The Effect of Interferon-γ and Tumor Necrosis Factor-α on COL1A2 Promoter Activity Elevated by Interleukin-4 in Human Skin Fibroblasts Cultures

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Background: **IL-4 is known as a potent stimulating factor and, TNF-α and IFN-γ suppress collagen synthesis of dermal fibroblasts. However, relatively little is known about interaction of these cytokines.**

Objective : **The purpose of this study was to evaluate the interactional effect of TNF-α and INF-γ for the type I collagen gene expression and promoter activation produced by IL-4.**

Methods : Using dermal fibroblasts from the normal skin cultured with three cytokines, IL-4, TNF-α and IFN-γ, we examined Northern blot analysis with each cDNA and assayed CAT activity with human pro α 2(I)collagen(COL1A2)/CAT reporter gene chimeric construct.

Results : **Compared to the control, treating with IL-4 resulted in prominent elevation of both type I procollagen mRNA levels and proα 2(I) collagen promoter activity. IL-4 with TNF-α suppressed the IL-4 induced elevations, whereas IL-4 with IFN-γ did not reveal the obvious suppression of the elevations produced by IL-4. Fibroblasts treated with IL-4 together with IFN-γ and TNF-α completely abolished the type I procollagen gene expression and the activation of COL1A2 promoter gene elicited by IL-4.**

Conclusion : **IL-4 induced enhancement of type I collagen gene expression was synergistically suppressed by TNF-α and IFN-γ on transcriptional level.(Ann Dermatol 9:(2) 108~115, 1997).**

Key Words : IL-4, TNF-α, IFN-γ, Type I collagen, Promoter activity

Fibroblasts are responsible for the production and maintenance of the connective tissue matrix of all organs including the dermis of the skin. Cultured fibroblasts derived from the early phases of traumatic wound tissue or from sites of pathological fibrosis, display activated phenotypes characterized by increased production of the connective tissue matrix components: collagen and fibronectin and retain their activated phenotypes for many generations *in vitro*[1-3]. Following tissue injury, some cytokines make the fibroblasts undergo a metabolic activation and exhibit increased growth and synthetic rates, or inhibit fibroblast growth and collagen production required to effect fibrotic repair of injured tissue.

A number of cytokines have been shown to modulate the growth and migration of connective tissue derived cells and the synthesis of extracellular matrix components, such as collagens[4]. Interleukin-4(IL-4) is a potent stimulating factor of collagen synthesis by fibroblasts especially in the initiation of the fibrotic process[5]. In contrast, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) suppress collagen synthesis[6-8]. Short-term

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exposure of cultured scleroderma fibroblasts to IFNs results in suppression of their abnormally elevated collagen production and in induction of a reduced/normalized-collagen-producing phenotype which is retained in the absence of IFN's[7-9] When interferons bind to specific cell surface receptors, there are changes in steady-state levels of multiple mRNAs[10]. The role of TNF-α, IFN-γ and IL-4 in the pathogenesis of the fibrotic condition and cultured fibroblast has been elucidated[11-12], but relatively little is known about modulation of the collagen gene expression and interaction of these cytokines. A synergistic action of IFN-α with TNF-γ has been shown for tumoricidal[13] and antiviral activity[14]. Several studies have provided evidence that activation of type I collagen gene expression by fibroblasts plays a crucial role in the development of dermal fibrosis in diseases, such as scleroderma, eosinophilic fasciitis, and keloids[1,15-16].

To investigate the mechanism by which cytokines affect remodeling processes, the potential interaction of IL-4 with IFN-γ and/or TNF-α on modulating type I collagen gene expression was studied using Nothern blot analysis, and type I collagen promoter gene activation was studied also by transfection experiments and chloramphenicol acetyltransferase (CAT) assay in cultured human skin fibroblasts. Human pro α 2(I)collagen/CAT reporter gene chimeric construct used in this study can mimic physiological expression of the gene when transiently expressed in human fibroblastic cells[17].

