J Periodont Res 2007; 42; 39–44

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00912.x

The up-regulation of type I plasminogen activator inhibitor in human gingival fibroblasts stimulated with cyclosporin A

H.-J. Lin[1], C.-H. Tsai[2], F.-M. Huang[3], Y.-C. Chang[1,4]
[1]Institute of Stomatology, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan, [2]Department of Oral Pathology, Chung Shan Medical University, Taichung, Taiwan, [3]School of Dentistry, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan, and [4]Department of Periodontics, Chung Shan Medical University Hospital, Taichung, Taiwan

Lin H-J, Tsai C-H, Huang F-M, Chang Y-C. The up-regulation of type I plasminogen activator inhibitor in human gingival fibroblasts stimulated with cyclosporin A. J Periodont Res 2007; 42: 39–44. © Blackwell Munksgaard 2007

Background and Objective: Cyclosporin A is used as an immunosuppressive agent and its prominent side-effect is the induction of fibrous gingival overgrowth. The progression of fibrous gingival overgrowth results from the accumulation of extracellular matrix. Type I plasminogen activator inhibitor (PAI-1) acts as the inhibitor of extracellular matrix degradation and is involved in some fibrotic diseases. However, little is known about the correlation between PAI-1 and cyclosporin A-induced gingival overgrowth. The aim of this study was to investigate the effects of cyclosporin A on the expression of PAI-1 mRNA and protein in human gingival fibroblasts human gingival fibroblasts *in vitro* and to compare PAI-1 expression in normal healthy gingival tissues and cyclosporin A-induced gingival overgrowth specimens *in vivo*.

Material and Methods: Quantitative reverse transcription–polymerase chain reaction and western blot assay were used to investigate the effects on human gingival fibroblasts exposed to cyclosporin A. In addition, 10 cyclosporin A-induced gingival overgrowth specimens and five normal gingival tissues were examined by immunohistochemistry.

Results: Investigations of the time dependence of PAI-1 mRNA expression in human gingival fibroblasts treated with 200 ng/ml of cyclosporin A revealed a rapid accumulation of the transcript: a significant signal was first detectable after 1 h of exposure and the signal remained elevated throughout the 24-h incubation period ($p < 0.05$). Cyclosporin A was also found to up-regulate PAI-1 protein in a time-dependent manner ($p < 0.05$). The PAI-1 staining in gingival tissue was stronger in the cyclosporin A-induced gingival overgrowth group than in the normal gingival group ($p < 0.05$). In the cyclosporin A-induced gingival overgrowth group, intensive staining for PAI-1 expression was observed mainly in the cytoplasm of fibroblasts, endothelial cells and inflammatory cells.

Conclusion: These findings suggest that the up-regulation of PAI-1 may play an important part in the molecular pathogenesis of cyclosporin A-induced gingival overgrowth.

Dr Yu-Chao Chang, School of Dentistry, College of Oral Medicine, Chung Shan Medical University, 110, Sec. 1, Chien-Kuo N. Road, Taichung, Taiwan
Tel: +886 4 24718668 ext. 55011
Fax: +886 4 24759065
e-mail:cyc@csmu.edu.tw

Key words: cyclosporin A; gingival fibroblasts; gingival overgrowth; type I plasminogen activator inhibitor

Accepted for publication March 30, 2006

Cyclosporin A is a cyclic endecapeptide immunosuppressant that is widely used to prevent organ transplant rejection and to treat various immunological diseases, such as rheumatoid arthritis and psoriasis (1). Cyclosporin A therapy is associated with a number of major side-effects, including nephrotoxicity, hepatotoxicity, neurotoxicity and gingival overgrowth (2,3). Cyclosporin A-induced gingival overgrowth affects the gingiva of 30% of treated patients (4) and is observed as a papillary enlargement on the labial aspects and coalesces of the gingiva (5). Histological studies of overgrowth tissues have indicated that an accumulation of the extracellular matrix within the gingival connective tissue is a central feature of overgrowth tissues (3,6). It is probably a consequence of imbalance between synthesis and degradation of extracellular matrix molecules by gingival fibroblasts.

