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Elevated levels of gene expression for collagen and decorin in human gingival overgrowth

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***Abstract*:**
Objectives: It has been demonstrated that extracellular matrix molecules are involved in cyclosporine-induced gingival overgrowth (GO). However, for many of these molecules, it remains unclear whether their abundance is modulated on the protein and gene expression level.
Material and Methods: To contribute to this clarification, we have analysed the protein and mRNA expression of type-I collagen (COL1) and decorin (DC) in native specimens obtained from five patients with GO, and matched normal tissue using indirect immunofluorescence (IIM), in situ hybridization (ISH) and quantitative polymerase chain reaction (PCR).
Results: IIF revealed a largely co-localized although remarkably increased abundance for COL1 and DC in GO. This increase coincided with an up-regulated gene expression observed for both molecules, as detected by ISH and quantitative PCR.
Conclusions: Analysis of our data clearly demonstrates elevated levels for COL1 and DC and shows for the first time in native human tissue that involvement of these genes in GO is not confined to the protein level but also includes the transcriptional level.

Key words: cyclosporin; decorin; extracellular matrix; gingival overgrowth; human; in vivo; type- I collagen; vimentin

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Cyclosporine A (CsA) is an immune suppressant, and gingival overgrowth (GO) as one of the reported side-effects has a prevalence ranging from 2% to 70% (Margiotta et al. 1996). However, the precise molecular mechanisms underlying the development of GO have still not been elucidated. Moreover, this process seems to be multifactorial in nature involving genetics, plaque control and gingival inflammation, extent of periodontal destruction, dosage and duration of the administered drug, cyclosporine blood concentration, age of the patient, and the underlying medical condition (Seymour & Jacobs 1992, Somacarrera et al. 1994). Histopathologically, GO is characterized by (i) thickening of the epithelium with prominent rete pegs (Mariani et al. 2004, Spolidorio et al. 2004), (ii) enlargement of the connective tissue with an accumulation of fibroblasts, and (iii) an increase in collagen fibril bundles and amorphous ground substance (Deliliers et al. 1986, Mariani et al. 1993). In addition, tissue specimens of patients with CsA-induced GO display an enormous infiltration of plasma cells (Deliliers et al. 1986, Mariani et al. 1993) and an enhanced vascularization (Wondimu et al 1995, Ayanoglou & Lesty 1999).

The gingival connective tissue consists of a dense network of type-I collagen (COL1) fibril bundles that provide firmness to the gingiva and attach the gingiva to the tooth and alveolar bone (Bartold 2000). COL1 is also one of the major extracellular matrix ligands that regulates functions of the connective tissue cells (White et al. 2004). Gingiva has a fast turnover rate of collagen, allowing it to adapt to changing functional demands (Sodek & Ferrier 1988). Experimental investigations indicate interstitial collagen (COL1) as the main target of CsA, and an altered COL1 turnover seems to be responsible for this accumulation (Bolzani et al. 2000, Kataoka et al. 2000, Hyland et al. 2003). Two complementary mechanisms appear to contribute to the accumulation of this matrix molecule. These involve a reduction of the levels or activities of matrix-degrading enzymes or elevation of matrix production. However, there is conflicting evidence

concerning extracellular collagen fibrils and the non-collagenous proteins, which have been shown in various in vitro studies to increase (Zebrowski et al. 1994), decrease or remain unchanged (Tipton et al. 1991).

Decorin (DC) belongs to the family of small leucine-rich proteoglycans (SLRPs), and previous studies have shown that it is expressed in human gingiva (Bartold 2000), and the periodontal ligament (Hakkinen et al. 2000). The major functions of DC in gingiva are the regulation of kinetics of COL1 fibrillogenesis and the diameter and the distance of collagen fibrils (Vogel et al. 1984, Hakkinen et al. 2000). Furthermore, DC is involved in the control of cell adhesion and migration (Winnemoller et al. 1992). Given the multiple functions of DC, it may play an important role in gingival homeostasis.

Vimentin (VIM) is an intermediate filament that is constitutively expressed by mesenchymal cells including gingival fibroblasts in normal tissues. As an intermediate filament, VIM is a major component of the cytoskeleton enhancing structural integrity, cell shape, and organelle motility (Wang & Stamenovic 2002).