MATERIALS AND METHODS

Fibroblasts culture and treatment of cytokines

For the collection of fibroblasts, skin specimens were taken from the clinically normal skin of identical anatomical sites on three normal subjects of unrelated cosmetic surgery. Normal human skin fibroblasts were cultured to confluency and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL Co., Grand Island, NY,USA) with 10% fetal bovine serum(FBS), streptomycin(100 μg/ml) and amphotericin B(1 μg/ml) in the humid atmosphere of 37℃ and 5% CO_2, and subcultured after trypsinization. Cultured fibroblasts were utilized in passages 3-6. The trypan blue test was used for the checking of cell viability. Confluent fibroblasts in 100 mm diameter petri-dishes were treated with cytokines for 48 hr in the absence of FBS. The following cytokines and concentrations were used for this study : 10 ng/ml of IFN-γ (Genzyme Co., Cambridge, MA, USA), 1μg/ml of TNF-α(Sigma Co., St.Lous, MO, USA), 5 ng/ml of IL-4 (Genzyme Co., Cambridge, MA, USA). Most experiments were performed in triplicate and repeated twice.

cDNA probe preparation

The following human-sequence-specific cDNAs were used for Northern hybridizations: for type I collagen, a 1.8-kb proα 1(I) cDNA ; for fibronectin, a 1.3-kb cDNA ; for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1.2-kb cDNA. The cDNAs were labelled with [^{32}P]-dCTP (NEG 036H, New England Nuclear, Boston, MA, USA) by nick translation[22] to a specific activity of approximately 1×10^8 counts/min/μg.

RNA isolation and Northern blot hybridization

Total RNA was isolated from cultured normal human skin fibroblasts by the methods of Chomczynski and Sacchi[19]. The RNA was subjected to Northern blot hybridization analysis as described. Briefly, total RNA was fractionated by 1% agarose gel electrophoresis (50 volt, 5 hr) after denaturing the samples with formaldehyde and formamide[20]. RNA transcripts obtained were transferred to the nitrocellulose filter (Trans-Blot, BioRad, Herculis, CA, USA) in 20 x SSC at 4℃ for 12 hr. After drying the filter at room temperature, RNA was immobilized by heating at 78℃ for 90 min under vacuum. The filter was prehybridized for 12-18 hours at 42℃ with prehybridization mixture (50% formamide, 0.1% sodium dodecylsulfate(SDS), 3x SSC, 1x Denhart's solution, 50μg/ml salmon sperm DNA(ss-DNA)) and hybridized with [^{32}P]-labeled cDNAs by nick translation at 42℃ for 24 to 36 hr. After hybridization, washing and autoradiography were performed.

Transfection experiments and CAT assay

The construct containing the 3.5-kb COL1A2 promoter fused to the CAT gene (pMS-3.5/CAT) was derived from a 3.5-kb EcoRI/SphI genomic subclone that spans from position -3,500 to +58 of the COL1A2 promoter[21]. Cells were trypsinized for 24 hr prior to transfection and seeded at a density

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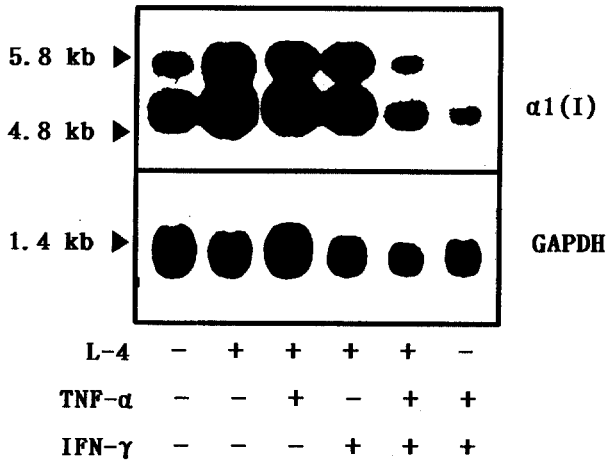