Type I plasminogen activator inhibitor (PAI-1) is a single-chain, 50-kDa glycoprotein (7). It is the major inhibitor of tissue-type plasminogen activator and urokinase-type plasminogen, which activate plasminogen to yield plasmin (8). Plasminogen activators and inhibitors are thought to be key participants in the balance of proteolytic and antiproteolytic activities that regulate extracellular matrix turnover. PAI-1 was dramatically up-regulated in some fibrotic diseases, including bleomycin-induced pulmonary fibrosis (9), carbon tetrachloride-induced liver fibrosis (10), coronary ligation-induced myocardial infarction (11) and areca quid chewing associated-oral submucous fibrosis (12). Importantly, bleomycin-induced fibrosis was severe in transgenic mice overexpressing PAI-1, but less so in PAI-1-deficient mice (13).

The levels of PAI-1 activities in the gingival crevicular fluid of cyclosporin A-treated renal transplant patients have been investigated (14). The findings suggest that the plasminogen-activating system might involve the pathogenesis of cyclosporin A-induced gingival overgrowth. However, there is limited information about the regulation of PAI-1 expression in cyclosporin A-induced gingival overgrowth, both *in vitro* and *in vivo*. Fibroblasts are the principal cell type residing in connective tissue and are the cells responsible for the formation and turnover of the extracellular matrix. Fibroblast function is, in turn, regulated by bioactive molecules acting in the local tissue environment. The purpose of this study was to investigate whether the expression of PAI-1 can be triggered in human gingival fibroblasts by cyclosporin A *in vitro*. In addition, the distribution of PAI-1 in normal gingival tissues and cyclosporin A-induced gingival overgrowth specimens was evaluated by using immunohistochemistry.

Material and methods

Tissue collection

The human biopsy materials used in this study were obtained under the guidelines of the Oral Medicine Center and the Joint Research and Ethics Committee of the Chung Shan Medical University Hospital. Normal gingival tissue samples were obtained from five healthy individuals undergoing routine surgical crown lengthening, with little, if any, evidence of inflammation and no systemic medication. Redundant hyperplasic gingival biopsy materials were obtained from 10 renal transplant patients receiving cyclosporin A therapy. These patients had been taking cyclosporin A for more than 1 yr and the dose had been adjusted to maintain stable serum levels of $\approx$200 ng/ml. No sign of graft rejection was detected in these renal transplant patients. The samples were obtained during surgical removal of diseased gingival tissue as part of their routine clinical management, which also included intensive plaque control.

Cell culture

Human gingival fibroblasts were cultured by using an explant technique, as described previously (15,16). Five healthy individuals were selected from the crown-lengthening procedure for this study. The normal gingival tissue samples were minced using sterile techniques and washed twice in phosphate-buffered saline supplemented with antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml of fungizone). Explants were placed into 60-mm Petri dishes and maintained in Dulbecco's modified Eagle's medium (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% fetal calf serum (Gibco Laboratories) and antibiotics, as described above. Cell cultures between the third and eighth passages were used in this study.

Effect of cyclosporin A on PAI-1 mRNA and protein in human gingival fibroblasts

Cells arrested in G0 by serum deprivation (0.5% fetal calf serum; 48 h) were used in the experiments (17). Near-confluent monolayers of human gingival fibroblasts were washed with serum-free Dulbecco's modified Eagle's medium and immediately exposed, for the indicated incubation times, to 200 ng/ml of cyclosporin A (Sigma, St Louis, MO, USA). Total RNA was collected at 0, 1, 3, 6 and 24 h for reverse transcription–polymerase chain reaction (RT–PCR). Cell extracts were collected at 0, 1, 2, 4, 8 and 24 h for western blotting.

