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Effect of Interleukin-10 on the Gene Expression of Type I Collagen, Fibronectin, and Decorin in Human Skin Fibroblasts: Differential Regulation by Transforming Growth Factor-β and Monocyte Chemoattractant Protein-1

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Interleukin-10 (IL-10) is a cytokine with many regulatory functions. In particular, IL-10 exerts neutralizing effect on other cytokines, and therefore IL-10 is thought to have important therapeutic implications. Recent reports suggest that IL-10 regulates not only immunocytes but also collagen and collagenase gene expression in fibroblasts. In this study, we investigated the effect of IL-10 on gene expression of extracellular matrix (ECM) proteins, such as type I collagen, fibronectin, and decorin, in human skin fibroblasts. Results of Northern blot analysis showed that both collagen I and fibronectin mRNAs were downregulated, while decorin gene expression was enhanced by IL-10 (10 ng/ml) time-dependently (6–24 h). α1(I) collagen and fibronectin mRNAs were decreased to one-third and one-fourth, respectively, by 50 ng/ml IL-10, whereas decorin mRNA was increased up to 2.7-fold by 50 ng/ml IL-10. Response to IL-10 by scleroderma fibroblasts was similar to that in normal dermal fibroblasts, with decreased expression levels of collagen and fibronectin and induced decorin mRNA levels. Transforming growth factor-β (TGF-β) is a crucial fibrogenic cytokine which upregulates the mRNA expression of collagen and fibronectin, whereas it downregulates decorin mRNA expression in fibroblasts. Monocyte chemoattractant protein-1 (MCP-1) has recently been shown to upregulate the type I collagen mRNA expression in cultured fibroblasts. We therefore examined whether IL-10 alters gene expression of ECM elicited by TGF-β and MCP-1. Our results demonstrated that IL-10 downregulated the TGF-β-elicited increase of mRNA expression of type I collagen and fibronectin, while partially recovering TGF-β-elicited decrease of decorin expression in normal skin fibroblasts. By contrast, IL-10 did not alter the MCP-1-elicited upregulation of mRNA expression of either α1(I) collagen and decorin. Our data indicate that IL-10 differentially regulates TGF-β and MCP-1 in the modulation of ECM proteins and therefore suggest that IL-10 plays a role in the regulation of tissue remodeling.

Key Words: **interleukin-10; extracellular matrix; transforming growth factor-β; monocyte chemoattractant protein-1; scleroderma.**

Systemic sclerosis (SSc) is characterized by excessive production and deposition of extracellular matrix (ECM), including collagen, fibronectin, or proteoglycans, in the skin, as well as various internal organs (1, 2). Although the pathogenesis of SSc remains still unknown, it is suggested that a number of cytokines released from inflammatory infiltrates in the dermis during the early phase play an important role in the induction of SSc. In particular, transforming growth factor-β (TGF-β), a multifunctional cytokine, is supposed to play a central role in tissue fibrosis. TGF-β is a strong chemoattractant for fibroblasts (3), and it increases the synthesis of collagen or fibronectin by many cell types *in vitro* (4–6). In addition, TGF-β modulates cell-matrix adhesion protein receptors (7, 8). TGF-β also regulates the production of proteins that can modify the ECM by proteolytic action, such as plasminogen activator, an inhibitor of plasminogen, or procollagenase (9–11). TGF-β is capable of stimulating its own synthesis by fibroblasts through autoinduction (12). Thus, maintenance of increased TGF-β production may

Abbreviations used: TGF-β, transforming growth factor-β; MCP-1, monocyte chemoattractant protein-1; IL-10, interleukin-10.

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lead to the progressive deposition of ECM, resulting in fibrosis.