Fig.1. Northern blot analysis of type I collagen mRNA transcripts from human skin fibroblasts cultures incubated for 48 hr in medium supplemented with IL-4(5 ng/ml), TNF- (10 ng/ml) and/or IFN-γ(1μg/ml). Abbreviations: IL-4, interleukin-4; TNF-α, tumor necrosis factor-α ; IFN-γ, interferon-γ ; α 1(I), proα 1(I) collagen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

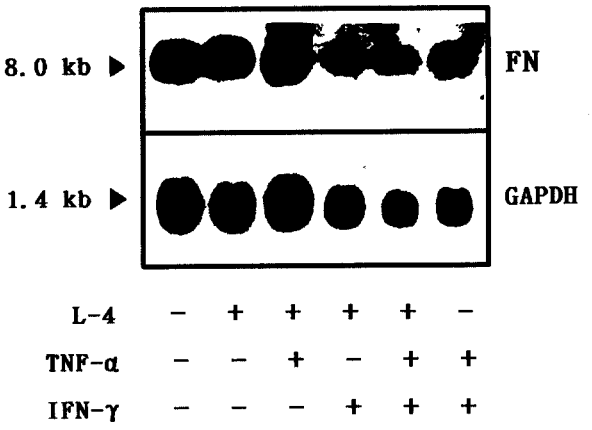

Fig. 2. Northern blot analysis of fibronectin(FN) mRNA transcripts from human skin fibroblasts cultures incubated for 48 hr in medium supplemented with IL-4(5 ng/ml), TNF- (10 ng/ml) and/or IFN-γ(1μg/ml). Abbreviations were described in fig. 1.

Table 1. Type I collagen and fibronectin(FN) mRNA abundance in human skin fibroblasts treated with IL-4 alone or in combination with TNF-α and/or IFN-γ

	Type I collagen/GAPDH	FN / GAPDH
Control	24.0±1.8 (1.00)	0.92±0.11 (1.00)
IL-4	67.8±2.1 (2.81)	0.97±0.09 (1.05)
IL-4 + TNF - α	35.8±3.9 (1.48)	0.71±0.11 (0.77)
IL-4 + IFN - γ	54.5±4.8 (2.26)	0.86±0.10 (0.94)
IL-4 + TNF - α + IFN-γ	20.3±1.9 (0.85)	0.75±0.15 (0.82)
IFN-γ + TNF - α	17.5±2.2 (0.73)	0.68±0.13 (0.74)

The values are mean SD from six separate experiments. Abbreviations were described in fig. 1. Values in the parentheses indicate fold induction.

of 1-3 × 10^5 cells/60 mm dish for the calcium chloride technique. The next day, cells were transfected with 20μg of reporter and 3.5μg of ß-galactosidase DNA by the calcium-chloride / DNA co-precipitation method, followed by a 1 min of glycerol(15%) shock[22]. After 48 hr incubation with or without 10 ng/ml of IFN-γ, 1μg/ml of TNF-α and 5 ng/ml of IL-4, the cells were harvested and lysed by three cycles of freeze-thawing in 100μl of 0.25 M Tris-HCl, pH 7.8. The protein concentration of each extract was used for determination of CAT activity using acetyl CoA and [^{14}C] chloramphenicol as substrate[23]. The acetylated and non acetylated forms of radioactive chloramphenicol were separated by thin layer chromatograp-hy(TLC) and visualized by autoradiography. The enzyme activity was quantified by cutting out pieces of TLC plates containing different forms of [^{14}C] chloramphenicol converted to its acetylated forms. Each piece of data was corrected by ß-galactosidase activity. The values were expressed as the percentage of the [^{14}C] chloramphenicol converted to its acetylated forms per 10μg of cell extraction protein.

