al. 2006). The pathogenesis of CsA-induced gingival overgrowth remains poorly understood at the cellular level (Chae et al. 2006). However, evidence suggests that self-performed plaque control, combined with professional subgingival instrumentation, is effective in the treatment of gingival overgrowth in transplant patients receiving CsA (Aimetti et al. 2005, Mavrogiannis et al. 2006).

Gingival overgrowth results from the deposition of excessive extracellular matrix, reflecting both overproduction of tissue and reduced levels of tissue breakdown (Rostock et al. 1986, Seymour et al. 1996). CsA appears to promote an abnormal accumulation of extracellular matrix components in the gingival connective tissue (Mariani et al. 1996, Ayanoglou & Lesty 1999, Vardar et al. 2005). Part of this effect results from reduced activity of the matrix metalloproteinases (MMPs) (Lohi et al. 1994, Sugano et al. 1998,

Fornoni et al. 2000, Silva et al. 2001, Dannewitz et al. 2006). There is evidence that CsA can act directly on oral tissues by affecting signalling in gingival fibroblasts (Bostrom et al. 2005). Many studies have measured cytokine production during gingival overgrowth (Ruhl et al. 2004) and many are known to be elevated, including interleukin-6 (IL-6), IL-1β, platelet-derived growth factor-B (PDGF-B), fibroblast growth factor-2 (FGF-2) and transforming growth factor-β (TGF-β) (Williamson et al. 1994, Nares et al. 1996, Plemons et al. 1996, Iacopino et al. 1997, Saito et al. 1997, Atilla & Kutukculer 1998, Hong et al. 1999, Buduneli et al. 2001, Uzel et al. 2001, Radwan-Oczko et al. 2006, Wright et al. 2006). One important cytokine that has not been studied in relation to CsA-induced gingival overgrowth is (IL-1α).

IL-1α is known to play a crucial role in the immunopathological responses involved with tissue destruction in chronic inflammatory diseases, such as periodontal diseases (Rasmussen et al. 2000). It has also been shown to stimulate collagen synthesis and proliferation in fibroblasts derived from scleroderma and nifedipine-induced gingival overgrowth tissues (Kahari et al. 1987, Sato et al. 2005). Nevertheless, IL-1α is also involved in tissue breakdown via the induction of MMPs (Saito et al. 1997, Flannery et al. 1999, Ijima et al. 2001). As IL-1α has both anabolic and catabolic effects on the extracellular matrix, regulation of IL-1α levels by CsA may disrupt the homeostatic balance in the gingival tissues. In addition, it is possible that variations in IL-1α levels between patients could be instrumental in determining some of the variability in the CsA responses between patients. Indeed, the carriage of polymorphic alleles of *IL-1A* has been previously associated with inflammatory diseases and increased levels of protein when considered alone or in conjunction with polymorphisms in other genes (McDowell et al. 1995, Kornman et al. 1997, Shirodaria et al. 2000).

In this study, we aimed to investigate whether susceptibility to gingival overgrowth in Turkish renal transplant patients treated with CsA is related to the carriage of the polymorphism -889 in the *IL-1A* gene promoter. In addition, we measured the levels of IL-1α, IL-1β, IL-6 and IL-8 in the gingival crevicular fluid (GCF) of renal transplant patients with and without gingival overgrowth, and assessed whether these cytokine levels could be related to genotype and extent of any gingival overgrowth.

Material and Methods

Subjects

A total of 80 subjects of Turkish origin were included in the present study. Written informed consent was obtained from each subject before their enrollment in the study. Twenty-five renal transplant patients (16 males and nine females, mean age 30 ± 9 years) receiving CsA therapy and exhibiting gingival overgrowth, and 26 other patients (14 males and 12 females, mean age 36 ± 10 years) with renal transplantations and using CsA, but exhibiting no sign of CsA-induced gingival overgrowth were monitored. Renal transplant patients who had been followed by the Nephrology Department at the Ege University were on CsA therapy for a minimum of 6 months and the CsA dose was adjusted to maintain stable serum levels between 80 and 300 ng/ml. CsA-treated patients also received azathioprine and prednisolone. Patients taking any other drugs such as calcium channel blockers reported to cause drug-induced gingival overgrowth were excluded. The non-medicated control group consisted of 29 systemically healthy subjects (12 males and 17 females, mean age 35 ± 16) with a clinically healthy periodontium and no clinical evidence of gingival overgrowth or history of medications associated with gingival overgrowth. These control subjects were healthy volunteers from Ege University. No subjects had taken medications such as antibiotics or contraceptives that could affect their periodontal status for at least 3 months before the study. Radiographic examination was also carried out to detect alveolar bone destruction. CsA-treated patients without any sign of alveolar bone loss were selected for the present study. Self-reported smoking status was recorded for each patient. They were classified as smokers (all smoked more than five cigarettes/day) and non-smokers (never smoked).

