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Cell interactions between human gingival fibroblasts and monocytes stimulate the production of matrix metalloproteinase-1 in gingival fibroblasts

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Background: Matrix metalloproteinase-1 (MMP-1) plays an important role in inflammatory diseases including periodontitis, which is characterized by tissue destruction and dense infiltration of mononuclear cells.

Objectives: The aim of this study was to investigate the effect of cell interactions between human gingival fibroblasts and human monocytes on the production of MMP-1 in a coculture model.

Methods: The fibroblasts were cultured in either cell-to-cell contact with monocytes or in separated cocultures using a microporous membrane to prevent cell-to-cell contact. The mRNA expression of MMP-1 was analyzed using reverse transcription–polymerase chain reaction (RT–PCR) and the protein levels of MMP-1 in the cell medium were measured using enzyme-linked immunosorbent assay (ELISA).

Results: Coculturing gingival fibroblasts with monocytes in cell-to-cell contact increased the mRNA expression of MMP-1 in both fibroblasts and monocytes. The protein levels of MMP-1 increased in the culture media of the cocultures and correlated to the number of fibroblasts as well as to the number of monocytes. When fibroblasts were cultured with monocytes in separated cocultures, the mRNA expression and protein level of MMP-1 increased in the fibroblasts. In addition, treatment of fibroblasts with conditioned medium from monocytes also stimulated the production of MMP-1 in the fibroblasts. Moreover, the levels of the MMP-1 inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), increased in cocultures with cell-to-cell contact, but not in fibroblasts of separated cocultures. The glucocorticoid dexamethasone and the tetracycline doxycycline reduced the enhanced level of MMP-1 in the cocultures with cell-to-cell contact.

Conclusion: The current study demonstrates that monocytes stimulate the production of MMP-1 in gingival fibroblasts by cell interactions, which may contribute to the maintenance of MMP-mediated tissue destruction in periodontitis.

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, which collectively are able to degrade essentially all components of the extracellular matrix. At present, at least 20 human members of the MMP family have been characterized and divided into subgroups of collagenases, gelatinases, stromelysins, membrane-type MMPs and other MMPs according to their substrate specificity (1). Collagenases including MMP-1 are involved in general turnover of extracellular matrix and play important roles in physiological processes such as wound healing and cell migration (2, 3). MMP-1 is also involved in tissue breakdown during pathological conditions such as cancer, rheumatoid arthritis, atherosclerosis and periodontitis (4–7).

In periodontitis, MMP-1 is reported to be involved in the gingival extracellular matrix degradation (8). Increased levels of MMP-1 have been detected in gingival tissue as well as in gingival crevicular fluid from periodontitis affected patients (7, 9). Gingival fibroblasts, the major constituents of gingival connective tissue, by producing MMP-1 (10), contribute to the enhanced levels of MMP-1. In addition, in periodontitis-affected gingival tissue, dense infiltration of mononuclear cells is observed and interactions between gingival fibroblasts and mononuclear cells such as monocytes/macrophages are suggested to play a major role in the modulation of the connective tissue (11–13).

MMP-1 is synthesized by a variety of cells including keratinocytes, endothelial cells, osteoblasts, monocytes and fibroblasts (1, 14, 15). The production of MMP-1 is stimulated by growth factors such as epidermal growth factor and monocyte-derived cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor α (TNFα) (10, 15). MMP-1 is also regulated by its inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1 16), by anti-inflammatory drugs such as the glucocorticoid dexamethasone and the tetracycline doxycycline (10, 17) as well as by cell-to-cell interactions (18). The induction of MMP-1 by cell-to-cell interactions of fibroblasts and monocytes/macrophages has previously been reported between rabbit skin fibroblasts and bone marrow macrophages as well as between human fetal lung fibroblasts and monocytes (19, 20). However, to our knowledge, there are no studies reporting the production of MMP-1 in gingival fibroblasts in connection with cell interactions with monocytes.

The aim of the study was to investigate the effect of cell interactions between gingival fibroblasts and monocytes on the production of MMP-1 in gingival fibroblasts using a coculture model. Furthermore, we investigated the regulation of MMP-1 with special regard to its inhibitor TIMP-1 as well as to anti-inflammatory agents such as the glucocorticoid dexamethasone and the tetracycline doxycycline.

Material and methods

Fibroblast cultures

Cultures of fibroblast cells were established from gingival biopsies obtained from eight healthy individuals between 3 and 18 years of age with no clinical signs of periodontal disease. The plan to take gingival tissue biopsies was accepted by the Ethical Committee of Karolinska Institutet. Minced pieces of the tissue were explanted to 25-cm^2 Nunc tissue cultures (Nunc, Naperville, IL, USA) containing 5 ml of Dulbecco's modified Eagle's medium (DMEM) (Gibco, Paisley, Scotland). The fibroblasts were obtained by trypsination of the primary outgrowth of cells as described previously (21). The cells were grown in DMEM supplemented with fetal calf serum (FCS) (5%) (Gibco) at 37°C in a humidified incubator gassed with 5% CO_2 in air and routinely passaged using 0.025% trypsin in phosphate-buffered saline (PBS) containing 0.02% EDTA (Gibco). Cells used for the experiment proliferated in logarithmic phase between the 7th and 12th passages.