RESULTS

Northern blot analysis of proα1(I) collagen and fibronectin mRNA

For Northern blot analysis of cultured normal skin fibroblasts with or without cytokines, ^{32}P la-

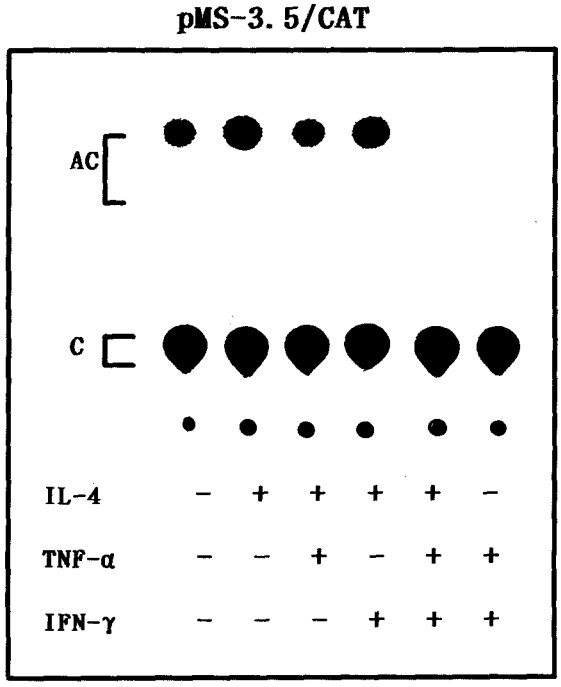

Fig. 3. Assay of COL1A2 promoter(pMS-3.5/CAT) activity in human skin fibroblasts cultured with IL-4 (5 ng/ml), TNF-α(10 ng/ml), and/or IFN-γ(1μg/ml). The figure shows CAT assay depicting the separation of acetylated (AC) and unacetylated (C) forms of [^{14}C]chloramphenicol by thin layer chromatography. Abbreviations were described in fig. 1.

beled Proα1(I) collagen, fibronectin, and GAPDH cDNA probes were hybridized with each mRNAs specifically. Proα1(I) collagen revealed two mRNA transcripts, the sizes of which were 5.8-kb and 4.8-kb(Fig.1). Fibronectin and GAPDH revealed one transcript each, and their sizes were 8.0-kb and 1.4-kb, respectively(Fig.1 & 2). There was no change of their sizes, which meant no alteration of quality. For the quantitative assay of proα1(I) collagen mRNA steady-state level, a densitometer was used.

Effects of IL-4 on the steady-state accumulation of type I collagen and fibronectin mRNA

To examine the effect of IL-4 on the endogeneous expression of type I collagen and fibronectin gene, the quantities of proα1(I) collagen and fibronectin mRNA were evaluated. Incubation of cultured human skin fibroblasts with IL-4(5 ng/ml) alone for a period of 48 hr resulted in a marked increase in the steady-state levels of type I collagen mRNA, which was the highest increase, 2.8-fold higher than the untreated control cells. This increase was selective in that the steady-state levels of fibronectin mRNA for GAPDH were not altered by IL-4(Table 1).

Effects of TNF-α and IFN-γ on type I collagen and fibronectin mRNA

Incubation with IL-4 together with TNF-α showed 1.5-fold increased proα1(I) collagen mRNA levels compared to the control, which showed suppressed levels by 47% compared to incubation with IL-4 alone. Incubation with IL-4 together with IFN-γ showed 2.3-fold increased proα1(I) collagen mRNA levels compared to the control, which showed suppressed levels by 19.6% compared to incubation with IL-4 alone. Incubation with IFN-γ together with TNF-α showed a suppressed mRNA production level, which was 73% of the control level. Co-treatment with all these three cytokines which completely abolished the expression of proα1(I) collagen gene increased by IL-4, revealed marked suppression to the level of 85% of

Table 2. Effects of TNF-α(10 ng/ml) and/or IFN-γ(1μg/ml) in combination with IL-4(5 ng/ml) on COL1A2 promoter activity in human skin fibroblasts

	CAT activity	Fold induction
Control	2.1±0.3	1.00
IL-4	5.2±0.6	2.48
IL-4 + TNF - α	2.8±0.2	1.33
IL-4 + IFN - γ	4.8±0.5	2.28
IL-4 + TNF - α + IFN-γ	0.8±0.3	0.38
IFN-γ + TNF - α	0.7±0.4	0.33

The values are mean SD from six separate experiments. Abbreviations were described in fig. 1. Values in the parentheses indicate fold induction.

the untreated control culture. Those increased by IL-4 and other decreases in fibronectin mRNA were not as sensitive as in type I collagen(Table 1).