Interleukin-10 (IL-10) is a cytokine that was initially described as a product of Th2 cells that inhibited cytokine production by Th1 cells (13). IL-10 inhibits the release of several cytokines including interferon-γ (IFN-γ), IL-1, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), as well as exerting a direct effect on the growth and function of T cells, B cells, and mast cells (14). Thus, IL-10 has the capacity to attenuate a wide range of effector immune and inflammatory responses. In addition, a recent report suggests that IL-10 downregulates mRNA expression of type I collagen, but upregulates gene expression of collagenase and stromelysin, suggesting that IL-10 modulates the expression of genes involved in the synthesis and breakdown of ECM in the connective tissue (15). This also raises a possibility that IL-10 is a therapeutic candidate for fibrotic diseases.

Monocyte chemoattractant protein-1 (MCP-1) is a specific and potent chemoattractant for monocytes (16) which has been implicated in a variety of inflammatory processes. Current observations demonstrated that MCP-1 upregulates type I collagen mRNA expression in cultured lung fibroblasts (17). *In vivo* studies have also shown the involvement of MCP-1 in experimental lung or kidney fibrosis (18, 19). In this study, we examined the direct effect of IL-10 on the expression of ECM genes, such as type I collagen, fibronectin and decorin in human skin fibroblasts. In addition we investigated the effect of IL-10 on TGF-β- and MCP-1-elicited expression of ECM genes.

MATERIALS AND METHODS

Cell cultures. Adult human skin fibroblasts, established by outgrowth of biopsies from healthy donors or scleroderma patients as previously described (20), were used in passage 3–6. Cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), 50 μg/ml sodium ascorbate, 300 μg/ml glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and grown in the atmosphere of a CO_2 incubator at 37°C.

Reagents. Recombinant human MCP-1 (R&D Systems, Minneapolis, MA), TGF-β (PeproTeck, UK), and IL-10 (PeproTeck) were diluted in 0.1% BSA in PBS.

Stimulation with IL-10. 1×10^6 fibroblasts were seeded in monolayers in DMEM with 10% FCS, and on the next day, medium was replaced by fresh DMEM with supplements as indicated above, but without FCS. Twenty-four hours later, cells were incubated with IL-10 at various concentrations ranging from 1 to 50 ng/ml for time periods varying 6–24 h. In a separate experiment, TGF-β (5 ng/ml) or MCP-1 (10 ng/ml) were concomitantly added with IL-10 (10 ng/ml) for 24 h. Optimal concentrations of TGF-β and MCP-1 were determined in a preliminary experiment.

RNA isolation and Northern blot hybridization. Total RNA was isolated from fibroblasts in monolayers using RNA isolation kit (Trizol, Life Technologies Inc., NY). RNA pellets were dissolved in water, precipitated with 0.3 M sodium acetate, and 2.5 vol of ethanol. For Northern blot hybridization, 5 μg of total RNA was separated by electrophoresis in 1% formaldehyde agarose gels and transferred to Gene Screen hybridization transfer membranes (New England Nuclear Research Products, Boston, MA). RNA was fixed by UV-crosslinking and hybridized to cDNA probes specific for α1(I) procollagen (Hf 677) (21), fibronectin (FN 711) (22), decorin (23), and GAPDH (24), which were labeled by random priming using α-^{32}P-dCTP. Filters were hybridized overnight at 42°C in 50% formaldehyde, 5 × SSC, 100 μg/ml denatured salmon sperm DNA, 5 × Denhardt's, washed twice at room temperature in 2 × SSC, 0.1% SDS, followed by a further washing step at high stringency (62–65°C in 0.1 × SSC, 0.1% SDS). Autoradiography was from 6 h to overnight at −80°C using intensifying screens (Kodak, Rochester, NY).

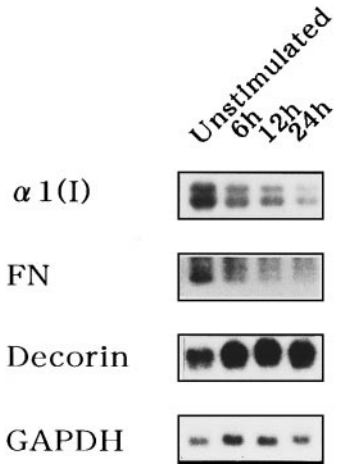

FIG. 1. Time-dependent expression of α1(I) collagen, fibronectin, and decorin mRNA levels in human skin fibroblasts after stimulation with IL-10. After replacement of the medium into serum-free DMEM, primary human dermal fibroblasts were further incubated with 10 ng/ml IL-10 for 6–24 h or without IL-10 for 24 h (unstimulated). Total RNA (5 μg/lane) was analyzed by Northern blot hybridization with cDNA probes.