RT–PCR

Total RNA was prepared using TRIzol reagent (Gibco Laboratories) following the manufacturer's instructions. Single-strand DNA was synthesized from RNA in a 15-μl reaction mixture containing 100 mg of random hexamer and 200 U of Moloney murine leukemia virus reverse transcriptase (Gibco Laboratories). The reaction mixture was diluted with 20 μl of water, and 3 μl of the diluted reaction mixture was used for PCR amplification. The PCR reaction mixture contained 10 pmol of forward and reverse primers and 2 U of *Taq* DNA polymerase. Amplification was performed for 25 cycles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and for 30 cycles for PAI-1 in a thermal cycle. Each cycle consisted of 1 min of denaturation at 94°C, 1 min of annealing at 57°C and 1 min of extension at 72°C. The

sequences of primers used were as follows (12): GAPDH forward: 5′-TCCTCTGACTTCAACAGCGAC-ACC-3′ and reverse: 5′-TCTCTCTT-CCTCTTGTGCTCTTGG-3′; PAI-1 forward: 5′-ATCACCATCTTCCAG-GAG-3′ and reverse: 5′-ATCACCAT-CTTCCAGGAG-3′.

The PCR products were analyzed by agarose-gel electrophoresis, and a 325-bp band for PAI-1 was observed. When the band densities were measured and compared with the density of the band obtained for the housekeeping gene, GAPDH, relative proportions of mRNA synthesis were determined within each experiment. The intensity of each band, after normalization with GAPDH mRNA, was quantified by the photographed gels using a densitometer (AlphaImager 2000; Alpha Innotech, San Leandro, CA, USA).

Western blot

Cell extracts were solubilized with sodium dodecyl sulfate solubilization buffer (5 mM EDTA, 1 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5, 0.5% Trition X-100, 2 mM phenylmethysulfonyl fluoride and 1 mM *N*-ethylmaleimide) for 30 min on ice. Then, cell lysates were centrifuged (12,000 *g* at 4°C for 5 min) and the protein concentrations determined with Bradford reagent using bovine serum albumin as a standard. Equivalent amounts of total protein per sample of cell extracts were run on a 10% sodium dodecyl sulfate polyacrylamide gel and immediately transferred to nitrocellulose membranes. The membranes were blocked for 2 h with phosphate-buffered saline containing 3% bovine serum albumin, rinsed, and then incubated with primary antibody (anti-PAI-1, diluted 1 : 500 in phosphate-buffered saline containing 0.05% Tween 20) for 2 h. After three washes with Tween 20 for 10 min, the membranes were incubated for 1 h with biotinylated secondary antibody diluted 1 : 1000 in the same buffer, washed again as described above, and treated with 1 : 1000 streptavidin–peroxidase solution for 30 min. After a series of washing steps, the reactions were developed using diaminobenzidine (Zymed Laboratories, San Francisco, CA, USA). The intensities of the obtained bands were determined using an AlphaImager 2000 (Alpha Innotech).

Immunohistochemistry

The surgically removed gingival tissues were fixed by overnight incubation in 10% buffered formalin, and the specimens were dehydrated in an ascending series of graded alcohols and embedded in paraffin. Five-micrometer sections were stained with the monoclonal anti-PAI-1 immunoglobulin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1 : 100 dilution) using a standard avidin-biotin-peroxidase complex method. 3-amino-9-ethylcarbazole (DAKO, Carpinteria, CA, USA) was then used as the substrate for localizing the antibody binding. Negative controls included serial sections from which either the primary or secondary antibodies were excluded. The preparations were counterstained with hematoxylin, mounted with Permount (Merck, Darmstadt, Germany) and examined by light microscopy.

Statistical analysis

Triplicate or more separate experiments were performed throughout this study. Statistical analysis was performed by one-way analysis of variance (ANOVA). Tests of differences of the treatments were analyzed by Duncan's test.

