Journal of
PERIODONTAL RESEARCH

J Periodont Res 2012; 47: 431–438

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01450.x

Cyclosporine-A inhibits MMP-2 and -9 activities in the presence of *Porphyromonas gingivalis* lipopolysaccharide: an experiment in human gingival fibroblast and U937 macrophage co-culture

P.-J. Kuo[1,2], H.-P. Tu[1,3], Y.-T. Chin[1,3], S.-H. Lu[4], C.-Y. Chiang[1], R.-Y. Chen[5], E. Fu[1]

Departments of [1]Periodontology, [2]Institute of Dental Sciences, [3]Department of Dental Hygiene, China Medical University, Taichung, Taiwan, [4]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan and [5]Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA, USA

Kuo PJ, Tu HP, Chin YT, Lu SH, Chiang CY, Chen RY, Fu E. Cyclosporine-A inhibits MMP-2 and -9 activities in the presence of Porphyromonas gingivalis *lipopolysaccharide: an experiment in human gingival fibroblast and U937 macrophage co-culture. J Periodont Res 2012; 47: 431–438. © 2012 John Wiley & Sons A/S*

Background and Objective: Studies have shown that bacterial plaque and the associated gingival inflammation increase the severity of gingival overgrowth induced by cyclosporine-A (CsA). This *in vitro* study aimed to evaluate the effect of CsA on the activities of MMPs from the co-culture of human gingival fibroblasts and U937 macrophages in the presence or absence of *Porphyromonas gingivalis* lipopolysaccharide (LPS).

Material and Methods: Activities of pro-MMP-2, MMP-2 and pro-MMP-9 in the supernatants of independent cultures and co-cultures were examined by zymography. RT-PCR was selected to evaluate the expression of mRNA for membrane type-1 (MT1) MMP in the co-cultures.

Results: Activities of MMPs in the co-cultures were significantly greater when compared with any of the independent cultures. Lipopolysaccharide significantly increased the MMP activities in a dose-dependent manner in the co-cultures, whereas CsA inhibited these activities. In the presence of both CsA and LPS, the MMP activities inhibited by CsA could still be observed in the co-cultures. In the individual cultures, in contrast, the CsA-inhibited MMP activities, in the presence of LPS, were minimally detected. The mRNA expression of MT1-MMP was significantly enhanced after LPS treatment; however, this enhancement was inhibited by CsA.

Conclusion: This study demonstrated that, in co-cultures of human gingival fibroblasts and U937 macrophages, CsA could inhibit MMP activities in the presence of *P. gingivalis* LPS. It might be part of the underlying reason for the persistent overgrowth of gingiva seen when bacterial plaque and local inflammation are present during CsA therapy.

Earl Fu, DDS, DScD, Department of Periodontology, School of Dentistry, National Defense Medical Center, PO Box 90048-507, Taipei, Taiwan
Tel: +886 2 87927150
Fax: +886 2 87927145
e-mail: dentalab@tpts5.seed.net.tw

Key words: cyclosporine; fibroblast; gingiva; macrophage; matrix metalloproteinase

Accepted for publication October 28, 2011

Cyclosporine-A (CsA) is a widely used immunosuppressant, with clinical applications ranging from organ transplants to chronic inflammatory diseases. One of the adverse effects associated with CsA treatment is the development of gingival overgrowth. Epithelial hyperplasia, interstitial fibrosis and focal inflammatory cell infiltration have been shown to be the histopathological signs/characteristics of CsA-induced gingival overgrowth (1), and focal inflammation is one of the major histological findings in the induced gingival overgrowth. Both human and animal studies have shown that the bacterial plaque, as well as the associated gingival inflammation, increases the overgrowth severity (2–5), although the exact mechanisms are still uncertain.

The direct and indirect effects of CsA on gingival fibroblasts and on epithelial cells have been investigated, and variable responses in the behavior of these cells in response to CsA treatment, including synthetic and metabolic activities, have been observed (6–10). The mechanism by which CsA suppresses the activity of MMPs has also been demonstrated (11–15). The MMPs are a family of structurally related proteins that degrade most of the components of the extracellular matrix and basal membranes in a zinc-dependent manner at physiological pH (16,17). They have been implicated in extracellular matrix remodeling in embryonic development, inflammation, tumor invasion, metastasis and fibrosis (18). Although complex interactions among the mediators of tissue remodeling and inflammation may be involved in the CsA-induced gingival overgrowth, the exact mechanism is yet not fully understood. Specifically, regarding the extracellular matrix, CsA can affect fibroblast proliferation, promote abnormal accumulation of extracellular matrix components in the gingival lamina propria and appears to affect the catabolic enzymes of the extracellular matrix (19–23).

Recent studies have shown that CsA can inhibit the production of MMP-2 in gingival fibroblasts (13,24) and the activities of MMP-2 and MMP-9 in human acute monocyte leukemia cell line monocytes (25); however, the lipopolysaccharide (LPS) of *Aggregatibacter actimocycetemcomintans* can augment the activities of MMP-2 in fibroblasts (26) and MMP-9 in fibroblasts and macrophages (27). As increased cytokine expressions and/or protease activities were observed in co-cultures of fibroblasts and macrophages (28–30), interactions between fibroblasts and inflammatory cells were suggested. Consequently, the present *in vitro* study was designed to evaluate the effect of CsA, in the presence or absence of LPS, on the activities of pro-MMP-2, MMP-2 and MMP-9 in individual cultures and in co-cultures of human gingival fibroblasts and U937 macrophages. In addition, the expression of mRNA for membrane type-1 MMP (MT1-MMP or MMP-14) in the co-cultures was determined in order to examine the possible role of MT1-MMP in the altered MMP-2 activities during CsA and LPS treatments (13).