RESULTS

IL-10 Downregulates Gene Expression of α1(I) Collagen and Fibronectin, But upregulates Decorin mRNA in Human Skin Fibroblasts

To study the effect of IL-10 on α1(I) collagen, fibronectin, and decorin gene expression, primary human dermal fibroblasts (1 × 106) were seeded in 10-cm tissue-culture plates. On the next day, culture medium was changed into fresh DMEM without FCS. Then, cells were incubated in the presence of IL-10 (10 ng/ml) for 6–24 h or in the absence of IL-10 for 24 h. IL-10 downregulated the expression of α1(I) collagen and fibronectin mRNA in monolayers as early as at 6 h, while decorin gene expression was upregulated with its peak between 6 to 24 h (Fig. 1). To examine the dose-dependent effect of IL-10, fibroblasts were stimulated with various concentrations (0–50 ng/ml) for 24 h in DMEM without FCS. As low as 1 ng/ml of IL-10 exhibited an inhibitory effect of α1(I) collagen and fibronectin mRNA expression. Densitometric quantification

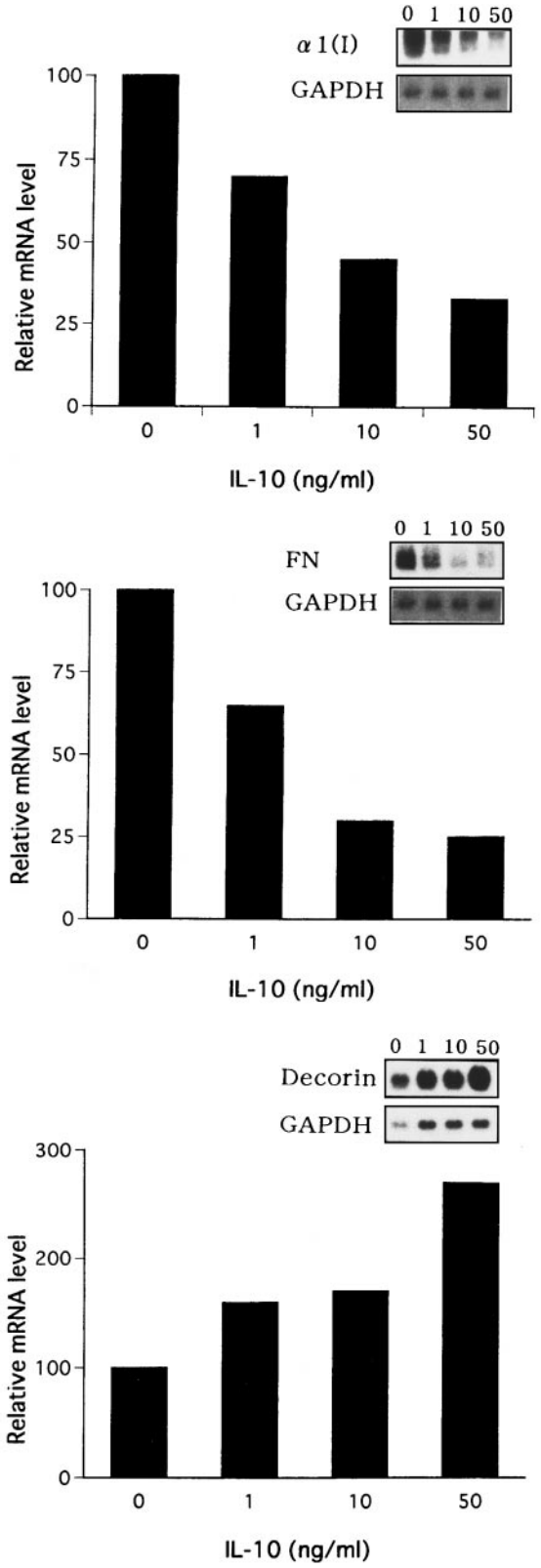

FIG. 2. Dose-dependent effects of IL-10 on the expression of α1(I) collagen, fibronectin, and decorin mRNA levels in human skin fibroblasts. Primary human dermal fibroblasts were incubated with various concentrations of IL-10 (0–50 ng/ml) for 24 h in medium without FCS. Total RNA was analyzed by Northern blot hybridization with cDNA probes.

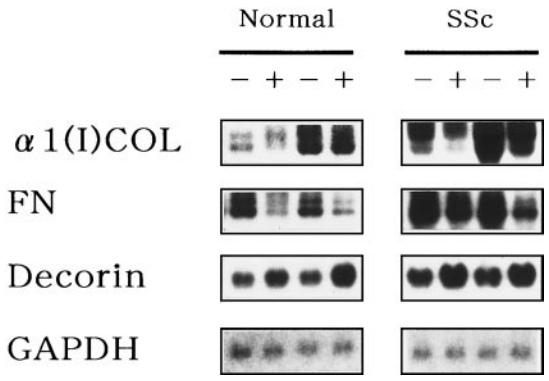

FIG. 3. Northern blot analysis of α1(I) collagen, fibronectin, and decorin mRNA levels of normal and scleroderma fibroblasts incubated with IL-10. Fibroblasts from normal donors and scleroderma patients were incubated with (+) or without (−) IL-10 (10 ng/ml) for 24 h. Total RNA (5 μg/lane) was analyzed by Northern blot hybridization with cDNA probes.