Peripheral blood monocytes

Peripheral blood mononuclear cells were isolated from buffy coats from healthy donors by gradient centrifugation ($1000 \times g$ for 30 min) on Lymphoprep (density: 1.077 g/ml) (Fresenius Kabi, Oslo, Norway). The cells were washed with DMEM and treated for 10 min with 1.5 M ammonium chloride containing potassium hydrogen carbonate (0.1 M) and EDTA (0.9 mM) for lysis of remaining erythrocytes. Monocytes were then purified from the peripheral blood mononuclear cells fraction by gradient centrifugation. Peripheral blood mononuclear cells (2×10^7) were collected

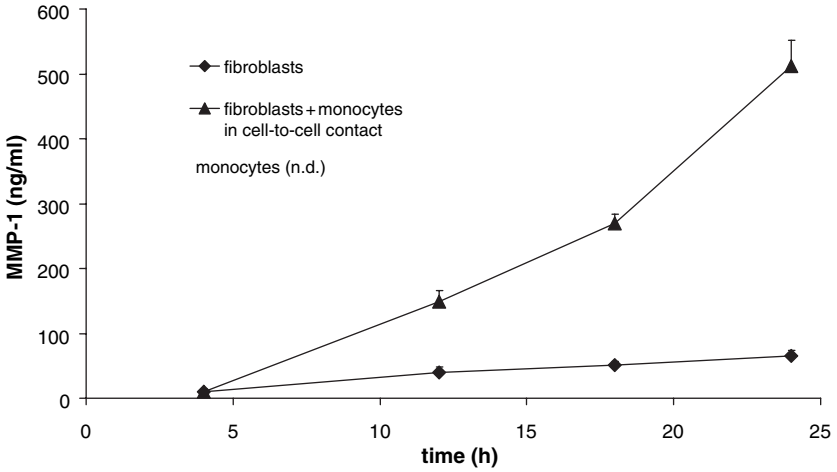

Fig. 1. Time-kinetics (4–24 h) of matrix metalloproteinase-1 (MMP-1) levels in the medium from cultures of gingival fibroblasts (3×10^4) alone or in cell-to-cell contact with monocytes (3×10^5). MMP-1 was not detected (n.d.) in cultures of monocytes alone. Mean value of triplicates with SD shown by vertical bars.

and resuspended in 2.5 ml 60% percoll (Amersham Biosciences, Little Chalfont, UK) (density 1.131, diluted with 10% PBS) in DMEM. Thereafter, 5 ml 47.5% and 2 ml 34% percoll in DMEM were subsequently layered upon the cell suspension. After centrifugation (1700 *g* for 40 min), the upper layer containing the monocytes was collected and washed with DMEM.

Coculture of gingival fibroblasts and monocytes

Gingival fibroblasts, at the cell numbers indicated in the legends to figures and tables, were seeded in 24-well plates (Nunc) with DMEM containing 5% FCS and incubated for 24 h at 37°C. The cell layer was rinsed three times with DMEM. The coculture experiments with DMEM, supplemented with 5% FCS, were carried out in two ways: (i) cocultures with cell-to-cell contact with monocytes layered onto confluent fibroblasts (600 µl); (ii) separated cocultures with a microporous membrane (pore size 0.4 µm) (Corning Incorporated, NY, USA) separating fibroblasts (600 µl) in the lower compartment from the monocytes (200 µl) in the upper compartment. The cocultures, at cell number indicated in the legends to figures, were incubated for 4–24 h. In some experiments dexamethasone (Sigma, St. Louis, MO, USA) or doxycycline hyclate (Nordic Drugs, Limhamn, Sweden) were added to the cocultures.

Confocal laser scanning microscopy of cocultures of fibroblasts and monocytes in cell-to-cell contact

Fibroblasts (3×10^4 cells) were cultured in 4-well glass slides (Nunc) with DMEM containing 5% FCS. After 24 h the medium was withdrawn and 1.0 ml 5% FCS DMEM containing monocytes (3×10^5 cells) were added to the fibroblasts and the cells were cocultured for 24 h. Thereafter the cells on the slides were washed three times with PBS and fixed with 4% paraformaldehyde for 15 min. After fixation, the cells were washed three times with PBS and incubated overnight at 4°C with CD90 fluorescein isothiocyanate-labeled mAb (clone 5E10, BD Biosciences, San Jose, CA, USA) recognized on fibroblasts (22) and CD14 phycoerythrin-labeled mAb (clone MØP9BD, BD Biosciences) recognized on monocytes. The cells were then washed three times with PBS and mounted with fluorescent mounting medium (DakoCytomation, Glostrup, Denmark). The cells were then viewed with Leica TCS-NT confocal laser scanning microscope equipped with Argon-Krypton laser (Leica Microsystems, Heidelberg, Germany).

Gingival fibroblasts cultured with conditioned medium from monocytes

Gingival fibroblasts (3×10^4) were seeded in 24-well plates as described above. Monocytes, at different cell numbers (1×10^5 and 3×10^5), were cultured in 24-well plates containing 600 µl DMEM supplemented with 5%