Material and methods

Cultures of human gingival fibroblasts and U937 macrophages

Human gingival fibroblasts were obtained as described elsewhere (8). In brief, the gingival specimens were immersed in Leibovitz L-15 medium containing 2 mg/mL dispase II (Roche Diagnostics, Indianapolis, IN, USA) and 10% fetal bovine serum at 4°C for 2 d. After separation from the outer epithelial layer, the connective tissue was minced and digested in medium containing 10% fetal bovine serum and 2 mg/mL collagenase for 24 h. Tissue was then placed in flasks containing 10% fetal bovine serum in Dulbecco's modified Eagle's medium–F-12 to enable the cells to migrate from the explants. Confluent fibroblasts were starved in serum-free medium for 24 h before experiments. In the experiment using U937 macrophages, 10^5 macrophages per milliliter were selected. Two serum-free media, either Dulbecco's modified Eagle's medium–F-12 or RPMI-1640 (Invitrogen, Grand Island, NY, USA), were used. In the co-cultures, the media were maintained in the same conditions as those for fibroblast cultures throughout the experiment. *Porphyromonas gingivalis* LPS (InvivoGen, San Diego, CA, USA), at concentrations ranging from 0 to 100 ng/mL in phosphate-buffered saline, and CsA (Sigma-Aldrich Inc., St Louis, MO, USA), at concentrations ranging from 0 to 10^4 ng/mL in dimethyl sulfoxide (Sigma-Aldrich Inc.), were used in the individual cultures and co-cultures. The gelatinolytic activities of pro-MMP-2, MMP-2 and pro-MMP-9 in supernatants of the cultures treated with LPS and CsA for 24 and/or 48 h were determined by using zymography. Using RT-PCRs, the mRNA expressions of MT1-MMP were examined at 0, 3, 10, 15 and 24 h after the co-culturing in order to determine the appropriate duration of culturing. The effects of CsA and/or LPS on the mRNA expressions in the co-cultures were then evaluated.

Gelatin zymography

The release of MMPs (pro-MMP-2, MMP-2,and pro-MMP-9) from cell cultures was evaluated using gelatin zymography. The proteins of the cells in the medium were separated in nonreducing conditions using a 10% sodium dodecyl sulfate–polyacrylamide gel containing 0.1% gelatin. Equal amounts of protein, measured using the BCA™ protein assay (Pierce, Rockford, IL, USA), were loaded into each lane of the gel. After electrophoresis, the gel was placed into renaturing buffer (2.5% Triton X-100), shaken gently to remove the sodium dodecyl sulfate and then incubated in developing buffer for 16 h. The gel was stained with 2.5% Coomassie Brilliant Blue. The latent and active forms of MMP-2 were detected as 72 and 66 kDa bands, respectively, while pro-MMP-9 was detected as a 92 kDa band. The gel images were then scanned directly (Transilluminator/SPOT; Diagnostic Instruments, Sterling Heights, MI, USA).

RT-PCRs

Total RNA was extracted from the cultured cells (homogenized gingival

fibroblasts and U937) and then reverse transcribed. The PCR conditions were as follows: an initial denaturation at 94°C for 2 min 30 s, followed by 30 or 40 cycles at 94°C for 30 s, an appropriate annealing temperature (58–60°C) for 30 s and then 72°C for 55 s. The PCR primers were as follows: MP1-MMP, sense (5′-CATCGCTGCCATGCAGAAGT-3′) and antisense (5′-GTCATCATCGGGCAGCAC-3′), with an expected product of 633 bp; and glyceraldehyde-3-phosphate dehydrogenase, sense (5′-AGCCGCATCTTCTTTTG-CGTC-3′) and antisense (5′-TCATATTTGGCAGGTTTTTCT-3′), with an expected product of 816 bp (13). Amplified RT-PCR products were analysed using 1% agarose gels and visualized using ethidium bromide staining and a camera system (Transilluminator/SPOT; Diag\nostic Instruments). The gel images were scanned directly (ONE-Dscan 1-D Gel Analysis Software; Scanalytic Inc., Fairfax, VA, USA), and the relative densities were determined as the ratio of sample signal intensity to the intensity of the glyceraldehyde-3-phosphate dehydrogenase band.

Statistical analysis

A one-way ANOVA and Duncan's test for *post hoc* analysis were used to evaluate the differences in the gelatinolytic activities of pro-MMP-2, MMP-2 and pro-MMP-9 among the independent cultures of U937 macrophages and human gingival fibroblasts and their co-cultures. Regression analysis was used to determine the dose effect of LPS or CsA on the MMP activities. One-way ANOVA and Duncan's test were also used either to compare the mRNA expressions of MT1-MMP during the 24 h of co-culturing or to evaluate the effects of CsA and LPS on the mRNA expressions. A value of $p < 0.05$ was considered to be significant.