Evidence for transcriptional regulation of the type I collagen gene at the promoter level

Fibroblasts were transfected with construct and cytokines were added to the culture media for 48 hr after glycerol shock. As the result of CAT assay, the percentage of acetylation were 2.1% in the untreated control, 5.2% in 5 ng/ml of IL-4, 2.8% in IL-4 with 10 ng/ml of TNF-α, 4.8% in IL-4 with 1μg/ml of IFN-γ, 0.8% in IL-4 with other two cytokines, and 0.7% in IFN-γ with TNF-α (Fig. 3). Compared to the control group, each promoter activity was increased by 2.5-fold, 1.3-fold, 2.3-fold, 0.4-fold and 0.3-fold in fibroblasts treated with IL-4 alone, IL-4 with TNF-α, IL-4 with IFN-γ, IL-4 with other two cytokines, and IFN-γ with TNF-α, respectively(Table 2).

DISCUSSION

Regulation of collagen production is an important step in biological situation such as tissue development, homeostasis, and wound healing. Collagen biosynthesis and collagen accumulation appears to result from increased production of collagen on a per-cell basis, and not from decreased turnover of the deposited collagen[1,2].

The activation of fibroblast collagen production has been found to be associated with increased steady-state cellular levels of proα1(I) and proα1(III) collagen mRNA, suggesting activation of type I and type III procollagen gene expressions at the pretranslational or transcriptional level[24-26]. But the precise mechanisms leading to activation of collagen gene expressions are unknown so far. The role of cytokines from tissue-infiltrated inflammatory cells in the pathophysiology of fibrosis has been suggested[27]. Mononuclear cells, predominantly lymphocytes and macrophages, secrete soluble factors capable of stimulating collagen synthesis by fibroblasts, suggesting that cytokines may stimulate collagen gene expression of fibroblasts[25,28].

A number of recently identified cytokines have shown significant influences on cell proliferation and collagen synthesis. The effect of IL-4 on fibroblast functions may be a "fibrogenic cytokine" that could be important in promoting biogenesis of extracellular matrix proteins in normal wound healing and in pathological fibrosis in which mast cells and T lymphocytes play a central role[29]. Elevation of steady-state levels of type I procollagen and fibronectin mRNAs in IL-4 treated cells suggests that this cytokine regulates extracellular matrix biogenesis by pretranslational mechanisms. IL-4 induced dose-dependent increase of collagen production, but non-collagen protein synthesis was not significantly altered, and a concomitatant increased steady-state level of proα1(I) collagen mRNAs was observed, showing that IL-4 acts at a pre-translational level[5,30]. Increased mRNAs may correspond either to an increased gene transcription or to an increased stability of the mRNAs. Incubation of cells transfected with proα2(I)collagen/CAT reporter gene chimeric construct with IL-4 resulted in marked up-regulation of the promoter activity in this study and other experiment[15].

The interactions of IL-4 with fibroblasts could be modulated by other cytokines produced by inflammatory cells, for instance IFN-γ or TNF-α, which inhibits collagen synthesis[8]. In this study, incubation of human skin fibroblast with IL-4(5 ng/ml) alone produced highest promoter activity, 2.5-fold higher increase than untreated control cells. Compared to the control, the steady-state levels of proα1(I) collagen mRNA showed a marked increase by 2.8 fold induction, but GAPDH were not affected. These results suggest that the expression of type I collagen gene can be up-regulated by IL-4 at the transcriptional level. Other two lymphokine factors, TNF-α and IFN-γ, have been shown to suppress collagen gene expression in cell cultures[6]. The decreased steady state levels of procollagenα(I) mRNA coupled with the decreased procollagen α1(I) gene transcriptional activity indicates that TNF-α inhibits collagen synthesis at the transcriptional or pretranslational level[6,31].