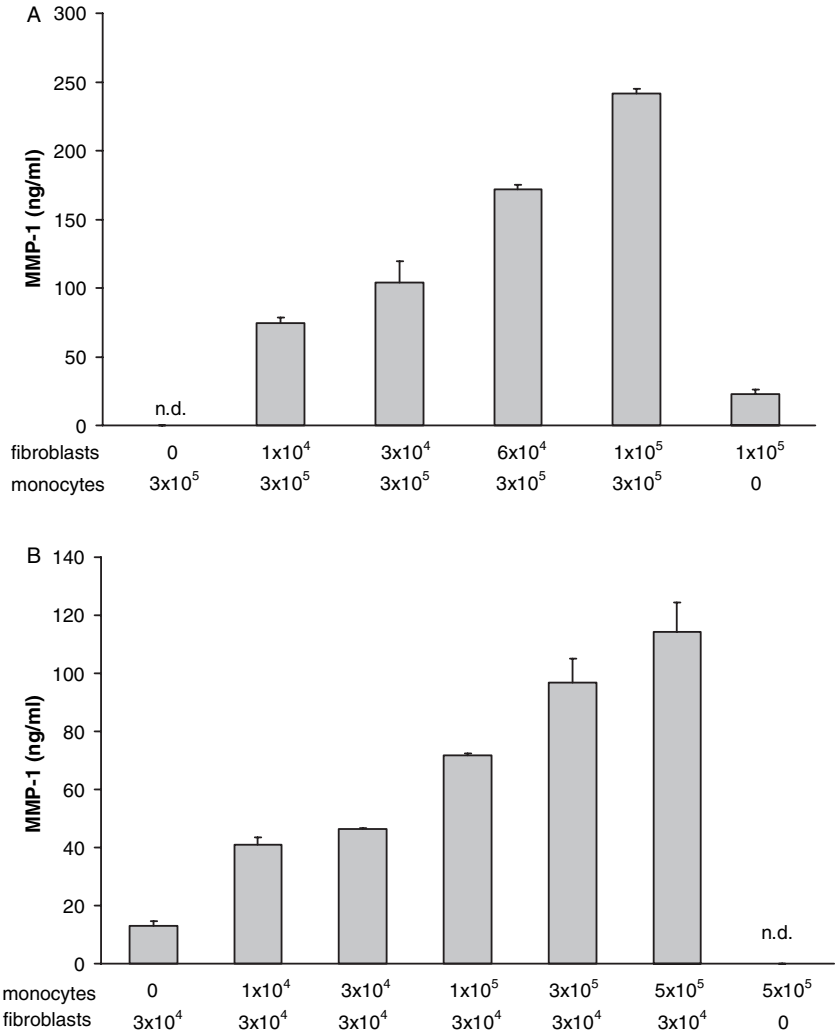

Fig. 2. The effect of different number of fibroblasts and monocytes on matrix metalloproteinase-1 (MMP-1) levels in cocultures with cell-to-cell contact. (A) The levels of MMP-1 in cocultures of increasing number of fibroblasts (1×10^4–1×10^5) and fixed numbers of monocytes (3×10^5). (B) The levels of MMP-1 in cocultures with increasing number of monocytes (1×10^4–5×10^5) and fixed number of fibroblasts (3×10^4). MMP-1 was not detected (n.d.) in cultures of monocytes alone. Mean value of triplicates with SD shown by vertical bars.

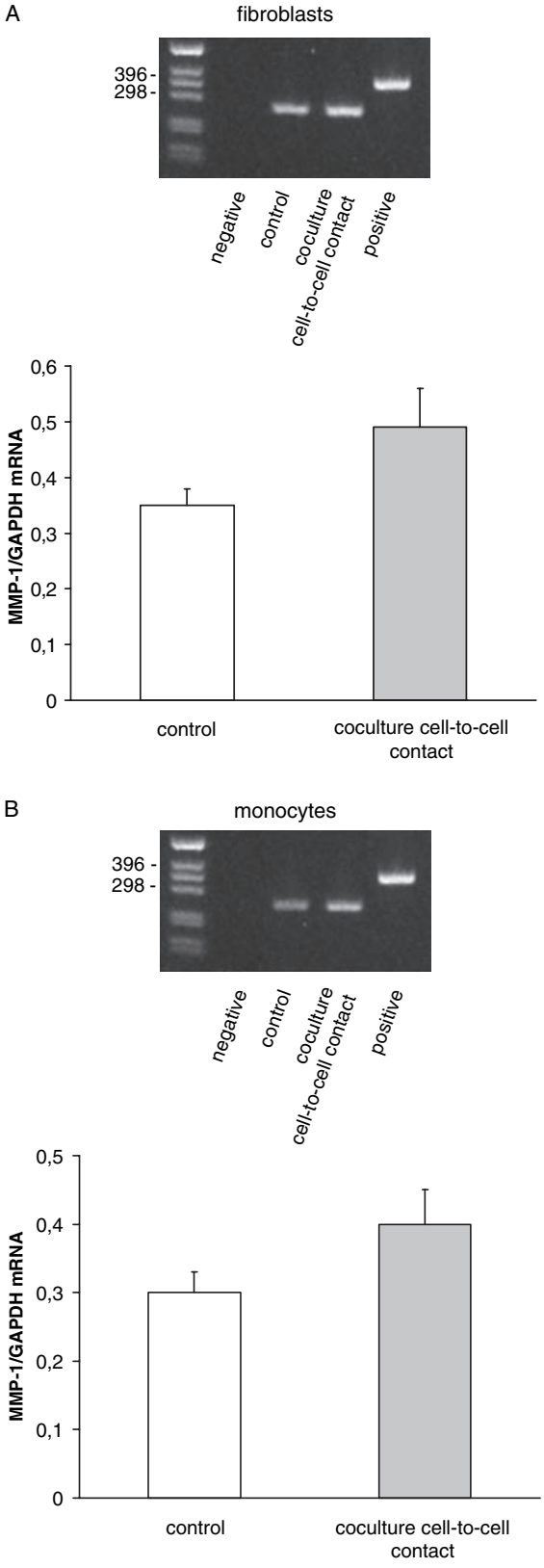

Fig. 3. The mRNA expression of matrix metalloproteinase-1 (MMP-1) in gingival fibroblasts (1×10^6) and monocytes (1×10^7), respectively, cultured alone or in cell-to-cell contact. (A) The mRNA expression in gingival fibroblasts cultured alone or in cell-to-cell contact with monocytes. (B) The mRNA expression in monocytes cultured alone or in cell-to-cell contact with fibroblasts. The intensity of the bands of MMP-1 mRNA, obtained from three experiments, is quantified and expressed relative to glyceraldehyde-3-phosphate dehydrogenase mRNA expression. Mean value of triplicates with SD shown by vertical bars.