Results

Investigations of the time dependence of PAI-1 mRNA expression in 200 ng/ml of cyclosporin A-treated human

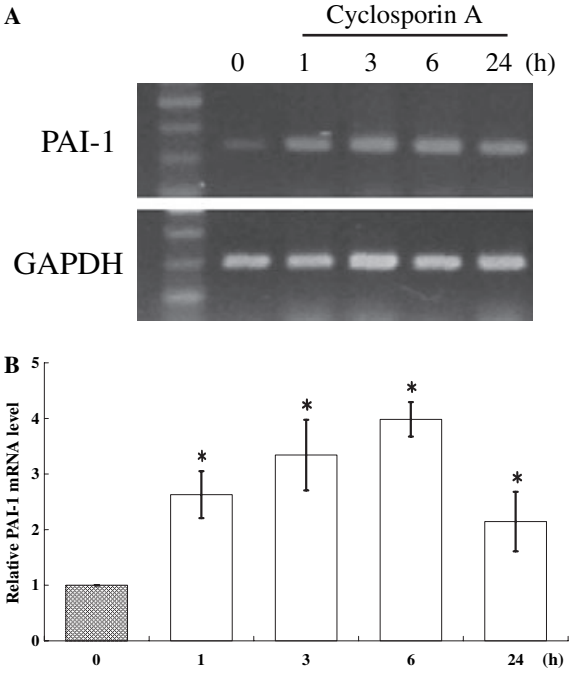

Fig. 1. (A) Induction of type I plasminogen activator inhibitor (PAI-1) mRNA expression in human gingival fibroblasts with 200 ng/ml cyclosporin A. Human gingival fibroblasts were exposed to cyclosporin A for the indicated times (0, 1, 3, 6 or 24 h). A DNA ladder of known base pairs was used for the identification of polymerase chain reaction (PCR) products. Expression of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used in order to monitor equal RNA loading. (B) Levels of PAI-1 mRNA after treatment of human gingival fibroblast with cyclosporin A were measured by AlphaImager 2000. The relative level of PAI-1 mRNA gene expression for each sample was normalized against the GAPDH mRNA signal and the control was set as 1.0. The absorbance values represent the means of five different human gingival fibroblast strains ± standard deviations. *Significant difference from control values with $p < 0.05$.

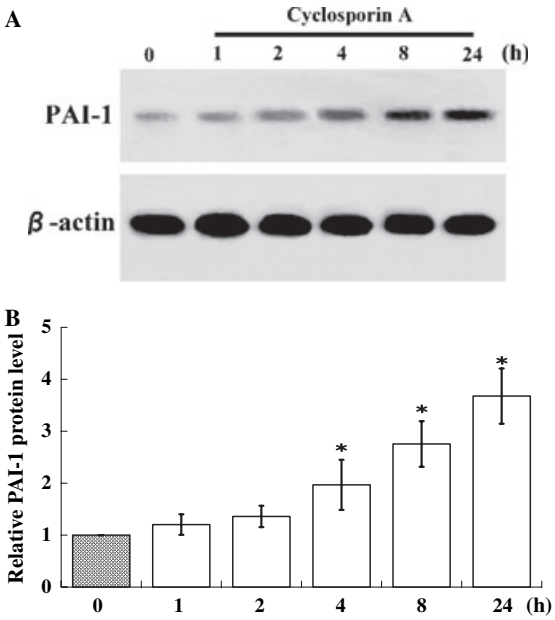

Fig. 2. (A) Induction of type I plasminogen activator inhibitor (PAI-1) protein expression in human gingival fibroblasts with 200 ng/ml cyclosporin A and analysis by western blot. Human gingival fibroblasts were exposed to cyclosporin A for the indicated times (0, 1, 2, 4, 8 or 24 h). Expression of β-actin was analysed in order to monitor equal protein loading. (B) Levels of PAI-1 protein after treatment of human gingival fibroblasts with cyclosporin A were measured by AlphaImager 2000. The relative level of PAI-1 protein expression was normalized against the β-actin signal and the control was set as 1.0. Absorbance values represent the mean ± standard deviation. *Significant difference from control values with $p < 0.05$.

gingival fibroblasts revealed a rapid accumulation of the transcript. A significant signal was first detectable after 1 h of exposure and the signal remained elevated throughout the 24-h incubation period (Fig. 1A). From the AlphaImager 2000, the levels of the PAI-1 mRNA increased ≈2.5-, 3.3-, 4.0- and 2.1-fold after exposure to cyclosporin A for 1, 3, 6 and 24 h, respectively (Fig. 1B).