Clinical parameters

Assessment of clinical periodontal parameters was made of the full dentition, excluding third molars. All subjects had a clinical periodontal examination including the measurement of probing depth using a Williams probe. Measurements were performed at six sites per tooth for the whole mouth. Dichotomous measurement of supragingival plaque accumulation and bleeding on probing and hyperplastic index scores were also recorded. The degree of gingival overgrowth was classified into four categories based on the criteria of Angelopoulos and Goaz (Angelopoulos & Goaz 1972) modified by Pernu et al. (1992). The patients were dichotomized into a gingival overgrowth-negative (GO$-$) group, those with no signs of gingival overgrowth (score 0), and a gingival overgrowth-positive (GO+) group, those with signs of overgrowth (scores 1–3) for analysis. Patients with $>50\%$ of sites positive for plaque were classified as plaque positive for the analysis. Patients with $>50\%$ of sites positive for bleeding were classified as bleeding-positive patients for the analysis. Radiographic examination was also carried out to detect alveolar bone destruction. All clinical measurements were performed by the same investigator (D. C. P.).

GCF collection

GCF samples were collected from 36 of the 51 renal transplant patients. GCF samples were collected from one proximal site of one tooth in an anterior sextant per subject. The selected sites were cleared of supragingival plaque, isolated with cotton rolls and dried with a gentle stream of air to prevent saliva contamination. A sterile Periopaper strip (ProFlow Inc., Amityville, NY, USA) was gently inserted into the periodontal pocket and left in place for 30 s. Mechanical irritation was avoided and strips contaminated with blood were discarded.

The GCF sample volume was measured with a calibrated Periotron 6000 (Harco Electronics Ltd., Winnipeg, Canada) before the transfer of each strip to a separate sterile polypropylene tube, before freezing at -40°C. The readings were converted to an actual volume (μl) by reference to the standard curve. All the samples were lyophilized and stored at -20°C until the laboratory procedures. On the day of analysis, 300 μl of phosphate-buffered saline (PBS, pH 7.2) was used to re-elute the samples. The tubes were shaken gently for 1 min. and then centrifuged at $2000 \times g$ for 5 min.

at 4°C. The amount of IL-1α, IL-1β, IL-6 and IL-8 in the GCF samples was determined using enzyme-linked immunosorbent assays (ELISA, R&D, Minnepolis, MN, USA).

The assays were carried out in accordance with the manufacturer's instructions. The results were expressed as pg/30 s sample and ng/ml.

Genotyping

Two milliliters of venous blood was taken by standard venepuncture and collected in tubes containing potassium ethylene diamine tetraacetic acid (EDTA). Genomic DNA was extracted using the QIAmp® DNA mini kit (Qiagen, Crawley, UK) according to the instruction manual and stored at −20°C until further use.

Primers were used to amplify a 1.1 kb region immediately 5′ to the coding sequence of the *IL-1A* promoter. The amplification reactions [polymerase chain reaction (PCR)] were carried out in a thermal cycler (Hybaid, Thermo Electron Corporation, Basingstoke, UK) and were preformed with 300–700 ng DNA in a reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, $MgCl_2$ (2.5 mM), deoxyribonucleotides (dNTPs) (200 μM each) and 4U Bio-X-Act Long Polymerase (Bioline, London, UK) (total reaction volume 50 μl). The reaction was incubated for 5 min. at 95°C, annealed at 60°C for 1 min., and this was followed by 30 cycles at 68°C for 5 min., at 95°C for 30 s and one cycle at 68°C for 30 s. PCR primers and dNTPs were removed before genotyping; 3.5 μl of PCR product was incubated with 1.5 μl of ExoSapIT (Amersham) for 37 min. at 37°C, followed by 20 min. at 80°C to inactivate the enzymes.