Results

The activity of pro-MMP-9 was significantly greater in U937 independent culture compared with that in human gingival fibroblast culture, while the pro-MMP-2 and MMP-2 activities were greater in human gingival fibroblast culture, both in the 24 and the 48 h cultures (Fig. 1). In the co-culture system, significantly greater MMP activities were observed compared with those in the independent cultures, although a lack of significance was noted for pro-MMP-2 at 24 h and for MMP-2 at 48 h in co-cultures when compared with the values in human gingival fibroblast culture. Moreover, the treatment with *P. gingivalis* LPS significantly increased the MMP activities in a dose-dependent manner in the co-cultures (Fig. 2).

Using the co-culture system without LPS, CsA significantly inhibited the activities of MMPs (pro-MMP-2, MMP-2 and pro-MMP-9) in a dose-dependent manner, regardless of whether the media were obtained at 24 or 48 h, although a lack of significance was observed for pro-MMP-9 at 48 h (Fig. 3).

In the presence of *P. gingivalis* LPS, the proMMP-9 activities in U937 independent cultures treated with CsA and dimethyl sulfoxide were not significantly different, although the pro-MMP-9 activities were significantly reduced in the cultures receiving CsA compared with those receiving dimethyl sulfoxide in the absence of LPS (Fig. 4, lower left panel; pro-MMP 9 in U937). In human gingival fibroblast independent culture, the pro-MMP-9 activities were similar among the conditions related to the CsA and LPS treatments (Fig. 4, lower left panel; pro-MMP9 in human gingival fibroblasts). In the co-culture system, however, LPS alone significantly enhanced the pro-MMP-9 activities, while CsA significantly inhibited the activities, regardless of the presence or absence of LPS (Fig. 4, lower left panel; pro-MMP-9 in U937 and human gingival fibroblast co-culture). Similar

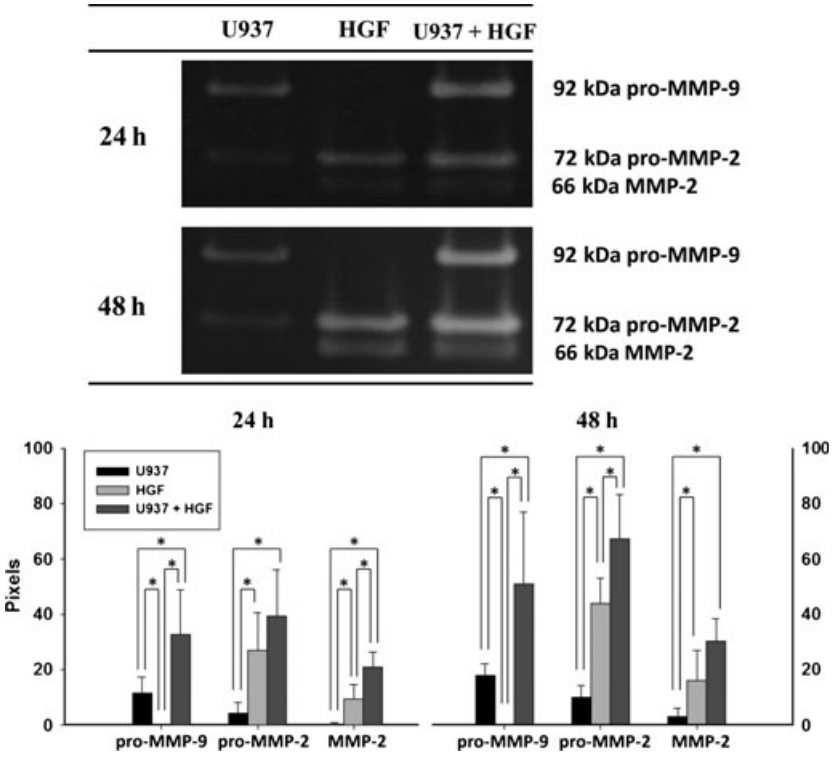

Fig. 1. Activities of pro-MMP-2, MMP-2 and pro-MMP-9 in independent cultures of U937 macrophages and human gingival fibroblasts (HGFs), as well as in their co-cultures. The top part of the figure shows the patterns of gelatinolytic activities at 92 (pro-MMP-9), 66 (MMP-2) and 72 kDa (pro-MMP-2) in the independent cultures and the co-cultures after 24 and 48 h. The bar graphs show comparisons of the optical intensities of the MMPs among the cultures. Data are expressed as means and SDs. The experiment was repeated four times. * Significantly different from the control, $p < 0.05$.