Expression of PAI-1 protein in human gingival fibroblasts challenged with cyclosporin A was directly assessed in cell lysates using western blotting. Figure 2A shows that 200 ng/ml of cyclosporin A up-regulated PAI-1 protein expression by human gingival fibroblasts. The kinetics of this response demonstrated that PAI-1 was first significantly detectable in cell lysates at 4 h post cyclosporin A challenge, and the maximum PAI-1 protein level induced by cyclosporin A occurred at 24 h. From the AlphaImager 2000, the levels of the PAI-1 protein increased by ≈1.2-, 1.4-, 2.0-, 2.8- and 3.7-fold after exposure to cyclosporin A for 1, 2, 4, 8 and 24 h, respectively (Fig. 2B).

The PAI-1 staining in gingival tissue was stronger in the cyclosporin A-induced gingival overgrowth group than in the normal gingival group. Figure 3A represents gingival tissue obtained from the normal gingival group with faint PAI-1 expression. In the cyclosporin A-induced gingival overgrowth group, intense red–brown staining for PAI-1 expression was observed mainly in the cytoplasm of fibroblasts, endothelial cells and inflammatory cells (Fig. 3B).

Discussion

The biochemical events involved in the development of the cyclosporin A-induced gingival overgrowth are not well understood at present, but the fibrinolytic pathway is believed to play an important role. Cyclosporin A-induced gingival overgrowth is thus probably a consequence of disturbances in the homeostatic equilibrium between the synthesis and degradation of extracellular matrix molecules. One of the key regulators of fibrinolysis is the plasminogen/plasmin system. Plasminogen is activated by tissue-type plasminogen activator and urokinase-type plasminogen to plasmin, which promote the degradation of extracellular matrix. This process is counterbalanced by PAI-1, which inactivates tissue-type plasminogen activator and urokinase-type plasminogen, resulting in a decreased production of plasmin and hence an accumulation of extracellular matrix (18). Impaired fibrinolysis may result from increased concentrations of the principal inhibitor of the fibrinolytic system, PAI-1.

PAI-1 is consistently and dramatically up-regulated in a variety of fibrotic diseases (9–13). In this study, we reported that the up-regulation of PAI-1 mRNA and protein expression occurred in human gingival fibroblasts stimulated with cyclosporin A. Our results are in agreement with Shihab *et al.* (19,20), who reported that PAI-1 was up-regulated in chronic cyclosporin A nephropathy. However, in gingival crevicular fluid samples, PAI-2, but not PAI-1, was up-regulated in patients with cyclosporin A-induced gingival overgrowth (14). The reasons for these differences are not clear. It may be a result of the different sources of specimens, as gingival crevicular fluid was analysed by Budueneli *et al.* (14), whereas human gingival fibroblasts and cyclosporin A-induced gingival overgrowth specimens were tested in this study. One previous study has demonstrated that elevated plasma PAI levels in cyclosporin A-treated patients induce hypofibrinolysis (21). This suggests that one of the pathogenetic mechanisms of cyclosporin A-induced gingival overgrowth may be the synthesis of PAI-1 expression by resident cells in response to cyclosporin A challenge. The accumulation of extracellular matrix components in gingival connective tissue may be caused by a simultaneous effect on PAI-1.