FCS. After 24 h of incubation, the media were collected, cells were removed by centrifugation and the resultant supernatants were immediately added to the fibroblasts and incubated for 24 h.

Analysis of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in the cell-medium

Medium from the cultures was withdrawn and centrifuged to remove the monocytes. The supernatants were stored at −80°C before analysis of MMP-1 and TIMP-1. The levels of MMP-1 (total) and TIMP-1 in the medium were determined by enzyme-linked immunosorbent assay (ELISA) purchased from Amersham and R & D systems (Minneapolis, MN, USA), respectively. The detection limit and sensitivity for MMP-1 was 6.2 ng/ml and 1.7 ng/ml, respectively. The detection limit and sensitivity for TIMP-1 was 0.156 ng/ml and 0.08 ng/ml, respectively.

RNA isolation and reverse transcription–polymerase chain reaction

Gingival fibroblasts (1×10^6) were seeded in cell culture dishes (100 mm) (Corning Incorporated) in DMEM medium supplemented with 5% FCS and cultured for 24 h at 37°C. The cell layers were then rinsed three times with DMEM followed by addition of 5.0 ml DMEM containing monocytes (1×10^7). The fibroblasts and monocytes were cocultured either in mixed cocultures (13 ml) or in separated cocultures with a microporous membrane (0.4 μm) (Corning Incorporated) separating fibroblasts in the lower compartment (13 ml) from the monocytes on the upper compartment (9 ml).

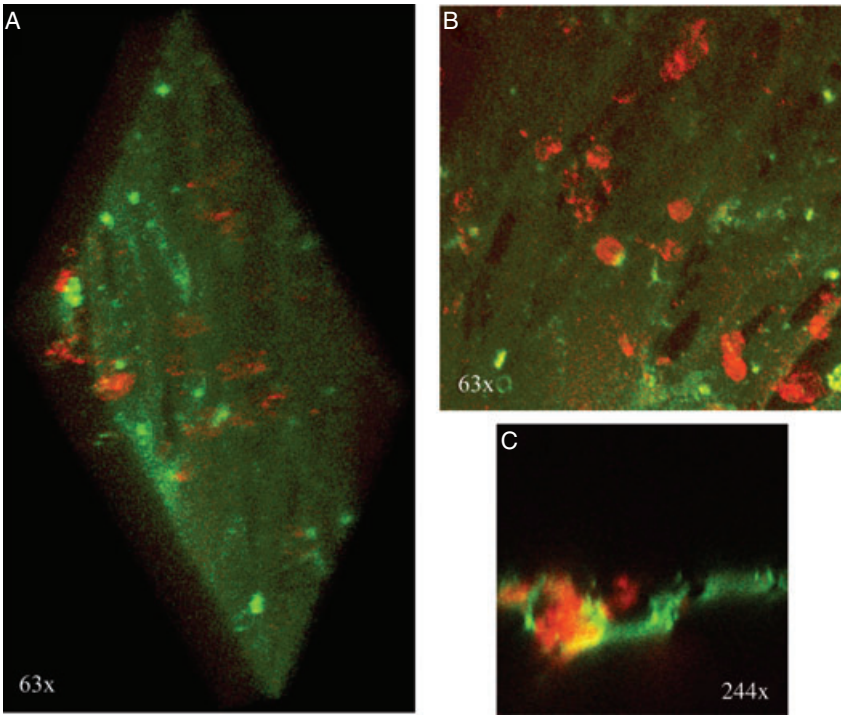

Fig. 4. Confocal laser scanning microscopy showing cell-to-cell contact between gingival fibroblasts (3×10^4) and monocytes (3×10^5) during coculture. The three dimensional arrangement of fibroblasts (green) and monocytes (red) demonstrates the contact between the cells where (A) shows the reconstituted three-dimensional picture in *x–y–z* plane, (B) shows the *y* plane and (C) shows the *z* plane.

After 16 h of incubation, the medium was removed and frozen. The cell monolayer of fibroblasts was washed twice with ice-cold PBS, frozen immediately in liquid nitrogen and stored at −80°C for isolation of total RNA. The monocytes in the cocultures were isolated with repeated washing with ice-cold PBS and by a short period of trypsination. Thereafter, the monocyte fraction was centrifuged and the pellet with monocytes was frozen immediately in liquid nitrogen and stored at −80°C for isolation of total RNA.

Total RNA from fibroblasts and monocytes was obtained by Qiagen RNA-isolating mini kit (Qiagen, Hilden, Germany) and quantified spectrophotometrically at 260/280 nm. To obtain first strand cDNA, 1.0 µg of total RNA was reverse transcribed using Superscript II (Invitrogen, Paisley, UK) in a total volume of 20 µl. The obtained cDNA (1 µl) was used in a final reaction volume of 50 µl for PCR amplification. The reactions were performed, using specific primers for MMP-1 and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (R & D systems) under the following conditions. Initial denaturation at 94°C for 4 min, followed by 30 cycles at 94°C, 55°C, 72°C for 45 s and finally an extension at 72°C for 10 min. For each experiment, PCR amplification was also performed without cDNA, negative control, as well as with positive control, indicated as negative and positive in the figures. A total of 10 µl of each PCR reaction product was run on 2% agarose gel containing ethidium bromide next to 1-kb DNA-ladder standard (Invitrogen). The relative amount of input RNA was analysed using GAPDH. The product sizes were 262 base pairs (bp) for MMP-1 and 576 bp for GAPDH. The product sizes for positive controls were 360 bp for MMP-1 and 340 bp for GAPDH. The intensity of the bands were quantified with Electrophoresis Documentation and Analysis System (EDAS) 290 (Eastman Kodak Company, Rochester, NY, USA).