Although COL1 appears to be essentially involved in CsA-induced GO, it still remains unclear whether changes in the abundance of COL1 are restricted to the protein level or also include the gene expression level. For this purpose, we have analysed the status of COL1 in conjunction with the COL1-associated proteoglycan DC in specimens of GO and normal tissue on both the protein and gene expression level. As the intermediate filament VIM is not known to be affected by CsA, it was used as a control molecule displaying similar gene expression in mesenchymal cells of normal and GO tissue.

Material and Methods

Patients

For tissue harvest, informed consent was obtained from the patients according to the Helsinki Declaration, and the protocol was approved by the institutional ethic committee. Five CsA-treated renal transplant patients (ages 29–61 years), four female and one male, who attended the Department of Operative Dentistry and Periodontology (University of Heidelberg, Germany) were selected for the study. Specimens were taken from the inter-dental area, where the tissue revealed characteristic signs of GO. Normal gingival tissue was obtained from five healthy patients (ages 18–55 years), four female and one male, who followed coronally advanced flap procedure aiming to cover recession defects (three patients) or during implant placement and implant uncovering (one patient in each case). No relevant pre-existing medical or drug histories were cited during the last 6 months, and the tissue was free from plaque-induced inflammation or enlargement.

Tissue preparation and indirect immunofluorescence

Indirect immunofluorescence (IIF) was performed on serial cryostat sections (10 μm) of normal gingiva and CsA-induced overgrown gingival tissue. In brief, after air drying, sections were fixed in 80% methanol and in acetone (5 min. each, 4°C), and incubated with primary antibodies overnight at 4°C following the protocols described previously (Tomakidi et al. 2003). Mouse monoclonal antibodies (mabs) against DC were purchased from R&D Systems (115402; R&D Systems Inc., Minneapolis, MN, USA) and against VIM from Monosan (VI-RE/1; Monosan®, Uden, the Netherlands). Primary rabbit polyclonal antibody against COL1 was obtained from Biodesign (N/A Catalog # T59103R; Biodesign International, Saco, ME, USA). All antibodies were adjusted to their final working concentration in PBT (phosphate-buffered saline (PBS) containing 0.5% BSA, 0.5% Tween-20, 0.02% NaN_3; anti- COL1 1:100; anti-DC 1:50; and anti-VIM 1:100). For IIF, samples were washed three times in PBS for 5 min and incubated with secondary fluorochrome-conjugated antibodies (1:100; Alexa Fluor®, MoBiTec GmbH, Göttingen, Germany) for 1 h at room temperature (RT). To allow total nuclei staining, propidium iodide (Sigma-Aldrich GmbH, München, Germany; 10 μg/ml) was added to secondary antibody. Specimens were embedded in mounting medium (Vectashield, Vector Laboratories Inc., Burlingame, CA, USA), and documented by a confocal laser scan microscope (Leica TCS/NTCLSM microscope, Leica Microsystems Inc., Bannockburn, IL, USA). To test the specificity of immunostaining, negative controls were run without primary antibodies. These specimens revealed no staining reaction (figures are not shown). The relative immunofluorescence intensity was scored by two experienced examiners (B. D. and P. T.), who were blinded to the status of specimens, using an arbitrary four-scale model (– represented no immunoreactivity; +++ represented the highest protein abundance).

Labelling of RNA probes and in situ hybridization (ISH)

After harvesting, gingival biopsies were immediately stored in RNA stabilization solution (RNAlater, Ambion, TX, USA) and the expression of COL1A1, and DC mRNA in tissue derived from normal gingiva and GO was assessed by ISH with a digoxigenin (DIG)-labelled sense/antisense riboprobe. The technique was adapted from the simplified ISH protocol developed by Braissant & Wahli (1998) with DIG-labelled riboprobes to detect abundant and rare mRNA on tissue sections.