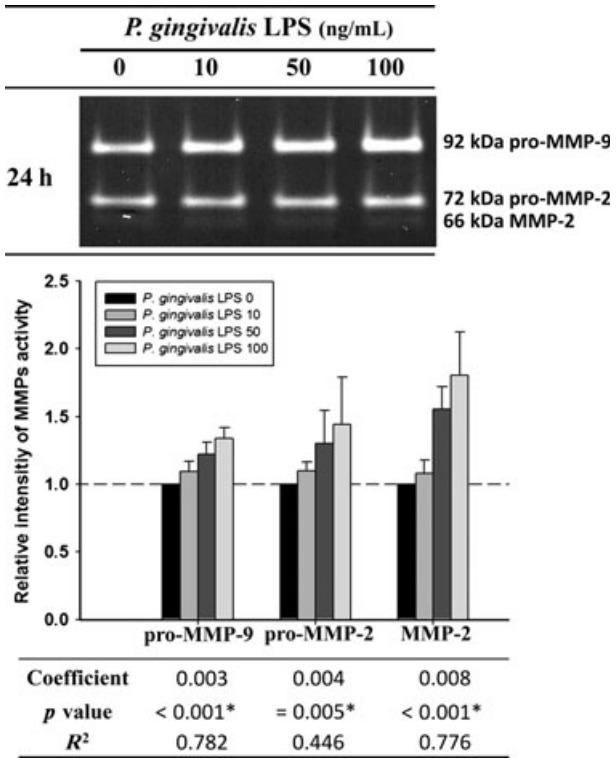

Fig. 2. The effect of *Porphyromonas gingivalis* lipopolysaccharide (LPS) on the MMP activities in co-cultures of U937 and human gingival fibroblasts. The top part of the figure shows the gelatinolytic activities of MMPs in the co-cultures receiving LPS at concentrations of 0, 10, 50 or 100 ng/mL, for 24 h. The lower plot shows the dose effect of LPS on the relative intensities of MMPs in the co-cultures determined by regression analysis. Data are expressed as means and SDs, and the values of coefficient, p and R^2 are listed underneath the plot. The experiment was repeated four times. * Significance at $p < 0.05$.

findings of CsA-reduced MMP activities in the absence and presence of LPS were observed repeatedly in the co-cultures for pro-MMP-2 and MMP-2 (Fig. 4, lower middle and lower right panels; MMP-2 and pro-MMP-2). For the individual cultures of human gingival fibroblasts, CsA-inhibited activities were statistically significant in the presence of LPS, but not in the absence of LPS.

The mRNA expression of MT1-MMP was significantly increased at 15 and 24 h after the co-culturing when compared with that at 0 h (Fig. 5, left panel). The expression of MT1-MMP was significantly enhanced after LPS treatment; however, the enhancement was inhibited by CsA (Fig. 5, right panel).

Discussion

Plaque-associated periodontitis exhibits local tissue breakdown caused by a series of plaque-induced inflammatory consequences; however, this induced breakdown seems not to be observed in the gingival overgrowth induced by CsA. An animal study showed that CsA decreased the initial periodontal breakdown in the presence of inflammatory stimulation by silk ligatures around teeth necks (31). Some other animal studies have also demonstrated that the overgrowth was reduced when a chlorhexidine rinse was used, but the overgrowth was exacerbated when plaque was retained by silk ligatures (2,4). A human study has shown that the gingival overgrowth may be improved through plaque control (5); however, intensive plaque control does not appear to prevent the overgrowth. Our results in the present study are the first to demonstrate *in vitro* that CsA can inhibit the activities of MMPs in the presence of *P. gingivalis* LPS by using the co-cultures of gingival fibroblasts and macrophages (Fig. 4), although LPS alone also enhanced the activities of MMPs (Fig. 3).

In the present study, the MMP activities were detected in the independent cultures of human gingival fibroblasts and U937 macrophages; however, the activities were significantly less than those in the co-culture conditions (Fig. 1). Moreover, the influence of the presence of LPS on CsA-inhibited MMP activities by using the individual cultures was inconsistent compared with those using the co-culture system (Fig. 4). The co-culture system also firmly demonstrates that CsA reduced, whereas LPS enhanced, the MMP activities (Figs 2 and 3). Based on the above findings, cross-talk between the two cell types is suggested. It is known that macrophages and fibroblasts are co-localized and express MMPs in diseased periodontal tissue, including CsA-induced gingival overgrowth. However, the effect of interaction between these two cell types on CsA-inhibited expression of MMPs was still unknonwn. Recently, the co-culture system of gingival fibroblasts and macrophages in normal or high-glucose environments was tested in order to examine whether hyperglycemia regulates MMP expression by affecting the cross-talk between the two cell types (28). In that study, co-culture led to an augmentation of MMP-1 expression and high glucose further enhanced this augmentation. As the interleukin-6 released by the gingival fibroblasts was essential for the augmentation of MMP-1 expression by the macrophages, cross-talk between the two types of cells exposed to high glucose was proposed by the authors. Another study, using co-culture, found that the release of interleukin-6 was increased 100-fold when compared with that using culture of macrophages alone (30).