Statistical analysis

Student's *t*-test was used in the statistical analysis. p-value < 0.05 was considered significant.

Results

Effect of cell-to-cell contact of fibroblasts and monocytes on the production of matrix metalloproteinase-1

The production of MMP−1 in interactions between human gingival fibroblasts and human monocytes was investigated in the media of cocultures. Gingival fibroblasts constitutively produced MMP-1, whereas no detectable levels of MMP-1 were found in the culture media of monocytes during the incubation period (4–24 h) (Fig. 1). Coculturing the fibroblasts and monocytes with cell-to-cell contact resulted in an enhanced production of MMP-1 in a time-dependent manner (Fig. 1). The MMP-1 production increased continuously during the incubation period (4–24 h) and was significant ($p < 0.001$) at 12 h (Fig. 1). Moreover, the production of MMP-1 correlated both to the number of fibroblasts (Fig. 2A) and to the number of monocytes (Fig. 2B) in 24 h of cocultures.

The mRNA expression of MMP-1 was studied in fibroblasts and monocytes, respectively, as well as in cocultures of fibroblasts and monocytes. RT–PCR results, relative to GAPDH mRNA expression, showed that both control fibroblasts (Fig. 3A) and control monocytes (Fig. 3B) constitutively express MMP-1 mRNA. When coculturing the fibroblasts in cell-to-cell contact with monocytes, the mRNA expression of MMP-1 increased ($p < 0.05$), both in gingival fibroblasts (approximately 1.3-fold) (Fig. 3A) and in monocytes (approximately 1.3-fold) (Fig. 3B).

To investigate whether gingival fibroblasts and monocytes were in cell-to-cell contact during coculture we used confocal laser scanning microscopy. The three-dimensional reconstitution of serial optical sections showed that fibroblasts and monocytes were in cell-to-cell contact (Fig. 4).