DNA templates were prepared from total RNA derived from human periodontal ligament cells (PDL) by reverse transcription polymerase chain reaction (RT-PCR). PCR primers for COL1 (COL1A1), DC, and VIM contained the sequence of the SP6 (GATTTAGGTGACACTATAG) or T7 (TAATACGACTCACTATA) promotor and the corresponding downstream target sequences (see Table 1). Riboprobes were labelled with DIG by in vitro transcription with SP6 or T7 polymerase and 4 μg DNA templates according to the manufacturer's protocol (Roche Diagnostics, Mannheim, Germany). Cryosections of the specimens were cut into 10 μm thickness, fixed in phosphate-buffered 4% paraformaldehyde and 0.5% glutaraldehyde for 10 min at RT, digested with proteinase K (1 μg/ml) at 37°C for 30 min, and post-fixed for with 4% phosphate-buffered paraformaldehyde for 5 min. at 4°C. The general precautions to prevent RNase contamination of samples and reagents, including baking of all glassware for 8 h at 180°C and DEPC treatment of all aqueous solutions, were taken as recommended previously. The hybridization experiment and the colorimetric detection were performed according to the manufacturer's protocol (Non-Radioactive In Situ Hybridization Application Manual and DIG-Detection kit; Roche Diagnostics). For detection of hybridization, sections were incubated with blocking buffer containing anti-DIG

Table 1. Oligonucleotide primers for quantitative real-time PCR and in situ hybridization

Genes	Primer sequences	Experiment	Amplicon size (bp)
Type-I collagen (COL1A1)	Forward: 5′-CGGAGGAGAGTCAGGAAGG-3′	qPCR	156
	Reverse: 5′-ACATCAAGACAAGAACGAGGTAG-3′		
	Sp6 forward: 5′-*GATTTAGGTGACACTATAG* GGAAACAGACAAGCAACCCAAACT-3′	ISH	142
	Sp6 reverse: 5′-*CATACGATTTAGGTGACACTATAG* GGTCATGTTCGGTTGGTCAAAGATAA-3′		
Decorin	Forward: 5′-TCTCCTACATCCGCATTG-3′	qPCR	335
	Reverse: 5′-GAAGTCACTTGATCC-3′		
	Sp6 forward: 5′-*CATACGATTTAGGTGACACTATAG* TCTCCTACATCCGCATTG-3′	ISH	335
	Sp6 reverse: 5′-*CATACGATTTAGGTGACACTATAG* GCAGAAGTCACTTGATCC-3′		
Vimentin	Forward: 5′-TTTTTCCAGCAAGTATCCAACC-3′	qPCR	59
	Reverse: 5′-GTTTTCCAAAGATTTATTGAA-3′		
	Sp6 forward: 5′-*CGATTTAGGTGACACTATAG* AGAACTTTGCCGTTGAAGC-3′	ISH	170
	T7 reverse: 5′-*CTAATTAATACGACTCACTATA* TCCAGCAGCAGCTTGTAGGT-3′		
β-actin	Forward: 5′-AGAGCTACGAGCTGCCTGAC-3′	qPCR	459
	Reverse: 5′-AAAGCCATGCCAATCTCATC-3′		

Promotor sequences for Sp6-/T7 polymerase are underlined.
qPCR, quantitative real-time PCR analysis; ISH, in situ hybridization.

conjugated with alkaline phosphatase (1:200, 150 mU/ml) for 2 h at RT. After the colour reaction, the sections were counterstained with 0.02% fast green FCF (Aldrich Chemical Corp., Milwaukee, WI, USA) for 1–2 min. Sections were mounted in Glycerin (glucerol-PBS 1:1) and documented by light microscopy. The relative intensity was scored by two experienced examiners (B. D. and T. S.), who were blinded to the status of the specimens, using an arbitrary four-scale model (− represented no mRNA transcripts; +++ represented the highest abundance of mRNA transcripts).