Recently, extracellular matrix metalloproteinase inducer (EMMPRIN), a highly glycosylated, plasma membrane-bound glycoprotein, was identified, and its expression is considered to be responsible for the induction of fibroblasts to produce or secrete MMPs (32). EMMPRIN (CD147) contains two immunoglobulin superfamily domains, a transmembrane and a stoplasmic

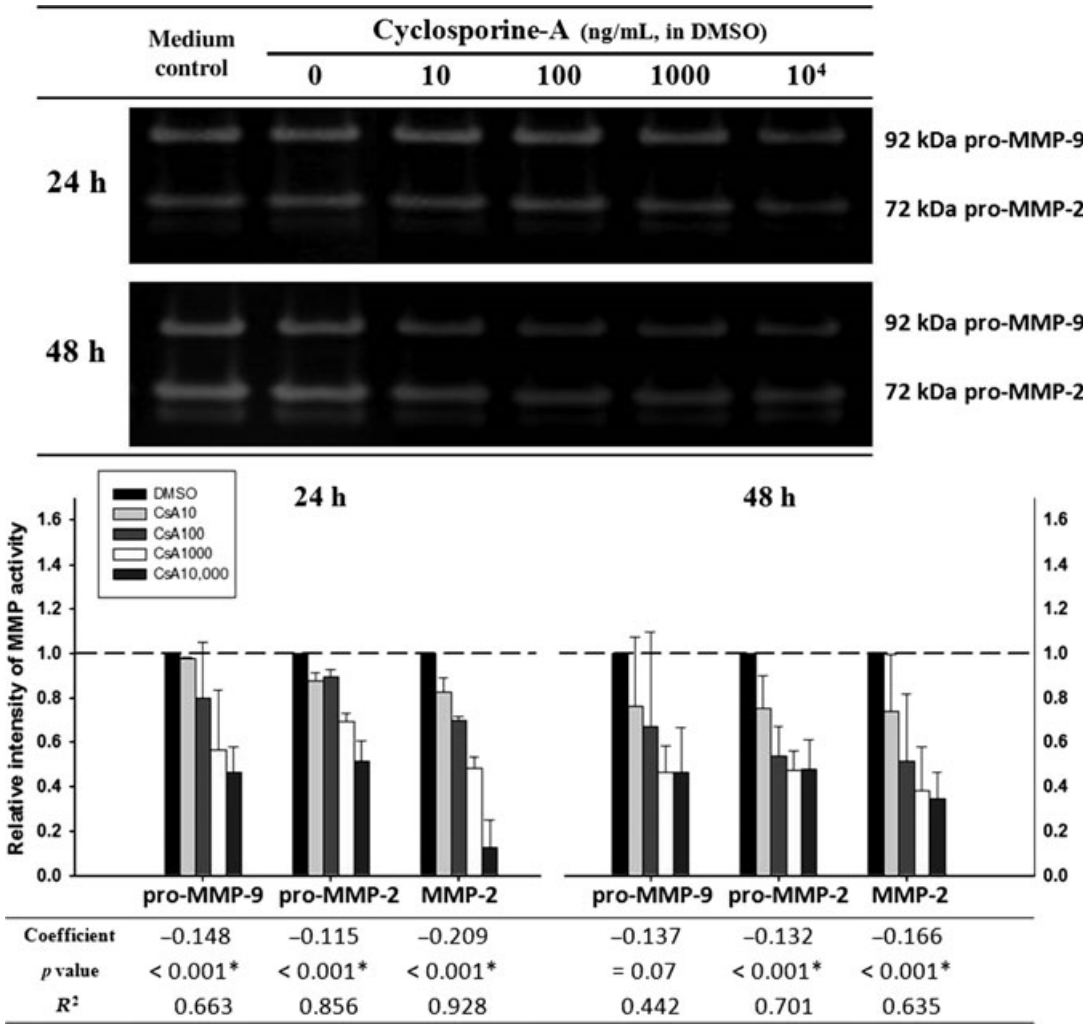

Fig. 3. The inhibitory effect of cyclosporine-A (CsA) on the pro-MMP-2, MMP-2 and pro-MMP-9 activities in the co-cultures of human gingival fibroblasts and U937 macrophages in the absence of *P. gingivalis* LPS. The top part of the figure shows the gelatinolytic activities of MMPs in the co-cultures receiving CsA at 0–10^4 ng/mL, for 24 h. The lower plot shows the dose effect of CsA on the relative intensities of MMPs in the co-cultures determined by regression analysis. Data are expressed as means and SDs, and the values of coefficient, p and R^2 are listed underneath the plot. The experiment was repeated four times. * Significance at $p < 0.05$.

domain (33), and was originally identified on the surface of tumor cells. Its expression on tumor cells may induce tumor progression and invasion by triggering the production or release of MMPs by fibroblasts and endothelial cells (34–36). In order to explore the association of MMP-2 and -9 activities and CD147 for the pathogenesis of rheumatoid arthritis, the co-culture of human fibroblasts and a human acute monocyte leukemia cell line was examined (29). Significantly elevated activation and release of MMP-9 and/or MMP-2 were seen in the co-culture of human monocytes/THP-1 cells and fibroblasts compared with cultures of the cells alone. Given the increased expression of CD147 on monocytes/macrophages in patients with rheumatoid arthritis, the authors suggested that the increased CD147 might be responsible for the elevated MMP secretion, cell invasion and cyclophilin A-mediated cell migration into the joints, all of which may contribute to the cartilage and bone destruction of rheumatoid arthritis. The expression of CD147 in gingival tissue was also evaluated in renal transplant recipients treated with CsA (37); however, there was no significant difference with respect to expression of CD147 in overgrown gingiva from patients receiving CsA and control gingiva from healthy subjects. Thus, the role of CD147 in the CsA-induced MMP activation in gingiva is still unknown and requires further detailed investigation.