In addition, PAI-1 immunostaining was shown to be up-regulated in cyclosporin A-induced gingival overgrowth specimens compared with normal gingival tissues. Strong

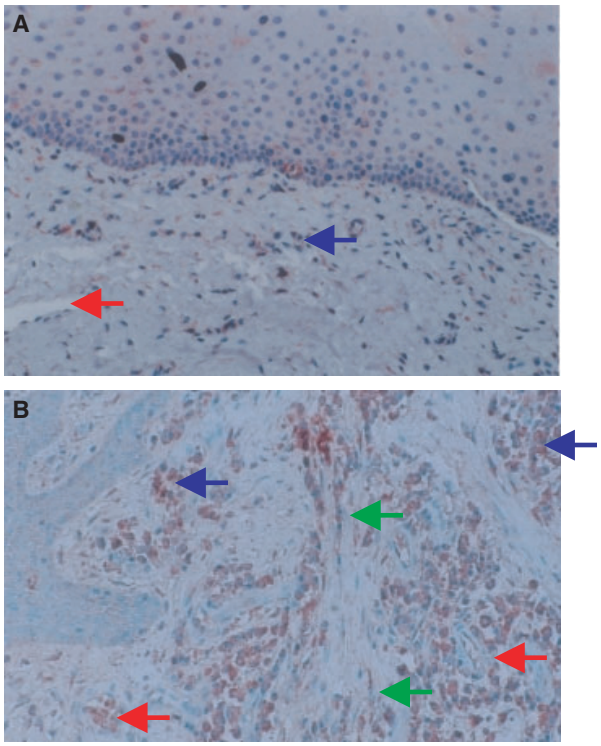

Fig. 3. (A) Very faint immunoreactivity of type I plasminogen activator inhibitor (PAI-1) was observed in normal human gingival tissues (magnification 200×). (B) Strong immunostaining for PAI-1 was noted in the cyclosporin A induced gingival overgrowth specimens. PAI-1 was evident as an intense red–brown color in the cytoplasm of fibroblasts, endothelial cells and inflammatory cells. (magnification 200×). Blue arrow, inflammatory cells; red arrow, endothelial cells; green arrow, fibroblasts.

immunostaining for PAI-1 was detected in fibroblasts, endothelial cells and inflammatory cells. PAI-1 deposition is associated with cyclosporin A-induced gingival overgrowth, suggesting that it could play an important role in the extracellular matrix turnover. These phenomena prompted us to elucidate that cyclosporin A-induced gingival overgrowth may be caused by increased synthesis and deposition of extracellular matrix proteins, altered fibrolysis, or both.

The other main extracellular proteolytic system comprises the matrix metalloproteinases (MMPs) and their inhibitors. Recently, studies have shown that a reduced production of MMPs or an increased production of MMP inhibitors by gingival fibroblasts, in response to cyclosporin A, may contribute to the extracellular matrix accumulation in the pathogenesis of cyclosporin A-induced gingival overgrowth (22–24). Plasmin degrades fibrin and several extracellular matrix and adhesion proteinases and, by activation of procollagenases, may contribute to collagen degradation (18). Indeed, the plasmin-dependent pathway is understood to be a significant alternative pathway for the initiation of extracellular matrix degradation by MMPs (25). PAI-1, as a potent inhibitor of urokinase-type plasminogen, was demonstrated to inhibit urokinase-type plasminogen-induced, MT1–MMP-mediated MMP-2 activation (26). Thus, PAI-1 regulates plasmin formation and fibrinolysis and, through several different mechanisms, plays an important role in the control of MMP activation. The interaction between MMPs and PAI-1 is worthy of further investigation. In addition, the local overall activity of the plasminogen/plasmin system depends on the interaction between activators and inhibitors. However, to what extent these molecules contribute to the pathogenesis of cyclosporin A-induced gingival overgrowth remains to be determined.

In the present study, we have demonstrated, for the first time, that PAI-1 is elevated in cyclosporin A-induced gingival overgrowth specimens as opposed to normal gingival tissues. Data from our *in vitro* experiments showed that cyclosporin A was capable of stimulating PAI-1 mRNA and protein expression in human gingival fibroblasts. This suggests that one of the pathogenetic mechanisms of cyclosporin A-induced gingival overgrowth *in vivo* may be the synthesis of PAI-1 by resident cells in response to cyclosporin A challenge (Fig. 4). However, the genetic and environmental determinants of PAI-1 expression are still incompletely understood. Further research is required, however, including detection of PAI-1 gene trancripts, specifically whether cyclosporin A-induced gingival overgrowth evolves solely as a result of increased/altered *de novo* synthesis and deposition of PAI-1 by cyclosporin A.