RNA extraction and quantitative real-time PCR analysis (qPCR)

Total RNA was extracted from sections of deep-frozen tissue using the RNeasy mini kit (Qiagen Inc., Valencia, CA, USA). Sufficient material was available for four patients with GO and normal gingival, respectively. RNA concentration was determined by absorbance at 260 nm. First-strand cDNA was synthesized from 1 μg total RNA aliquot in a reaction mixture containing random hexamer primer by performing the RevertAid™ reverse transcription protocol (#K1622, Fermentas Inc., Hanover, MD, USA). cDNA concentration was determined by fluorometry using a fluorescent dye (PicoGreen; Molecular Probes, Invitrogen, Karlsruhe, Germany) and adjusted to 5 ng/μl. PCR analysis was performed with the iCycler real-time PCR-Detection System (BioRad Laboratories, Philadelphia, PA, USA) according to the manufacturer's instructions. The qPCR amplification reactions were conducted with a reaction mixture containing 25 μl SYBR green PCR mastermix (iQ SYBR Green Supermix; BioRad Laboratories), 1 μl of template cDNA (5 ng), an appropriate amount of paired primers (300nM for COL1A1, 150 nM for DC, 300 nM for VIM, and 300 nM for β-actin) in a final volume of 50 μl. The standard temperature profile included initial denaturation at 94°C for 3 min., followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 52–57°C (primer-dependent) for 30 s, and extension at 72°C for 40 s. The results of the qPCR were analysed as relative expression levels of CsA-affected tissue in relation to normal gingiva. The relative expression levels of each mRNA were analysed using a modification of the $\Delta\Delta C_T$ equation, which allows counting for differences in efficiencies ($E = 10^{-1/\text{slope}}$) between the PCR reactions (Livak & Schmittgen 2001). The data were calculated using the software Gene Expression Macro provided with the iCycler. The data were normalized to the C_T of the housekeeping gene (HKG) β-actin.

Statistical Analysis

The arbitrary scores were translated into numbers (− = 0; + = 1, ++ = 2, and +++ = 3). For descriptive analysis of the data, the median and range of scores for both examiners and each aspect were calculated. The relative expression levels were subjected to Student's *t*-test; *p*-values less than 0.01 were considered statistically significant.

Results

IIF discriminates normal from CsA-induced gingival overgrown tissue

To screen for differences in the protein expression levels of the extracellular matrix (ECM) constituents COL1 and DC, we analysed tissue specimens from normal gingiva and CsA-induced GO by IIF. Generally, no apparent discrepancies in histoarchitecture, i.e. structure and thickness of the epithelium, were visible between the normal gingiva and GO. This analogy in the epithelial phenotype was substantiated by propidium iodide (PI) total nuclei staining (Fig. 1a–f). Moreover, no specific fluorescence signals for COL1 (Fig. 1a, b), DC (Fig. 1c, d), and VIM (Fig. 1e, f) were noted in the epithelial compartment (Table 2).

In normal tissue, IIF revealed a homogeneous distribution of COL1 in the gingival connective tissue, with a slight emphasis on the papillary projections (Fig. 1a, Table 2). In marked contrast, COL1 exhibited a very intense staining in CsA-affected tissue (Fig. 1b, Table 2). This became visible in the entire connective tissue and was most pronounced at the epithelium-lamina propria interface (Fig. 1b, b_1, Table 2). The topography of DC staining showed largely a co-localization with COL1 in the connective tissue of both normal gingiva (Fig. 1c, Table 2) and CsA-induced GO (Fig 1d, Table 2). Interestingly, as already seen for COL1, DC also displayed an elevated presence in the connective tissue of GO (Fig. 1d, Table 2). Similar to COL1, DC showed its highest abundance in the papillary