Our results also showed that the activities of pro-MMP-2 and MMP-2 in human gingival fibroblast culture were more clearly observed than that of pro-MMP-9 (Fig. 1), and CsA reduced their activities in the independent fibroblast culture and in the co-culture with U937 (Fig. 4, lower middle and lower right panels; MMP-2 and pro-MMP-2), which further confirmed our findings in a previous study (13). In that study, an inhibitory effect

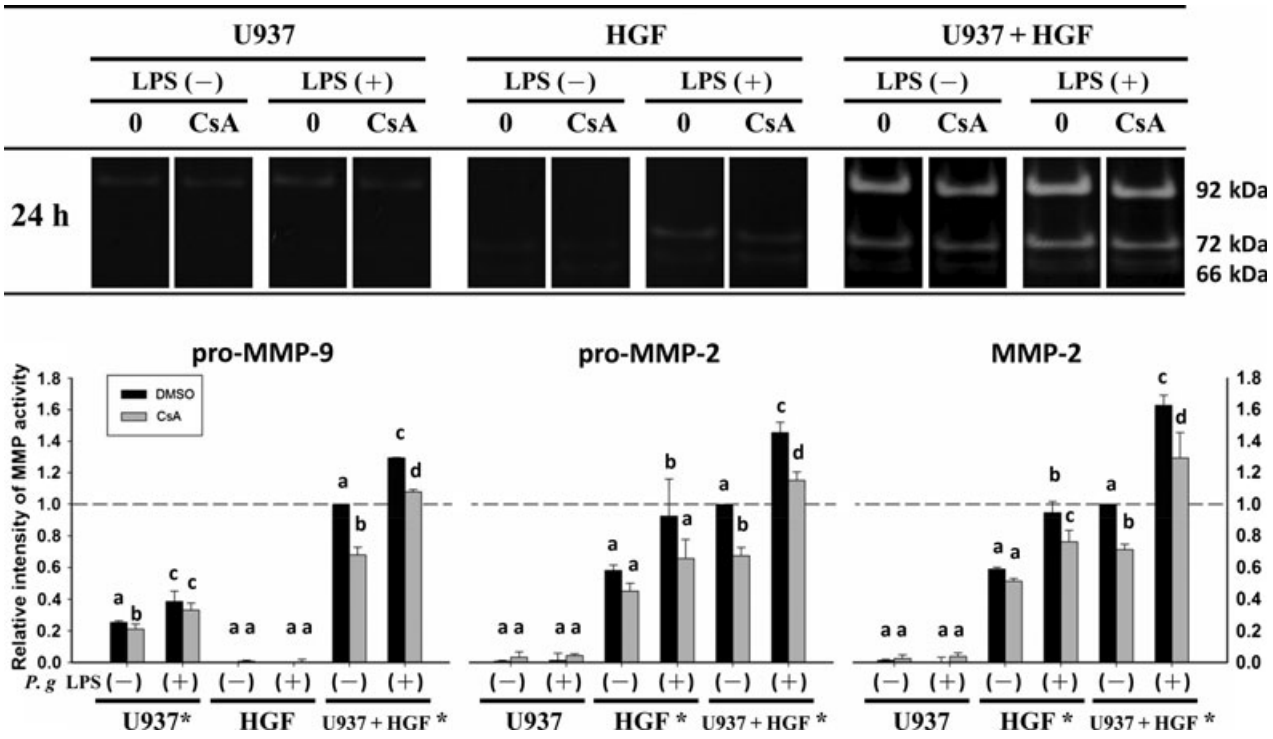

Fig. 4. Effect of CsA on the activities of pro-MM-2, MMP-2 and pro-MMP-9 from the independent cultures and co-cultures of U937 and human gingival fibroblasts (HGFs) in the absence and presence of *P. gingivalis* LPS (CsA, 100 ng/mL; LPS, 100 ng/mL). The top part of the figure shows the pattern of gelatinolytic activities of the MMPs in the cultures at 24 h. The lower part of the figure shows the activities of pro-MMP-2, MMP-2 and pro-MMP-9 related to those from the co-cultures that received neither LPS nor CsA. The experiment was repeated four times. Data are expressed as means and SDs. * Significance was achieved if $p < 0.05$ by one-way ANOVA; and a–d, the subsets obtained after the *post hoc* analysis.

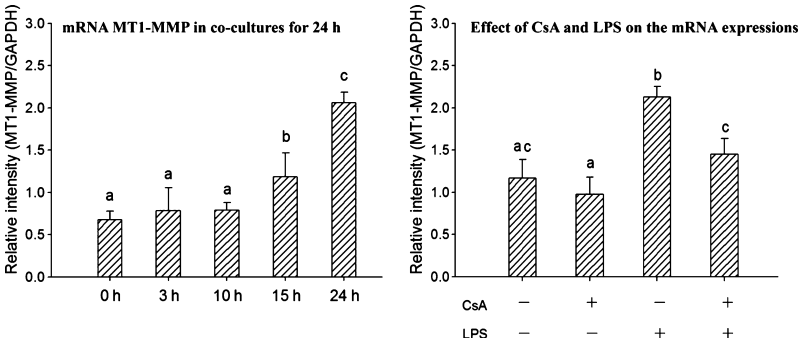

Fig. 5. Effects of CsA and LPS on the mRNA expression of membrane type-1 MMP (MT1-MMP) in the co-cultures of U937 and human gingival fibroblasts. The left plot shows the mRNA expressions of MT1-MMP in the co-cultures of U937 and human gingival fibroblasts at 0, 3, 10, 15 and 24 h. The right plot shows the effects of CsA (100 ng/mL) and LPS (100 ng/mL) on the mRNA expression of MT1-MMP in the co-cultures for 15 h. Data are expressed as means and SDs; and a–c, the subsets obtained by the *post hoc* analysis if statistical significance was achieved by one-way ANOVA.