Moreover, the mechanism behind the cyclosporin A-induced expression of PAI-1 and other signal proteins still remains to be elucidated. Cyclosporin A has been found to increase transforming growth factor-β (27) and AP-1

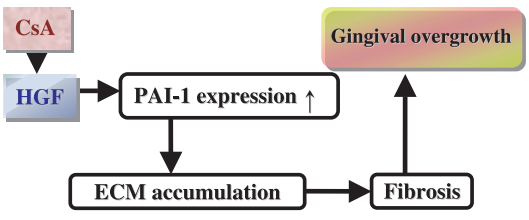

Fig. 4. One of the pathogenic mechanisms of cyclosporin A-induced gingival overgrowth *in vivo* may be the synthesis of type I plasminogen activator inhibitor (PAI-1) by resident cells in response to cyclosporin A challenge. ECM, extracellular matrix; HGF, human gingival fibroblasts, human gingival fibroblast.

activity (28) in human gingival fibroblasts. In addition, angiotensin II, very-low-density lipoprotein and phorbol ester, require the activity of mitogen-activated protein kinase to induce PAI-1 mRNA expression (29,30). The signal transduction pathway involved in PAI-1 expression may prove versatile. Thus, the mechanisms and signal transduction pathways involved in the production of PAI-1 triggered by cyclosporin A might be one important issue for advanced studies.