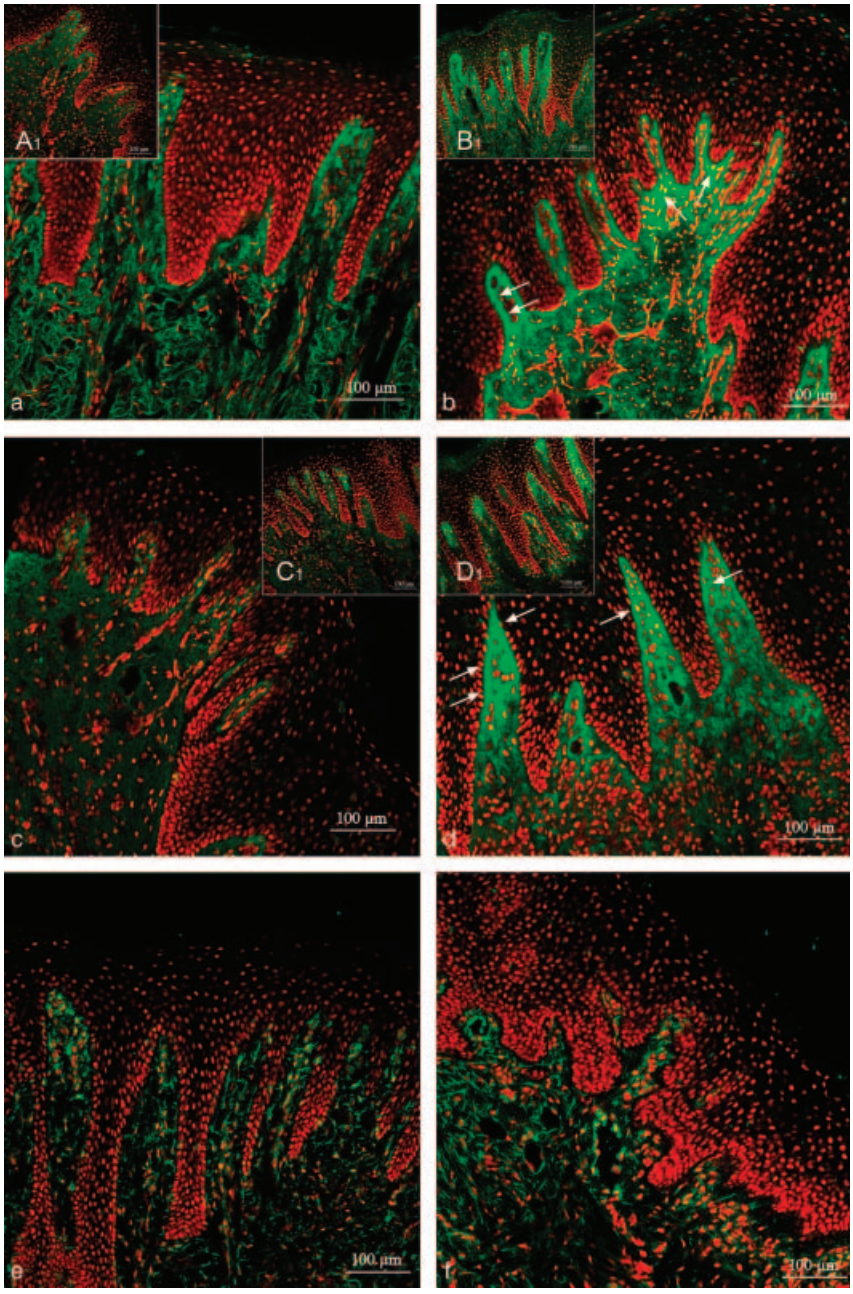

Fig. 1. Detection of protein expression by IIF in human gingival frozen sections of normal tissue (left panel) and of CsA-induced GO (right panel) of type-l collagen (COL1) (top, **A**, **A1**-**B**, **B1**), decorin (middle, **C, C1-D, D1**) and vimentin (bottom, **E-F**). Tissue compartments are indicated epithelium (EP) and connective tissue (CT). Arrows indicate the papillary connective tissue; bars represent 100 µm.

connective tissue adjacent to the epithelium (Fig. 1d, d_1, Table 2).

This coinciding pattern detected for both ECM components in GO tissue suggests that CsA is associated with the increased protein amount detected for these molecules.

To analyse whether this immunosuppressive drug affects the expression of structural components of connective tissue cells such as fibroblasts, we investigated the status of VIM, a component of the intermediate filament of mesenchymal cells.

In both the normal and pathologically affected tissues, VIM apparently showed no difference in its amount and distribution (Fig. 1e,f, Table 2).