According to Kähäri et al.[15], the exposure of human skin fibroblasts transfected with pMS-3.5/CAT to human recombinant TNF-α resulted in inhibition of the proα2(I) collagen promoter activity, while TNF-α did not have a significant effect on the expression of the cells transfected with pSV2CAT. This observation suggested that TNF-α exerts its inhibitory effects on type I collagen gene expression at the transcriptional level. Inhibition of collagen production caused by TNF was

dose and time-dependent[6]. In this study, TNF-α had a more potent suppressive effect on the gene expression and CAT activity for the collagen gene than IFN-γ. On the effect of TNF-α on gene expression of fibronectin, normal dermal fibroblasts demonstrated decreased levels of fibronectin mRNA production. The effects of INF-γ on human skin fibroblasts has demonstrated the decreased mRNA levels in type I collagen and fibronectin, and reduction of collagen accumulation in experimental fibrosis[32]. It is interesting how these cytokines affect the promoter activity and whether these cytokines modulate the *cis*-acting DNA sequences controlling the transcription of the type I collagen genes or not.

In this study, the modulation of type I procollagen promoter activity was examined by a variety of cytokines, including IL-4, TNF-α, and IFN-γ. The approach was made possible by previous studies which established that the human proα2(I) collagen/CAT reporter gene chimeric construct is able to mimic physiological expression of the gene when transiently expressed in human fibroblastic cells[17]. This construct contains -3.5 kb of 5' flanking DNA from human proα2(I) collagen gene, cloned in front of the structural gene for CAT. Incubation of cells transfected with this chimeric construct with IL-4 resulted in marked up-regulation of the promoter activity in cultured normal human skin fibroblasts, which was approximately 2.5-fold increase compared to the control. The increase or decrease noted in the promoter activity was in parallel with proα1(I) collagen mRNA levels according to combination of cytokines. Therefore, the increase noted in the type I procollagen mRNA steady-state levels appeared to reflect primarily activation of transcription for the gene[33].

Both TNF-α and IFN-γ showed counteraction to the IL-4-induced enhancement of type I procollagen gene expression. However, the mechanism of inhibition is known to be different. According to the observation of Kähäri et al[15], TNF-α appeared to affect type I procollagen gene expression on the transcriptional level by suppressing the pro α2(I)collagen promoter activity, while IFN-γ appeared to exert its effect primarily through destabilization of the mRNA and had only a minimal effect at the transcriptional level. This study showed that IL-4 with IFN-γ did not apparently decrease the IL-4 induced CAT activity, suggesting that IFN-γ has the minimal effect on the transcriptional level. Treating with IL-4 alone showed the highest promoter activity, but the remarkable decreased activity, 38% of the control, was revealed when TNF-α and IFN-γ were added to IL-4. The activation of proα2(I) promoter gene produced by IL-4 was completely abolished by treating with IL-4 together with IFN-γ and TNF-α. The synergistic suppressive effect of TNF-α and IFN-γ on proα2(I)collagen promoter activity can be explained by the previous report which IFN-γ could increase the number of TNF-α receptors without causing a significant change in the apparent binding affinity in cultured cells, thus making these cells more susceptible to TNF-α modulation[34]. IFN-γ leads to direct stimulation of TNF-receptor synthesis, possibly due to increased transcription of its mRNA. But the mRNA for collagenase was increased by TNF-α and no synergism with IFN-γ was obvious[35].