Forward primer: TTACATATGAGCCTTCCATG;
Reverse primer: CTGCAGTGGAGGACAATACCTT; and
Sequencing primer: TTCTACCACCTGAACTAGGCT

The *IL-1A* polymorphism at position −889 (McDowell et al. 1995) was determined using the sequencing primer using ABI PRISM® BigDye™ v2 (Applied Biosystems, Foster City, CA, USA). This was according to the manufacturer's instructions using an ABI PRISM 377 DNA sequencer (Applied Biosystems) and chromatographs were recorded at a wavelength of 260 nm. The sequences were compared with known human genomic DNA sequences using the BLAST online search engine (www.gene-regulation.com) to confirm amplification and sequencing of the correct region.

Statistical analysis

The comparison of variables in Table 1 between the two groups (i.e. those with GO and those without) was made using the unpaired *t*-test, the χ^2 statistic and the Mann–Whitney's test as appropriate.

The distribution of genotypes in renal transplant patients with and without gingival overgrowth statistically was evaluated by the use of the χ^2 test with Yate's correction. The frequencies of genotypes were given with their 95% confidence intervals (95% CIs).

To investigate the relationship of different patient-based variables with the incidence of gingival overgrowth, the importance of each variable was assessed using logistical regression with the SPSS 12.0 software. Variables that had probabilities $p < 0.2$ were then subjected to further multiple regression analysis to allow for potential interactions. Table 4 shows the probabilities that each of the variables is related to gingival overgrowth when analysed alone (unadjusted) or in combination (adjusted).

Results

The demographic and periodontal clinical details of the patients are shown in Tables 1 and 2. Among the 51 patients on CsA treatment, 10 subjects were classified as score 1 of gingival overgrowth, 10 patients were assigned score 2, while five patients exhibited overgrowth covering more than two-thirds of the anatomic crown and were scored 3. The mean gingival overgrowth score was 1.8 ± 0.8 according to Pernu's scoring system. Twenty-six patients without gingival overgrowth were scored 0. Patients with gingival overgrowth had significantly greater ($p < 0.001$, $r = 0.64$) probing depths of 2.7 mm ± 0.5 compared with 2 mm ± 0.5 in those without overgrowth. There was a statistically significant difference between GO+ and GO− groups in terms of bleeding on probing ($p < 0.0001$). The duration of cyclosporin therapy ranged from 6 to 173 months. Only 12% of the patients (six patients) on CsA were smokers and of these only a single patient showed gingival overgrowth.

Table 3 shows the carriage of the *IL-1A* C/T SNP −889 for the 50 patients and 29 healthy controls (sequencing for one CsA-treated patient sample failed due to poor DNA quality and was excluded). Renal transplant patients receiving CsA therapy were characterized by genotypes similar to the non-medicated controls in distribution of *IL-1A*. There were significant differences of genotypes −889 C/T or TT and −889 C/C between patients with and without gingival overgrowth. The carriage of the polymorphic allele (T) differed significantly (χ^2 $p = 0.02$) between the gingival overgrowth-positive 32% (8/25 patients) compared with gingival overgrowth-negative patients 64% (16/25). Carriage of the *IL-1A* −889T allele was strongly associated with absence of gingival overgrowth in CsA-treated patients (odds ratio = 0.26 with 95% CI 0.08–0.85).

Table 1. Demographic details of control and CsA-treated renal transplant patients (mean ± SD)

	GO+ ($n = 25$)	GO− ($n = 26$)	Controls ($n = 29$)
Age (year)	31(10)	36(10)	35(9)
Gender (F:M)	9:16	12:14	17:12
Smoking (%)	4	19	32
Duration of therapy (months)	64(45)	44(38)	–

GO+, gingival overgrowth positive; GO−, gingival overgrowth negative; CsA, Cyclosporin A.

Table 2. Clinical features of CsA-treated renal transplant and control patients (mean ± SD)

	GO+ ($n = 25$)	GO− ($n = 26$)	Controls ($n = 29$)
Gingival overgrowth score	1.8 (0.8)	0	0
Probing depth (mm)	2.7 (0.5)	2 (0.5)	<3
Bleeding positive (%)	84*	26	0
Plaque positive (%)	100	89	0

*$p < 0.0001$

GO+, gingival overgrowth positive; GO−, gingival overgrowth negative; CsA, Cyclosporin A.

To assess the extent to which patient-based factors might be correlated with gingival overgrowth, simple regression analysis was used (Table 4). Variables including age, gender, smoking, genotype and duration of treatment were first analysed independently. This approach confirmed that gender and smoking status appeared to be unrelated to gingival overgrowth. However, this initial analysis suggested possible associations between age, genotype and duration of therapy to gingival overgrowth. Further analysis of these factors in combination showed that duration was not significantly related to overgrowth ($p = 0.06$) but that age and genotype were significantly associated ($p = 0.031$ and $p = 0.02$). The analysis suggests that increased age had a protective effect (95% CI 0.862–0.993) while carriage of the *IL-1A* −889T allele was strongly protective (95% CI 0.046–0.77).