ISH indicates elevated mRNA levels of COL1 and DC in GO

To assess whether the increase in abundance of protein observed for COL1 and DC coincides with an increased gene expression, we investigated the transcription level of these molecules on frozen sections of four tissues derived from different CsA-treated patients and matched normal tissue byISH. Compared with normal tissue (Fig. 2a), ISH demonstrates that the gene expression for COL1 was clearly elevated in GO (Fig. 2b, Table 2). While in GO the transcripts were preferentially seen in the papillary junctions between the epithelium and the connective tissue (Fig. 2b, Table 2), COL1 gene expression was uniform in the normal connective tissue (Fig. 2a, Table 2). For DC, we could show a similar signal distribution in GO in the connective tissue and there again especially concentrated in the papillary region (Fig. 2d, Table 2), whereas in control gingival tissue sections an evenly distributed mRNA signal has been observed (Fig. 2c, Table 2). For each molecule under study, hybridization with the sense riboprobes yielded a faint background signal, exemplified in Fig. 2e. The coinciding pattern seen for COL1 and DC on the gene expression and on the protein level strongly suggests that the high abundance detected for both proteins in GO is correlated with an increase in their gene expression. Concerning VIM, for which we expected in analogy to IIF an unchanged situation, ISH revealed equal levels of transcripts with homogeneous distribution in the connective tissue of normal gingiva and GO (data not shown).

Quantitative analysis of the mRNA expression

To confirm the results from ISH, the gene expression levels for COL1, DC, and VIM were analysed by quantitative real-time PCR. Table 2 shows the relative gene expression levels calculated for COL1, DC, and VIM in GO and matched normal gingival tissue. This calculation demonstrated a significant increase in the gene expression for COL1 (2.57 ± 0.517, $p < 0.01$) and DC (1.36 ± 0.112, $p < 0.01$), whereas VIM remained almost unchanged.

Discussion

In GO, a disturbance in connective tissue homeostasis has been observed, leading to an accumulation of ECM components, in particular COL1 (Spolidorio et al. 2002). For this purpose, we

Table 2. Relative expression of type-I collagen, decorin, and vimentin in human gingiva detected by IIF and ISH and relative gene expression analysed by qPCR

Molecule	Tissue area in normal gingiva			Tissue area in gingival overgrowth		
	epithelial compartment	papillary connective tissue	deep connective tissue	epithelial compartment	papillary connective tissue	deep connective tissue
IIF						
Type-I collagen	–	++	++	–	+++	++
	(0)	(2.1; 1–3)	(2; 1–3)	(0)	(2.8; 2–3)	(2.3; 2–3)
Decorin	–	+	–	–	+++	+
	(0)	(1.1; 1–2)	(0.3; 0–1)	(0)	(2.8; 2–3)	(1.2; 1–2)
Vimentin	–	+	+	–	+	+
	(0)	(1.1; 1–2)	(1.2; 1–2)	(0)	(1.2; 1–2)	(1.3; 1–2)
ISH						
Type-I collagen	–	++	++	–	+++	++
	(0)	(1.8; 1–2)	(1.6; 1–2)	(0)	(2.7; 2–3)	(2.2; 2–3)
Decorin	–	+	–	–	++	+
	(0)	(0.8; 0–1)	(0.2; 0–1)	(0)	(1.6; 1–2)	(0.8; 0–2)
Vimentin	–	+	+	–	+	+
	(0)	(0.8; 0–1)	(0.9; 02)	(0)	(0.7; 0–1)	(0.9; 0–2)
Quantitative real-time PCR						
Molecule (reaction efficiency)	Normal gingival relative expression ± SEM			Gingival overgrowth relative expression ± SEM		
Type-I collagen (82.9%)	1 ± 0.247			2.57 ± 0.517*		
Decorin (85.2%)	1 ± 0.241			1.36 ± 0.112*		
Vimentin (95.5%)	1 ± 0.363			1.01 ± 0.321		

Relative expression of type-I collagen, decorin, and vimentin in IIF and ISH was scored on an arbitrary scale:
+++/3, strong; ++/2, intermediate variable; +/1, low; –/0, no. The listed results are the median scores and the range of scores of both independent expert examiners for all specimens.
Expression of type-I collagen, decorin, and vimentin mRNA was examined by quantitative real-time PCR. Values were normalized with the expression level of β-actin and denoted as mRNA expression relative to normal samples. Mean ± SEM, $n = 4$,
*$p < 0.01$.
qPCR, quantitative real-time PCR analysis; ISH, in situ hybridisation; IIF, indirect immunofluorescence.

have investigated the status of COL1 and the COL1-associated proteoglycan DC on the protein and gene expression level in human specimens of gingival overgrown and matched normal gingival tissue.