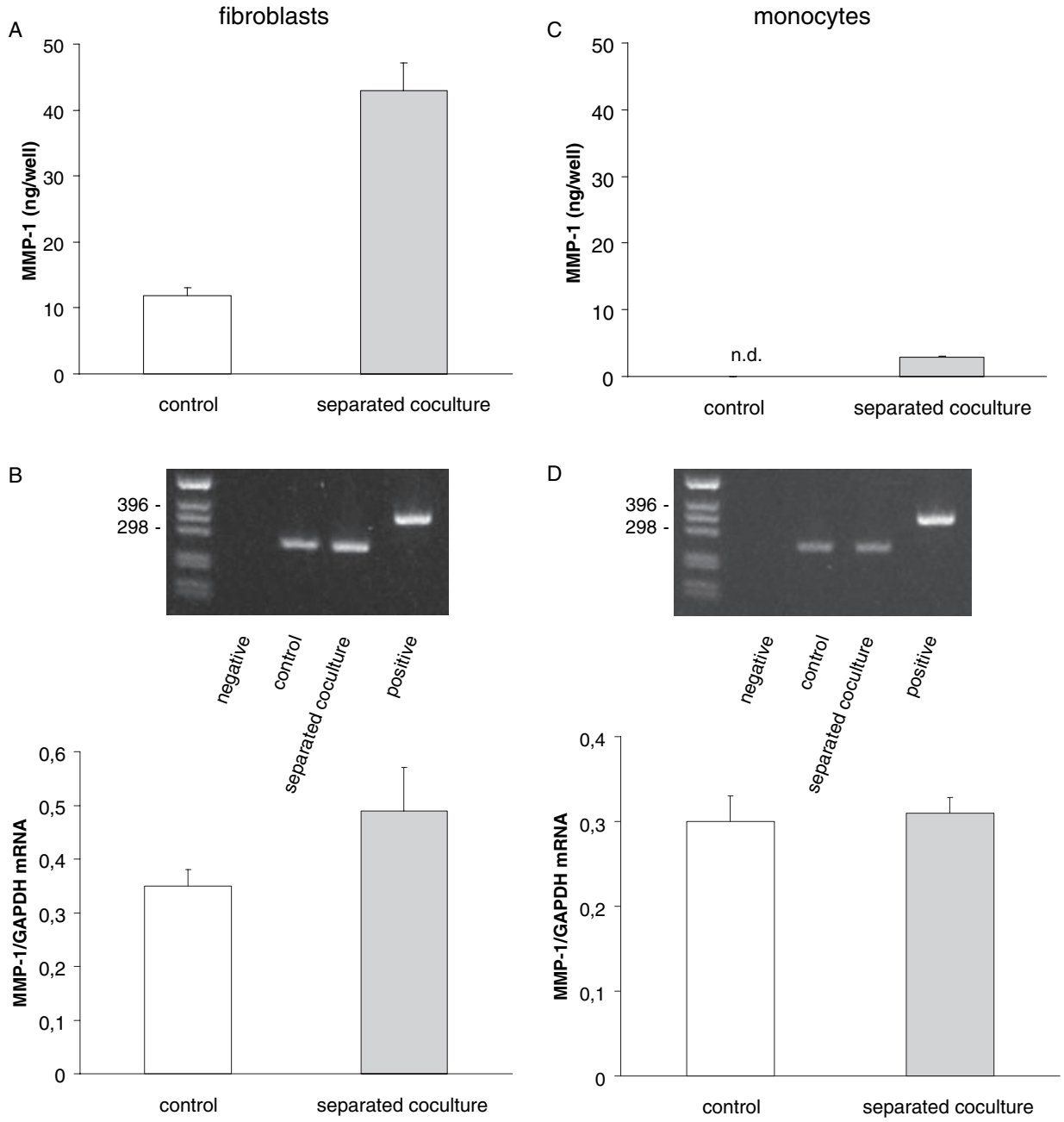

Fig. 5. The mRNA and protein expression of matrix metalloproteinase-1 (MMP-1) in fibroblasts and monocytes cultured alone or in separated cocultures. (A) The levels of MMP-1 in gingival fibroblasts (3×10^4) alone or in separated cocultures with monocytes (3×10^5). (B) The mRNA expression of MMP-1 in gingival fibroblasts (1×10^6) cultured alone or in separated coculture with monocytes (1×10^7). (C) The levels of MMP-1 in monocytes (3×10^5) alone or in separated cocultures with fibroblasts (3×10^4). (D) The mRNA expression of MMP-1 in monocytes (1×10^7) cultured alone or in separated coculture with fibroblasts (1×10^6). The levels of MMP-1, expressed in triplicates with SD, are related to the volumes in the transwell system as described in Material and methods. The intensity of the bands for MMP-1 mRNA, obtained from three experiments, is quantified and expressed relative to glyceraldehyde-3-phosphate dehydrogenase mRNA expression. Mean value of triplicates with SD shown by vertical bars.

Matrix metalloproteinase-1 production in separated coculture of fibroblasts and monocytes

Gingival fibroblasts were also cultured with monocytes in a transwell system with a microporous membrane (0.4 μM) placed between the cells, separating the monocytes in the upper compartment from the fibroblasts in the lower compartment, allowing only soluble mediators to pass, i.e. separated cocultures. The level of MMP-1 was enhanced ($p < 0.001$) in gingival fibroblasts (Fig. 5A) as well as in monocytes (Fig. 5C) in separated cocultures. The enhanced level of MMP-1 in gingival fibroblasts was also

Table 1. Effects of conditioned medium from monocytes, with different cell numbers on the production of matrix metalloproteinase-1 in gingival fibroblasts

	MMP-1 (ng/ml)	
	–	+
– conditioned medium	n.d.	34 ± 2
+ conditioned medium from monocytes (1×10^5)	n.d.	112 ± 11
+ conditioned medium from monocytes (3×10^5)	n.d.	174 ± 5

Conditioned medium was collected from 24 h culture of monocytes (1×10^5 and 3×10^5 cells) and immediately added to gingival fibroblasts (3×10^4) and cultured for 24 h. The levels of matrix metalloproteinase-1 (MMP-1) in the conditioned media from monocytes and in the media from fibroblasts were measured. MMP-1 was not detected (n.d.) in conditioned medium from monocytes. Mean value of triplicates with SD.

reflected by an increased ($p < 0.05$) MMP-1 mRNA expression (approximately 1.3-fold) (Fig. 5B). However, in contrast to gingival fibroblasts, the mRNA level of MMP-1 in monocytes was not enhanced (Fig. 5D).

Effect of conditioned medium from monocytes on the production of matrix metalloproteinase-1 in gingival fibroblasts

The effect of soluble factors from monocytes on the production of MMP-1 in gingival fibroblasts was studied using conditioned medium from monocytes. Notably, the production of MMP-1 significantly ($p < 0.001$) increased in gingival fibroblasts incubated with conditioned medium from monocytes (Table 1). The stimulatory effect of conditioned medium on MMP-1 production in fibroblasts increased with enhanced number of monocytes (1×10^5, 3×10^5). In contrast to fibroblasts, MMP-1 production in monocytes was not affected by conditioned medium from fibroblasts (data not shown).

Effect of interactions of fibroblasts and monocytes on the production of tissue inhibitor of metalloproteinase-1

The levels of TIMP-1, the endogenous inhibitor of MMP-1, were also determined after cell-to-cell contact as well as in separated cocultures of fibroblasts and monocytes. As shown in Fig. 6A, both fibroblasts and monocytes constitutively produced TIMP-1. Co-culturing the fibroblasts with monocytes in cell-to-cell contact resulted in an increased ($p < 0.001$) level of TIMP-1 as compared to the fibroblasts and monocytes alone (Fig. 6A). However, when fibroblasts were cultured with monocytes in separated cocultures, the level of TIMP-1 did not increase in the fibroblasts (Fig. 6B). In contrast to gingival fibroblasts, the level of TIMP-1 in the monocytes increased ($p < 0.001$) in separated cocultures (Fig. 6C).

Effect of dexamethasone and doxycycline on the production of matrix metalloproteinase-1

The effect of the glucocorticoid dexamethasone and the tetracycline doxycycline on MMP-1 production in cocultures of fibroblasts and monocytes with cell-to-cell contact was studied. Treatment of the cells with dexamethasone (1.0 μM) resulted in a reduction ($p < 0.001$) of MMP-1 levels in the cocultures (Fig. 7A). Furthermore, treatment of the cocultures with doxycycline (30 μM) also significantly ($p < 0.001$) reduced the increased MMP-1 levels (Fig. 7B).