To understand how collagen transcription is selectively controlled under the physiological conditions, a number of studies have recently focused on the identification and characterization of *cis*-acting DNA regulatory elements in mammalian genes. Collagen expression is known to be regulated by unique arrangement of complex networks of interacting sequences which reside both downstream and upstream of the transcription initiation sites[36]. The synergistic inhibition of collagen gene expression induced by TNF-α in combination with IFN-γ is already known in other systems[13,14]. The effects of TNF-α and IFN-γ are also synergistic and potentiated the specific reduction of collagen synthesis. A better understanding of synergistic effects of cytokines might not only shed light on the pathogenetic event in fibrosis or wound healing defects, but also open new concepts for therapeutic approaches. This result suggests that combination of cytokines would provide a potential approach for a more efficient pharmacological suppression or enhancement of collagen formation than afforded by either one of the peptide factors alone.

REFERENCES

1. Uitto J, Bauer EA, Eisen AZ: Scleroderma: Increased biosynthesis of triple-helical type I and type III procollagens associated with unaltered expression of collagenase by skin fibroblast in culture. J Clin Invest 64: 921-930,1979.

2. Fleischmajer R, Perlish JS, Krieg T, et al: Variability in collagen and fibronectin synthesis by scleroderma fibroblast in primary culture. J Invest Dermatol 76: 400-403, 1981.
3. Berman B, Duncan MR: Short-term keloid treatment in vivo with human interferon-alpha2b results in a selective and persistent normalization of keloidal fibroblast collagen, glycosaminoglycan and collagen production in vitro. J Am Acad Dermatol 81: 694-706, 1989.
4. Roberts AB, Sporn MB, Assoian RK, et al: Transforming growth factor type : rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA 83: 4167-4171, 1986.
5. Gillery P, Fertin C, Nicolas JF, et al: Interleukin-4 stimulates collagen gene expression in human fibroblast monolayer cultures. Potential role in fibrosis. FEBS Lett 302: 231-234, 1992.
6. Solis-Herruzo JA, Brenner DA, Chojkier M: Tumor necrosis factor inhibits collagen gene transcription and collagen synthesis in cultured human fibroblasts. J Biol Chem 263: 5841-5845, 1988.
7. Duncan MR, Berman B: Persistence of a reduced collagen-producing phenotype in cultured scleroderma fibroblasrts after short-term exposure to interferons. J Clin Invest 79: 1318-1324, 1987.
8. Jimenez SA, Freundlich B, Rosenbloom J: Selective inhibition of diploid fibroblast collagen synthesis by interferons. J Clin Invest 74: 1112-1116, 1984.
9. Rosenbloom J, Feldman G, Freundlich B, et al: Inhibition of exessive scleroderma fibroblast collagen synthesis by recombinant gamma interferon. Arthritis Rheum 29: 851-856, 1986.
10. Czaja MJ, Weiner FR, Eghbali M, et al: Differential effects of γ-interferon on collagen and fibronectin gene expression. J Biol Chem 262: 13348-13351, 1987.
11. Lee KS, Ro YJ, Ryoo YW, et al: Regulation of interleukin-4 on collagen gene expression by systemic sclerosis fibroblasts in culture. J Der Sci 12: 110-117, 1996.
12. Fertin C, Nicholas JF, Gillery P: Interleukin-4 stimulates collagen synthesis by normal and scleroderma fibroblasts in dermal equivalents. Cell Mol Biol 37: 823-829,1991.
13. Price G, Brenner MK, Prentice HG, et al: Cytotoxic effects of tumor necrosis factor and gamma-interferon on acute myeloid leukaemia blasts. Br J Cancer 55: 287-290, 1987.
14. Wong GH, Goeddel DV: Tumor necrosis factors alpha inhibit virus replication and synergize with interferons. Nature 323: 819-822, 1986.
15. Kähäri V-M, Heino J, Niskanen L, et al: Eosinophilic fascitis: Increased collagen production and type I procollagen mRNA levels in fibroblasts cultured from involved skin. Arch Dermatol 126: 613-617, 1990.
16. Abergel PR, Pizzurro D, Meeker CA, et al: Biochemical composition of the connective tissue in keloids and analysis of collagen metabolism in keloid fibroblast cultures. J Invest Dermatol 84: 384-390, 1985.
17. Boast S, Su M-W, Ramirez F, et al: Functional analysis of *cis*-acting DNA sequences controlling transcription of the human type I collagen genes. J Biol Chem 265: 13351-13356, 1990.
18. Rigby PWJ, Dieckmann M, Rhodes C, et al: Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113: 237-251, 1977.
19. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium, thiocyanate-phenolchloroform extraction. Anal Biochem 162: 156-159, 1987.
20. Wahl GM, Stern M, Stark GR: Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci USA 76(8): 3683-3687,1979.
21. Dickson LA, de Wet W, Di Liberto M, et al: Analysis of the promoter region and the N-propeptide domain of the human proα2(I) collagen gene. Nuc Acids Res 13: 3427-3438, 1985.
22. Graham FL, Van Der Eb AJ: A new technique for the assay of infectivity of human adenovirus 5 DNA. Virol 52: 456-467, 1973.
23. Gorman CM, Moffat LF, Howard BH: Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol 2: 1044-1051, 1982.
24. Kähäri V-M, Chen YQ, Su MW, et al: Tumor necrosis factor-α and interferon-γ suppress the activation of human type I collagen gene expression by transforming growth factor-ß1. Evidence for two distinct mechanisms of inhibition at the transcriptional and posttranscriptional levels. J Clin Invest 86: 1489-1495, 1990.
25. Scharffetter K, Lankat-Buttgereit B, Krieg T: Localization of collagen mRNA in normal and scleroder-