Our results from IIF analysis demonstrate a clear increase in the abundance of COL1 in GO, particularly seen in the papillary projections compared with normal gingiva. This increase in COL1 is in agreement with the results obtained from animal models and human tissue (Spolidorio et al. 2002, Paik et al. 2004). Therefore, our data suggest an increased synthesis of COL1 in the gingival connective tissue in patients having CsA-induced GO.

Numerous studies have examined the effect of CsA on the expression of COL1 on protein as well as on the mRNA level, but the results are conflicting. In cultures of gingival fibroblasts, an up-regulation of COL1 gene expression and protein level was demonstrated upon CsA-treatment (Gagliano et al. 2004). On the other hand, no change in the steady-state level of mRNAs for collagen was noted in CsA-affected gingiva in rats. However, most studies evaluating the mRNA expression level of COL1 were carried out in cultured fibroblasts or tissue samples obtained from animals and the results are not fully applicable to the in vivo situation in humans.

To evaluate whether the excessive amount of COL1 protein in GO is assigned to an increased gene expression, we analysed the synthesis of mRNA for COL1 and DC qualitatively and quantitatively.

ISH revealed clearly elevated levels of COL1 transcripts, and quantitative real-time PCR corroborated their significance ($p < 0.01$). These results obtained from the gene expression studies indicate that the high abundance of COL1 in GO is caused by an increased expression, including both the protein and gene expression level. In addition to the increased expression, another factor contributing to the high collagen abundance in GO is the reduction of components responsible for ECM turn-over.

This appears possible as we have observed decreased mRNA levels for the collagenolytic MMPs (MMP-1, 13) and increased levels for TIMP-1 in CsA-treated gingival fibroblasts derived from GO (unpublished data from real-time PCR). Thus, a combination of an up-regulation of ECM synthesis and down-regulation of ECM turnover appears to contribute to the phenotypic ECM-pattern, characteristic for GO. This hypothesis is backed up by studies demonstrating a down-regulation of MMP-1, -2, and -3 in CsA-treated rats and gingival cells (Bolzani et al. 2000, Kataoka et al. 2000, Hyland et al. 2003, Martelli-Junior et al. 2003).

Development and differentiation of dental and periodontal tissues are characterized by spatially and temporally regulated deposition of different extracellular matrix components, including proteoglycans (Alimohamad et al.