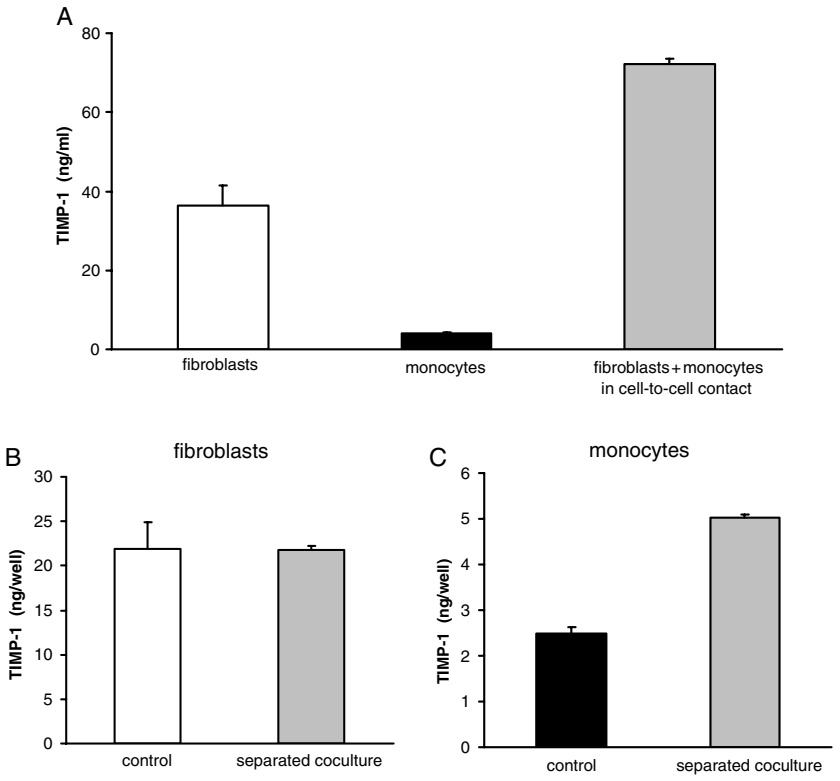

Fig. 6. The effect of cell interactions between fibroblasts (3×10^4) and monocytes (3×10^5) on tissue inhibitor of metalloproteinase-1 (TIMP-1) production. (A) The levels of TIMP-1 in fibroblasts and monocytes cultured alone or in coculture with cell-to-cell contact. (B) The levels of TIMP-1 in gingival fibroblasts alone or in separated cocultures with monocytes. (C) The levels of TIMP-1 in monocytes alone or in separated cocultures with fibroblasts. The levels of TIMP-1 in separated cocultures are related to the volumes in the transwell system as described in Material and methods. Mean value of triplicates with SD shown by vertical bars.

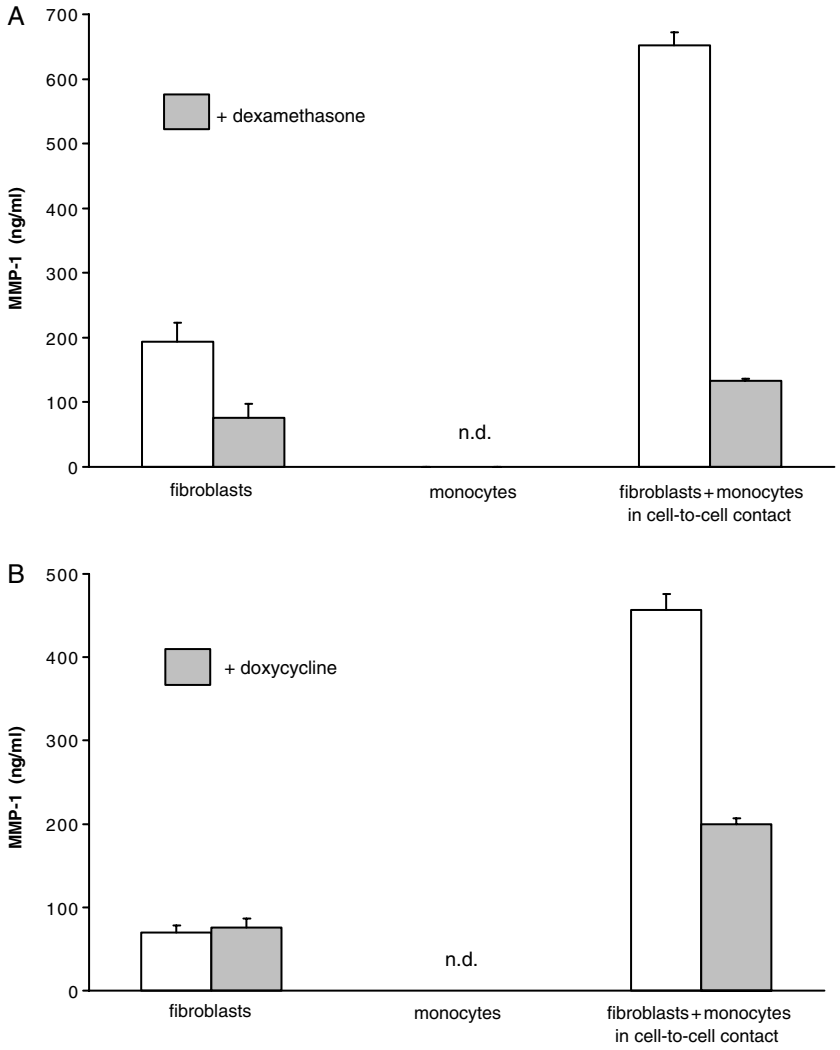

Fig. 7. The effect of the dexamethasone and doxycycline on the levels of matrix metalloproteinase-1 (MMP-1) in cocultures with cell-to-cell contact of fibroblasts (3×10^4) and monocytes (3×10^5). (A) The levels of MMP-1 in cocultures treated with dexamethasone (1.0 μM). (B) The levels of MMP-1 in cocultures treated with doxycycline (30 μM). MMP-1 was not detected (n.d.) in cultures of monocytes alone. Mean value of triplicates with SD shown by vertical bars.