Table 5 details the demographic and clinical data from the 36 patients for whom GCF samples were taken. The mean gingival overgrowth score for the overgrowth-positive group was 1.7 ± 0.7. There were no significant differences in mean GCF volumes between samples obtained from the GO+ and GO− patients. As there were only three smokers in the GCF sampled group, no reliable conclusions about differences in cytokine levels can be made between smokers and non-smokers.

The mean levels of IL-1α and IL-1β at GO+ sites were lower than those of the GO− group (Table 6). In contrast, GCF levels of IL-8 were higher in the GO+ group than that of the GO− group; however, these differences were not statistically significant ($p > 0.05$). There were correlations between the amount of IL-1α and IL-1β, and IL-1α and IL-8 ($p < 0.01$, $R = 0.60$, 0.48, respectively) (data not shown). In contrast, there was no correlation between the levels of IL-1β and IL-8. IL-6 levels were below the 2 pg/ml detection limit of the assay.

Levels of IL-1α protein in the GCF were compared with patient genotype. The *IL-1A* −889 genotype was not significantly associated with IL-1α protein levels in GCF (pg/30 s sample or ng/ml) in either groups ($p = 0.46$ and $p = 0.77$, respectively) (Table 7).

Table 3. IL-1 A −889 Genotype and allele frequencies in patients (GO+ and GO−) and non-medicated healthy controls

	GO+ ($n = 25$)		GO− ($n = 25$)		Controls ($n = 29$)	
	n	%	n	%	n	%
Genotype						
CC	17	68	9	36	17	59
CT/TT	8	32*	16	64*	12	41
Allele frequency						
C	40	80	34	68	44	76
T	10	20	16	32	14	24

*$p = 0.02$ (OR = 0.26 with 95% CI 0.082–0.85).
GO+, gingival overgrowth positive; GO−, gingival overgrowth negative; CsA, Cyclosporin A; CI, confidence interval; OR, odds ratio.

Table 4. Logistic regression of CsA-treated (GO+ and GO−) patient variables as possible correlates of gingival overgrowth

	Unadjusted *P*	95% CI	Adjusted P	95% CI
Age	0.064	0.892–1.003	0.031	0.862–0.993
Gender	0.462	0.496–4.685	–	–
Smoking	0.487	0.311–11.607	–	–
Genotype	0.023	0.074–822	0.020	0.046–0.770
Duration of therapy	0.104	0.998–1.026	0.060	0.999–1.034

Initial analysis of potential risk factors was performed using single variables. Those variables that had a $p < 0.2$ were subjected to further analysis in combination. The results of the final analysis are shown as adjusted *P* and adjusted OR.
GO+, gingival overgrowth positive; GO−, gingival overgrowth negative; CsA, Cyclosporin A; CI, confidence interval; OR, odds ratio.

Table 5. Demographic and clinical values of GCF-sampled CsA-treated patients (mean ± SD)

	GO+ ($n = 16$)	GO− ($n = 20$)
Age (years)	29 (9)	34 (10)
Gender (F:M)	8:8	8:12
Duration of therapy (months)	52 (48)	56 (50)
Probing depth (mm)	4 (1.5)	2.5 (0.5)
Bleeding positive (%)	81	19
Plaque positive (%)	100	85
Gingival overgrowth score	1.7 ± 0.7	0
GCF volume (μl)	0.5 (0.2)	0.6 (0.4)

GO+, gingival overgrowth positive; GO−, gingival overgrowth negative; CsA, Cyclosporin A; GCF, gingival crevicular fluid.

Table 6. Levels of IL-1α, IL-1β and IL-8 in GCF of CsA-treated patients (mean ± SD)

	IL-1α		IL-1β		IL-8	
	ng/ml	pg/sample*	ng/ml	pg/sample*	ng/ml	pg/sample*
GO+	15.5 ± 21.4	5.6 ± 13.8	2.5 ± 1.0	1.1 ± 0.9	58.3 ± 34.3	21.3 ± 15.9
GO−	21.4 ± 21.7	9.6 ± 5.3	5 ± 5.2	2.3 ± 3.4	48 ± 41.6	17.9 ± 8.6

*(pg/30 s sampling).
GO+, gingival overgrowth positive; GO−, gingival overgrowth negative; CsA, Cyclosporin A; IL, interleukin; GCF, gingival crevicular fluid.