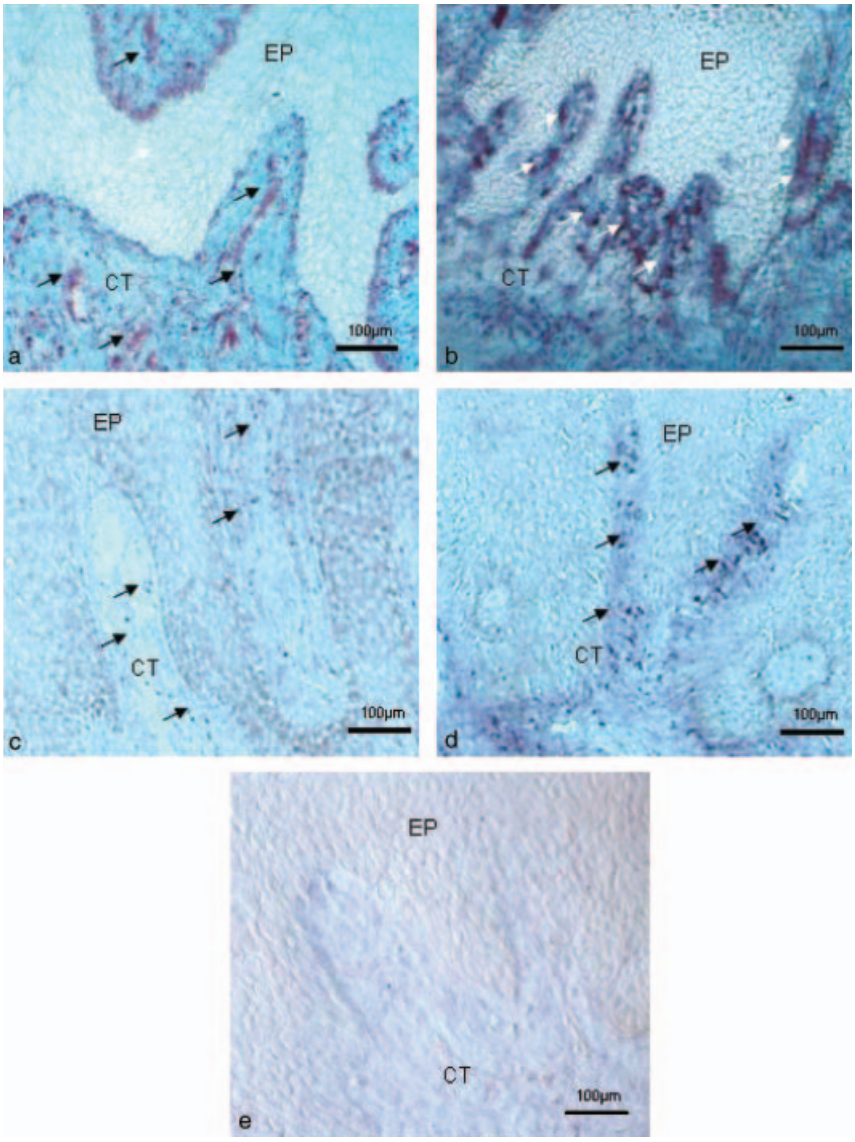

Fig. 2. Non-radioactive *in situ* hybridization (ISH) on frozen sections of tissue from patients with CsA-induced GO and control patients (without CsA treatment). ISH was performed with antisense riboprobes from human PDL cDNA. Tissue compartments are indicated epithelium (EP) and connective tissue (CT). **B.** The signal for COL1 in GO sections showed an increased mRNA transcription level with a mainly papillary distribution in the gingival connective tissue, compared with control tissue **(A). D.** The hybridization signal for DC showed a similar increased mRNA transcription, again concentrated in the papillary region (arrows), whereas in a normal tissue section, a much more homogeneously distrubuted DC mRNA signal was detected **(C).** For negative control the respective sense riboprobe has been used and showed only signals on background level (for example DC sense riboprobe, **E**); bars represent 100 µm.

2005). In the present study, we found co-localization of enhanced protein expression detected by IIF for COL1 with DC in the connective papillary tissue of CsA-induced GO compared with normal gingiva. Although we have not analysed the status of DC degradation, we could determine this enhanced protein abundance as elevated expression of DC. Similar to those of COL1, the significantly ($p<0.01$) increased levels of DC gene expression were confirmed qualitatively by ISH and quantitatively by real-time PCR (Table 2). The accordance in the up-regulation of COL1 and DC in GO may be due to the function of DC in fibril assembly of collagen molecules, including COL1 (Zimmerman et al. 2001). Another reason for the high levels of COL1 seen in GO may be the ability of DC to inhibit internalization of COL1, thereby affecting collagen phagocytosis (Bhide et al. 2005). Irrespective of the status of ECM turnover, the anti-phagocytotic, protective ability of DC may be considered as a further aspect for the observed excessive abundance of COL1 in GO.

Our data strongly suggest that VIM, representing the intermediate filament of fibroblasts, is not affected in CsA-induced GO. This observation is in line with the findings elaborated in CsA-treated human gingival fibroblasts that demonstrated unchanged protein patterns (Stabellini et al. 1991).

In summary, the work described here provides in vivo evidence of a causal correlation between the excessive protein abundance of COL1 and DC and the significant up-regulation of their gene expression in CsA-induced GO. This observation is of interest, as in addition to a putative disturbance of ECM turnover, an increase in gene and protein expression of these ECM constituents is involved in matrix alterations typical for this disease.

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Clinical Relevance

Scientific rationale for this study: In GO, the turnover of the extracellular matrix components including COL1 is disturbed. However, it remains unclear whether the detected accumulation of COL1 is caused by an elevated synthesis.

Principal finding: The elevated protein levels of COL1 and its associated proteoglycan DC in GO result from an enhanced synthesis as proven by an increase in the mRNA expression for both molecules.

Practical implication: COL1 and DC are associated with the pathogenesis of GO. Characterization of molecules regulating their turnover may help to generate perspective therapeutical strategies in molecular terms.