of CsA on MT1-MMP, which may further regulate pro-MMP-2 and MMP-2, was observed in rats and in human gingival fibroblast cultures. Another study reported that LPS activated monocytes and resulted in the induction of MT1-MMP (38). In the present study, we showed that LPS did enhance the mRNA expression of MT1-MMP; however, the enhancement was inhibited by CsA (Fig. 5). For the CsA-induced overgrown gingiva, the local tissue not only confronts the drug stimulation but also deals with the plaque-induced tissue inflammation. In the present study, the influence of LPS on CsA-inhibited MMP activities was evaluated in a co-culture of gingival fibroblasts and macrophages, which mimics plaque-associated inflammation using an *in vitro* study design.

In conclusion, this study demonstrated that CsA, in the presence of *P. gingivalis* LPS, inhibited MMP activities by using a co-culture system of human gingival fibroblasts and U937 macrophages. Our results may partly explain the enduring or even enhanced CsA-induced gingival overgrowth, when dental plaque is retained or local inflammation is persistent.

Acknowledgements

This study was partially supported by research grants from the Department of National Defence (DOD-C101-12-4)

and the National Science Council (NSC-100-2314-B-039-022), Taiwan.

References

1. Doufexi A, Mina M, Ioannidou E. Gingival overgrowth in children: epidemiology, pathogenesis, and complications. A literature review. *J Periodontol* 2005;**76:**3–10.
2. Fu E, Nieh S, Wikesjo UM. The effect of plaque retention on cyclosporine-induced gingival overgrowth in rats. *J Periodontol* 1997;**68:**92–98.
3. Guo J, Wang W, Yao L, Yan F. Local inflammation exacerbates cyclosporine a-induced gingival overgrowth in rats. *Inflammation* 2008;**31:**399–407.
4. Pilatti GL, Sampaio JE. The influence of chlorhexidine on the severity of cyclosporin A-induced gingival overgrowth. *J Periodontol* 1997;**68:**900–904.
5. Seymour RA, Smith DG. The effect of a plaque control programme on the incidence and severity of cyclosporin-induced gingival changes. *J Clin Periodontol* 1991;**18:**107–110.
6. Schincaglia GP, Forniti F, Cavallini R, Piva R, Calura G, del Senno L. Cyclosporin-A increases type I procollagen production and mRNA level in human gingival fibroblasts in vitro. *J Oral Pathol Med* 1992;**21:**181–185.
7. Zebrowski EJ, Pylypas SP, Odlum O, Johnson RB. Comparative metabolism of 3H-glucosamine by fibroblast populations exposed to cyclosporine. *J Periodontol* 1994;**65:**565–567.
8. Bartold PM. Regulation of human gingival fibroblast growth and synthetic activity by cyclosporine-A in vitro. *J Periodontal Res* 1989;**24:**314–321.
9. Tipton DA, Stricklin GP, Dabbous MK. Fibroblast heterogeneity in collagenolytic response to cyclosporine. *J Cell Biochem* 1991;**46:**152–165.
10. Tipton DA, Pabst MJ, Dabbous MK. Interleukin-1 beta- and tumor necrosis factor-alpha-independent monocyte stimulation of fibroblast collagenase activity. *J Cell Biochem* 1990;**44:**253–264.
11. Bolzani G, Della Coletta R, Martelli Junior H, Martelli Junior H, Graner E. Cyclosporin A inhibits production and activity of matrix metalloproteinases by gingival fibroblasts. *J Periodontal Res* 2000;**35:**51–58.
12. Silva HC, Coletta RD, Jorge J, Bolzani G, de Almeida OP, Graner E. The effect of cyclosporin A on the activity of matrix metalloproteinases during the healing of rat molar extraction wounds. *Arch Oral Biol* 2001;**46:**875–879.
13. Chiu HC, Lu YT, Chin YT *et al*. Cyclosporine A inhibits the expression of membrane type-I matrix metalloproteinase in gingiva. *J Periodontal Res* 2009;**44:** 338–347.
14. Sukkar TZ, Thomason JM, Cawston TE *et al*. Gingival fibroblasts grown from cyclosporin-treated patients show a reduced production of matrix metalloproteinase-1 (MMP-1) compared with normal gingival fibroblasts, and cyclosporin down-regulates the production of MMP-1 stimulated by pro-inflammatory cytokines. *J Periodontal Res* 2007;**42:**580–588.
15. Gagliano N, Moscheni C, Dellavia C *et al*. Effect of cyclosporin A on human gingival fibroblast collagen turnover in relation to the development of gingival overgrowth: an in vitro study. *Biomed Pharmacother* 2004;**58:**231–238.
16. Bode W, Gomis-Ruth FX, Stockler W. Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'. *FEBS Lett* 1993;**331:**134–140.