Discussion

Drug-induced gingival overgrowth remains a significant problem in patients medicated with CsA. According to previous reports, gingival overgrowth occurs in about 30% of CsA-treated patients, with prevalence ranging from 6% to 81% (King et al. 1993, Thomason et al. 1996, 2005). These variations may be attributed to several factors including age, gender and duration of therapy, oral hygiene and genetic pre-disposition (Thomason et al. 1995, Cebeci et al.

Table 7. Levels of IL-1α protein in the GCF of CsA-treated patients with different IL-1A genotypes (mean ± SD)

Genotype	*n*	%	IL-1α (ng/ml)	IL-1α (pg/sample)
GO+				
CC	12	75	17.33 ± 24.41	10.2 ± 4.1
CT/TT	4	25	10.22 ± 8.22	4.95 ± 7.04
GO −				
CC	7	37	21.81 ± 14.04	10.2 ± 6.27
CT/TT	12	63	22.75 ± 26.03	11.09 ± 17

GO+, gingival overgrowth positive; GO − , gingival overgrowth negative; CsA, Cyclosporin A; IL, interleukin; GCF, gingival crevicular fluid.

1996). However, there is no clear consensus on the relative role of these factors in the development of gingival overgrowth (Hefti et al. 1994, Montebugnoli et al. 2000, Radwan-Oczko et al. 2003).

Gingival overgrowth usually develops within the first 3–6 months of CsA treatment and subsequently stabilizes (Seymour et al. 1994, Somacarrera et al. 1994). In the present study, no significant correlation between the occurrence of gingival overgrowth and duration of CsA treatment was observed, in agreement with previous studies of renal transplant patients (Pernu et al. 1992, Thomas et al. 2000, Afonso et al. 2003).

Earlier studies have suggested that dental plaque is an important determinant of CsA-induced gingival overgrowth (Seymour & Jacobs 1992, Somacarrera et al. 1994), while more recent studies have shown that it has no role in gingival overgrowth, but suggest that the presence of gingivitis may be a pre-disposing factor (Khoori et al. 2003, Romito et al. 2004). In the present study, the presence of dental plaque was not associated with the incidence of gingival overgrowth, as all GO+ as well as 89% of GO − subjects showed plaque accumulation. Therefore, it appears from the present study that the presence of dental plaque alone cannot account for the differential response to CsA. Nevertheless, it is possible that plaque may participate indirectly by maintaining chronic inflammation, creating conditions permissive for gingival overgrowth. A recent analysis of different risk factors of CsA-induced gingival overgrowth identified the bleeding index as the only periodontal variable related to the increased risk (Thomason et al. 2005), in agreement with the present data.

During infection or inflammation, neutrophils and monocytes invade the gingival tissues and produce inflammatory cytokines such as IL-1α. Many investigators have suggested a role for IL-1α in the initiation and progression of periodontal diseases (Delima et al. 2001, Dayan et al. 2004). This cytokine is intimately involved in inflammatory, immune and reparative responses, and any perturbation of its levels could have widespread effects, not necessarily restricted to periodontal disease. A recent report has indicated that IL-1α enhances the proliferative effect of nifedipine on gingival fibroblasts derived from patients with nifedipine-induced gingival overgrowth (Sato et al. 2005). However, there are no data available on the involvement of IL-1α in CsA-induced gingival overgrowth. Polymorphisms in the *IL-1A* (−889) gene appear to be associated with an increased risk of developing inflammatory diseases either when considered alone (McDowell et al. 1995) or in conjunction with polymorphisms in other genes (Kornman et al. 1997). In addition, the carriage of T allele at position −889 of *IL-1A* has been correlated with measurable differences in the levels of IL-1α protein in the GCF of patients with severe periodontal disease (Shirodaria et al. 2000). Moreover, the same polymorphism has also been associated with altered response to oral cyclophosphamide in patients having scleroderma (Beretta et al. 2006). CsA promotes an abnormal accumulation of gingival extracellular matrix components, particularly collagen (Mariani et al. 1996, Silva et al. 2001). It may be possible that MMPs responsible for collagen degradation during homeostatic regulation of tissue resorption and remodelling are inhibited or even lacking. IL-1α has been shown to modulate both collagen production and synthesis of MMPs (Fini et al. 1994, Saito et al. 1997, Chang et al. 2002). Clearly, if polymorphisms in the *IL-1A* gene can modulate protein levels, they could have a significant effect on the local inflammatory responses and consequently in the development of gingival overgrowth.