References

1. Faulds D, Goa KL, Benfield P. Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs* 1993;**45:**953–1040.
2. Kahan BD, Flechner SM, Lorber MI, Golden D, Conley S, Van Buren CT. Complications of cyclosporine-prednisone immunosuppression in 402 renal allograft recipients exclusively followed at a single center for from one to five years. *Transplantation* 1987;**43:**197–204.
3. Wysocki GP, Gretzinger HA, Laupacis A, Ulan RA, Stiller CR. Fibrous hyperplasia of the gingiva: a side effect of cyclosporin A therapy. *Oral Surg Oral Med Oral Pathol* 1983;**55:**274–278.
4. Thomason JM, Seymour RA, Rice N. The prevalence and severity of cyclosporin and nifedipine-induced gingival overgrowth. *J Clin Periodontol* 1993;**20:**37–40.
5. Tyldesley WR, Rotter E. Gingival hyperplasia induced by cyclosporin-A. *Br Dent J* 1984;**157:**305–309.
6. Rostock MH, Fry HR, Turner JE. Severe gingival overgrowth associated with cyclosporine therapy. *J Periodontol* 1986;**57:**294–299.
7. Sprengers ED, Kluft C. Plasminogen activator inhibitors. *Blood* 1987;**69:**381–387.
8. Collen D. Natural inhibitors of fibrinolysis. *J Clin Pathol* 1980;**14:**24–30.
9. Barazzone C, Belin D, Piguet PF, Vassalli JD, Sappino AP. Plasminogen activator inhibitor-1 in acute hyperoxic mouse lung injury. *J Clin Invest* 1996;**98:**2666–2673.
10. Zhang LP, Takahara T, Yata Y, Furui K, Jin B, Kawada N, Watanabe A. Increased expression of plasminogen activator and plasminogen activator inhibitor during liver fibrogenesis of rats: role of stellate cells. *J Hepatol* 1999;**31:**703–711.
11. Takeshita K, Hayashi M, Iino S *et al*. Increased expression of plasminogen activator inhibitor-1 in cardiomyocytes contributes to cardiac fibrosis after myocardial infarction. *Am J Pathol* 2004;**164:**449–456.
12. Yang SF, Hsieh YS, Tsai CH, Chou MY, Chang YC. The upregulation of type I plasminogen activator inhibitor in oral submucous fibrosis. *Oral Oncol* 2003;**39:**367–372.
13. Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, Ginsburg D, Simon RH. Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest* 1996;**97:**232–237.
14. Buduneli N, Buduneli E, Ciotanar S, Atilla G, Lappin D, Kinane D. Plasminogen activators and plasminogen activator inhibitors in gingival crevicular fluid of cyclosporin A-treated patients. *J Clin Periodontol* 2004;**31:**556–561.
15. Chang YC, Tsai CH, Yang SH, Liu CM, Chou MY. Induction of cyclooxygenase-2 mRNA and protein expression in human gingival fibroblasts stimulated with nicotine. *J Periodont Res* 2003;**38:**496–501.
16. Chang YC, Lai CC, Lin LF, Ni WF, Tsai CH. The upregulation of heme oxygenase-1 expression in human gingival fibroblasts stimulated with nicotine. *J Periodont Res* 2005;**40:**252–257.
17. Chang YC, Hsieh YS, Lii CK, Huang FM, Tai KW, Chou MY. Induction of c-*fos* expression by nicotine in human periodontal ligament fibroblasts is related to cellular thiol levels. *J Periodont Res* 2003;**38:**44–50.
18. Vassalii JD, Sappino AP, Belin D. The plasminogen activator/plasmin system. *J Clin Invest* 1991;**88:**1067–1072.
19. Shihab FS, Andoh TF, Tanner AM, Nobel NA, Border WA, Franceschini N, Bennett WM. Role of transforming growth factor-beta 1 in experimental chronic cyclosporin nephropathy. *Kiney Int* 1996;**49:**1141–1151.
20. Shihab FS, Yi H, Bennett WM, Andoh TF. Effect of nitric oxide modulation on TGF-beta 1 and matrix protein in chronic cyclosporin nephrotoxicity. *Kidney Int* 2000;**58:**1174–1185.
21. Verpooten GA, Cools FJ, Van Gaal LF, DeBroe ME. Elevated plasminogen activator inhibitor levels in cyclosporine-treated renal allograft recipients. *Nephrol Dial Transplant* 1996;**11:**347–351.
22. Sugano N, Ito K, Murai S. Cyclosporin A inhibits collagenases gene expression via AP-1 and JNK suppression in human gingival fibroblasts. *J Periodont Res* 1998;**33:**448–452.
23. Bolzani G, Coletta RD, Matelli JH, Almeida OP, Graner E. Cyclosporin A inhibits production and activity of matrix metalloproteinases by gingival fibroblasts. *J Periodont Res* 2000;**35:**51–58.
24. Hyland PL, Traynor PS, Myrillas TT *et al*. The effects of cyclosporine on the collagenolytic activity of gingival fibroblasts. *J Periodontol* 2003;**74:**437–445.
25. Birkedal-Hansen H, More WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, Decrol A, Engler JA. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;**4:**197–250.
26. Baricos WH, Cortez SL, EL-Dahr SS, Schnaper HW. extracellular matrix degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. *Kidney Int* 1996;**47:**1039–1047.
27. Cortrim P, de Andrade CR, Martelli-Junior H, Graner E, Sauk JJ, Coletta RD. Expression of matrix metalloproteinases in cyclosporin-treated gingival fibroblasts is regulated by transforming growth factor (TGF)-β1 autocrine stimulation. *J Periodontol* 2002;**73:**1313–1322.
28. Bostrom A, Bharath H, Saulewicz A, Narayanan AS. Cyclosporin A affects signal events differentially in human gingival fibroblasts. *J Dent Res* 2005;**84:**532–536.
29. Mucsi I, Skorecki KL, Goldberg HJ. Extracellular signal-regulated kinase and the small GTP-binding protein, Rac, contribute to the effects of transforming growth factor-beta 1 on gene expression. *J Biol Chem* 1996;**271:**16567–16572.
30. Chen HC, Feener EP. MEK1,2 response element mediates angiotensin II-stimulated plasminogen activator inhibitor-1 promoter activation. *Blood* 2004;**103:**2636–2644.