17. Stocker W, Grams F, Baumann U *et al*. The metzincins –topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases. *Protein Sci* 1995;**4:**823–840.
18. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. *J Pathol* 2003;**200:**448–464.
19. Ono M, Hatamochi A, Arakawa M, Ueki H. Effects of cyclosporin A on cell proliferation and collagen production by human skin fibroblasts. *J Dermatol Sci* 1991;**2:**274–280.
20. Willershausen-Zonnchen B, Lemmen C, Schumacher U. Influence of cyclosporine A on growth and extracellular matrix synthesis of human fibroblasts. *J Cell Physiol* 1992;**152:**397–402.
21. Ayanoglou CM, Lesty C. Cyclosporin A-induced gingival overgrowth in the rat: a histological, ultrastructural and histomorphometric evaluation. *J Periodontal Res* 1999;**34:**7–15.
22. Mariani G, Calastrini C, Carinci F, Bergamini L, Calastrini F, Stabellini G. Ultrastructural and histochemical features of the ground substance in cyclosporin A-induced gingival overgrowth. *J Periodontol* 1996;**67:**21–27.
23. Thomason JM, Sloan P, Seymour RA. Immunolocalization of collagenase (MMP-1) and stromelysin (MMP-3) in the gingival tissues of organ transplant patients medicated with cyclosporin. *J Clin Periodontol* 1998;**25:**554–560.
24. Abe M, Yokoyama Y, Syuto T, Ishibuchi H, Ishikawa O. Interleukin-6 counteracts effects of cyclosporin A on extracellular matrix metabolism by human dermal fibroblasts. *Cell Tissue Res* 2008;**333:**281–288.
25. Yang Y, Lu N, Zhou J, Chen ZN, Zhu P. Cyclophilin A up-regulates MMP-9 expression and adhesion of monocytes/macrophages via CD147 signalling pathway in rheumatoid arthritis. *Rheumatology (Oxford)* 2008;**47:**1299–1310.
26. Bodet C, Andrian E, Tanabe S, Grenier D. Actinobacillus actinomycetemcomitans lipopolysaccharide regulates matrix metalloproteinase, tissue inhibitors of matrix metalloproteinase, and plasminogen activator production by human gingival fibroblasts: a potential role in connective tissue destruction. *J Cell Physiol* 2007;**212:**189–194.
27. Bodet C, Chandad F, Grenier D. Inhibition of host extracellular matrix destructive enzyme production and activity by a high-molecular-weight cranberry fraction. *J Periodontal Res* 2007;**42:**159–168.
28. Sundararaj KP, Samuvel DJ, Li Y, Sanders JJ, Lopes-Virella MF, Huang Y. Interleukin-6 released from fibroblasts is essential for up-regulation of matrix metalloproteinase-1 expression by U937 macrophages in coculture: cross-talking between fibroblasts and U937 macrophages exposed to high glucose. *J Biol Chem* 2009;**284:**13714–13724.
29. Zhu P, Ding J, Zhou J, Dong WJ, Fan CM, Chen ZN. Expression of CD147 on monocytes/macrophages in rheumatoid arthritis: its potential role in monocyte accumulation and matrix metalloproteinase production. *Arthritis Res Ther* 2005;**7:**R1023–R1033.
30. Lind M, Trindade MC, Yaszay B, Goodman SB, Smith RL. Effects of particulate debris on macrophage-dependent fibroblast stimulation in coculture. *J Bone Joint Surg Br* 1998;**80:**924–930.
31. Nassar CA, Nassar PO, Abi Rached RS, Holzhausen M, Marcantonio E Jr, Spolidorio LC. Effect of cyclosporin A on alveolar bone homeostasis in a rat periodontitis model. *J Periodontal Res* 2004;**39:** 143–148.
32. Foda HD, Rollo EE, Drews M *et al*. Ventilator-induced lung injury upregulates and activates gelatinases and EMMPRIN: attenuation by the synthetic matrix metalloproteinase inhibitor, Prinomastat (AG3340). *Am J Respir Cell Mol Biol* 2001;**25:**717–724.
33. Biswas C, Zhang Y, DeCastro R *et al*. The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Res* 1995;**55:**434–439.
34. Bordador LC, Li X, Toole B *et al*. Expression of emmprin by oral squamous cell carcinoma. *Int J Cancer* 2000;**85:**347–352.

35. Kanekura T, Chen X, Kanzaki T. Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. *Int J Cancer* 2002;**99:**520–528.
36. Zucker S, Hymowitz M, Rollo EE *et al*. Tumorigenic potential of extracellular matrix metalloproteinase inducer. *Am J Pathol* 2001;**158:**1921–1928.
37. Bulut OE, Sokmensuer LK, Bulut S, Tasman F, Muftuoglu S. Immunohistochemical study of cyclosporin-induced gingival overgrowth in renal transplant recipients. *J Periodontol* 2004;**75:**1655–1662.
38. Shankavaram UT, Lai WC, Netzel-Arnett S *et al*. Monocyte membrane type 1-matrix metalloproteinase. Prostaglandin-dependent regulation and role in metalloproteinase-2 activation. *J Biol Chem* 2001;**276:**19027–19032.