In the present study, carriage of the polymorphic allele at position −889 of *IL-1A* was found in a significantly higher proportion in GO − patients (64%) than GO+ patients (32%). Interestingly, the carriage of the same *IL-1A* allele, in conjunction with other polymorphisms (Kornman et al. 1997), has previously been associated with increased severity of periodontitis but appears to be protective for the development of gingival overgrowth. It is tempting to suggest that carriage of the polymorphic allele might act via elevating IL-1α protein levels. In the study, we were unable to positively correlate GCF IL-1α protein levels to genotype in either GO+ or GO − groups. This may be a consequence of the small number of samples available and the variety of confounding factors such as the presence of plaque, which will influence the levels of cytokines measured at a single time point.

Originally, sample numbers were not determined by a formal Power Calculation, but were determined by availability of appropriate subjects for inclusion. Retrospectively, power calculations show that with 25 subjects per group, the study had 80% power to detect a reduction of 35% in frequency of T allele carriage (CT/TT) with $\alpha = 0.05$. Consequently, given the relatively small sample size, there is an increased risk of a type II statistical error, although despite this a significant difference was still found in the *IL-1A* genotype between groups.

CsA has previously been shown to regulate the expression of a number of cytokines and growth factors including TGF-β, TNF-α, IL-6, IL-8 and IL-15 (Atilla & Kutukculer 1998, Myrillas et al. 1999, Buduneli et al. 2001, 2003, Wright et al. 2004, Drozdzik et al. 2005, Chin et al. 2006, Radwan-Oczko et al. 2006). The present study shows that the inflammatory cytokines IL-1α, IL-1β and IL-8 are present in GCF of CsA-treated patients, but IL-6 could not be detected. The relative amounts of IL-8 in GCF were much higher than the other cytokines measured, but their levels were highly variable between patients and, moreover, were not significantly associated with gingival overgrowth. In the present study, the mean levels of IL-1α and IL-1β (expressed as either ng/ml or total pg/30 s sample) were not significantly different between the GO+ and

GO − groups, which is in agreement with previous findings (Atilla & Kutukculer 1998). The results of this study support the view that CsA therapy does not have a significant effect on GCF cytokine levels. Instead, it seems that gingival inflammation may be the primary determinant of elevated levels of cytokines in the GCF of CsA-treated patients.

The role that genetic factors have in determining susceptibility to a range of periodontal conditions is well recognized (Shapira et al. 2005). Specific genetic characteristics may contribute to exacerbated gingival inflammation in response to plaque accumulation (Goodson et al. 2000). Several studies have investigated specific polymorphisms as risk factors for the development of gingival overgrowth. While studies on IL-6 and the drug transporter MDR1 gene have failed to identify any association with gingival overgrowth (Drozdzik et al. 2004, 2005), other studies have identified clear associations between polymorphisms in α2 integrin and TGF*β*-1 (Linden et al. 2001, Ogino et al. 2005, Radwan-Oczko et al. 2006) and gingival overgrowth. In the case of TGF*β*-1, serum levels have been shown to be a significant indicator of the risk of developing gingival overgrowth (Ellis et al. 2004). It is difficult to make direct comparisons between the results presented here and other studies. In most cases, the patients in these studies received both CsA and a calcium channel blocker while patients in the present studies received only CsA.

In conclusion, drug-induced gingival overgrowth remains a significant problem in renal transplant patients medicated with CsA. This study supports the possibility that the *IL-1A* genotype might be an important factor related to the development of gingival overgrowth. In view of the relatively small sample size, it would be useful to carry out further larger studies of *IL-1A* genotype and its functional role in CsA-induced gingival overgrowth.

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Clinical Relevance

Scientific rationale for study: The key factors that determine an individual's risk of developing gingival overgrowth as a complication of immunosuppressive cyclosporin therapy are only partially characterized.

Principal findings: This study investigated whether polymorphisms in the IL-1A gene alter the risk of gingival overgrowth. Within a small patient group, certain *IL-1A* alleles are associated with increased risk of gingival overgrowth.

Practical implications: These findings might help identify those patients at increased risk of developing gingival overgrowth. The indication that IL-1α may be an important risk factor suggests that it could provide a suitable avenue for further therapeutic approaches.