ma skin by in situ hybridization. Eur J Clin Invest 18: 9-17, 1988.
26. Vuorio T, Mäkelä JK, Kähäri V-M, et al : Coordinated regulation of type I and type III collagen production and mRNA levels of proα1(I) and proα2(I) collagen in cultured morphea fibroblasts. Arch Dermatol Res 279: 154-160,1987.
27. Fleischmajer R, Perlish JS, Duncan M: Scleroderma:a model for fibrosis. Arch Dermatol 119: 957-961, 1983.
28. Wahl SM, Wahl LM, McCarthy JB: Lymphocyte-mediated activation of fibroblast proliferation and collagen production. J Immunol 121: 942-946, 1978.
29. Postlethwaite AE, Holness MA, Katai H, et al: Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin-4. J Cli Invest 90: 1470-1485, 1992.
30. Kim TH, Kim SW, Kwon HJ, et al: Effects of interleukin-4 on extracellular matrix gene expression in normal skin fibroblasts cultures. Annal Dermatol 8: 90-97, 1996.
31. Lee KS: Regulation of the collagen gene expression. Kor J Dermatol 33: 1005-1013, 1995.
32. Granstein RD, Murphy GF, Margolis RJ, et al: Gamma- interferon inhibits collagen synthesis in vivo in the mouse. J Clin Invest 79: 1254-1758, 1987.
33. Rossi P, Karsenty G, Roberts AB, et al: A nuclear factor 1 binding site mediates the transcriptionl activation of a type I collagen promoter by transforming growth factor-ß. Cell 52: 405-414, 1988.
34. Tsujimoto M, Vilcek J: Tumor necrosis factor receptors in HeLa cells and their regulation by interferon-γ. J Biol Chem 261: 5384-5388, 1986.
35. Scharffetter K, Heckmann M, Hatamochi A, et al: Synergistic effect of tumor necrosis factor-α and interferon-γ on collagen synthesis of human skin fibroblasts in vitro. Exp Cell Res 181: 409-419, 1989.
36. Schmidt A, Rossi P, DeCrombrugghe B: Transcriptional control of the mouse α2(I) collagen gene: Functional deletion analysis of the promotor and evidence for cell specific expression. Mol Cell Biol 6(2): 347-354, 1986.