Discussion

In this study we investigated the effect of cell interactions between gingival fibroblasts and monocytes on the production of MMP-1 in gingival fibroblasts using a coculture model. The fibroblasts were cultured in either cell-to-cell contact with monocytes or in separated cocultures using a microporous membrane to prevent cell-to-cell contact. The novel findings are that MMP-1 expression is enhanced in gingival fibroblasts by cell-to-cell contact with monocytes as well as by soluble factors from the monocytes. The enhanced expression of MMP-1 in the cocultures with cell-to-cell contact was reduced in the presence of dexamethasone and doxycycline.

To our knowledge, this is the first report demonstrating that cell interactions between human gingival fibroblasts and monocytes result in increased MMP-1 expression. The increased MMP-1 correlated to the number of both fibroblasts and monocytes, suggesting that both types of cells contribute to the increased MMP-1. Our finding that cell-to-cell contacts between fibroblasts and monocytes stimulate the mRNA expression of MMP-1 in gingival fibroblasts is compatible with the *in vivo* study demonstrating that gingival fibroblasts close to inflammatory sites express MMP-1 (23). Furthermore, our results are in line with *in vitro* findings demonstrating the stimulation of MMP-1 by cell-to-cell interactions between rabbit skin fibroblasts and bone marrow macrophages as well as between human fetal lung fibroblasts and monocytes (19, 20).

The increased MMP-1 production in cocultures with cell-to-cell contact may be mediated by soluble factors such as cytokines including IL-1β and TNFα (10, 15) or cell adhesion molecules such as intercellular adhesion molecule-1 or β3-integrin (24, 25). The influence of soluble factors on the stimulated MMP-1 production was investigated by culturing fibroblasts with monocytes in a transwell system to prevent direct cell contact. Our finding that the production of MMP-1 also increased in a transwell system indicates that the stimulatory effect of monocytes on MMP-1 production in fibroblasts may, in addition to cell-to-cell contact, also be due to soluble factors derived from monocytes. This assumption is supported by the fact that conditioned medium from monocytes stimulated the production of MMP-1 in gingival fibroblasts, which has previously also been demonstrated with conditioned medium from monocytes stimulated with lipopolysaccharide (26, 27). The involvement of soluble factors in the stimulation of MMP-1 in gingival fibroblast suggests that inflammatory mediators such as IL-1β and TNFα secreted from monocytes/macrophages in inflamed gingival tissue can stimulate the fibroblasts to produce MMP (28, 29). The importance of the monocyte/macrophage-derived cytokines IL-1β and TNFα on MMP-1 production has previously been reported in cocultures of smooth muscle cells and monocytes (30).

MMP-1 is regulated by its endogenous inhibitor TIMP-1 (16). Co-culturing the fibroblasts with monocytes in cell-to-cell contact increased the

levels of TIMP-1, which may be due to a feedback mechanism to compensate for the enhanced MMP-1. Interestingly, coculturing fibroblasts and monocytes in separated cocultures increased the levels of TIMP-1 in monocytes, but not in fibroblasts, suggesting that the stimulation of TIMP-1 in the cocultured fibroblasts are dependent on cell-to-cell contact. The fact that TIMP-1, in contrast to MMP-1, did not increase in gingival fibroblasts in separated cocultures may result in an imbalance between MMP-1 and its endogenous inhibitor TIMP-1, in favour of MMP-1, leading to collagen degradation and destruction of the gingival tissue. This is compatible with clinical findings demonstrating decreased TIMP-1 and increased MMP-1 levels in gingival tissue and gingival crevicular fluid from periodontitis affected patients (9). The imbalance between MMP-1 and TIMP-1 may also stimulate the infiltration of inflammatory cells such as monocytes to migrate from the vasculature into the tissue, which is a prerequisite for cell interactions. In the tissue, the monocytes differentiate into macrophages and notably interactions of fibroblasts and monocytes can stimulate the differentiation of monocytes into macrophages (31). In our coculture experiments, however, the incubation time (4–24 h) is not sufficient for differentiation of monocytes into macrophages, which is reported to require several days of incubation (32).

In gingival fibroblasts, we have previously shown that MMP-1 production is reduced by the glucocorticoid dexamethasone (10). We herein demonstrate that dexamethasone also reduces MMP-1 levels in cocultures of fibroblasts and monocytes with cell-to-cell contact. The regulatory effect of dexamethasone on MMP-1 may be explained by an inhibition of MMP-1 at the transcriptional level (5) or by an inhibition of cell-to-cell contact between fibroblasts and monocytes. Notably, dexamethasone has been reported to inhibit the adhesion of monocytes to TNFα activated synovial fibroblasts by inhibiting the induced surface expression of intercellular adhesion molecule-1 (33). However, further studies elucidating the mechanisms of the interactions of gingival fibroblasts and monocytes on the production of MMP-1 are required.

Finally, we also investigated the effect of the anti-inflammatory tetracycline doxycycline (34) on MMP-1 production, as doxycycline is used as an adjunct to conventional treatment of periodontitis (17, 35). Interestingly, we found that doxycycline reduced the enhanced MMP-1 levels in the cocultures with direct cell-to-cell contact, which may partly explain the beneficial clinical effects of doxycycline (35). The reducing effect of doxycycline on MMP-1 levels in the cocultures might be due to suppressed MMP-1 transcription, as previously reported in corneal epithelial cells (36).

Taken together, our *in vitro* findings demonstrate that the production of MMP-1 in gingival fibroblasts is stimulated by direct cell-to-cell contact with monocytes as well as by soluble factors derived from the monocytes. The interactions between gingival fibroblasts and monocytes may contribute to the maintenance of MMP-mediated tissue destruction in periodontitis.

Acknowledgements

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