showed that α1(I) collagen and fibronectin were reduced to one-third and one-fourth, respectively, by 50 ng/ml IL-10, whereas decorin mRNA was increased up to 2.7-fold by 50 ng/ml IL-10 (Fig. 2).

Comparison of Gene Expression Altered by IL-10 between Normal and Scleroderma Fibroblasts

Next, we compared the mRNA levels of α1(I) collagen, fibronectin and decorin in normal dermal ($n = 2$) and scleroderma fibroblasts ($n = 2$). Fibroblasts were cultured with or without 10 ng/ml IL-10 for 24 h in DMEM without FCS, and total RNA was isolated. As is shown in Fig. 3, α1(I) collagen and fibronectin gene expression was decreased by stimulation with IL-10 in both normal and scleroderma fibroblasts. By contrast, decorin gene expression was upregulated by IL-10. Quantification by densitometry was summarized in Table I.

IL-10 Downregulates Gene Expression of α1(I) Collagen and Fibronectin Induced by TGF-β, and Recovers Decorin Expression Reduced by TGF-β in Fibroblasts

As is consistent with previous observations (4–6), mRNA levels of α1(I) collagen and fibronectin were upregulated by stimulation of TGF-β (5 ng/ml) for 24 h in normal dermal fibroblasts. IL-10 (10 ng/ml) abrogated this TGF-β-elicited increase of α1(I) collagen and fibronectin mRNA expression in fibroblasts (Fig. 4A). In

TABLE I

Effect of IL-10 on mRNA Levels of α1(I) Collagen, Fibronectin, and Decorin in Normal and Scleroderma Fibroblasts

	α1(I) Collagen (-fold)		Fibronectin (-fold)		Decorin (-fold)	
No.	Normal	SSc	Normal	SSc	Normal	SSc
1	0.85	0.6	0.4	0.5	2.2	3.1
2	0.7	0.5	0.3	0.3	4.0	2.5

Note. Fibroblasts were incubated with or without 10 ng/ml IL-10 for 24 h in DMEM without FCS. Total RNA was isolated and α1(I) collagen, fibronectin, and decorin mRNA levels were determined by Northern blot hybridization. Quantification was performed by densitometric scanning. The data were normalized with corresponding GAPDH mRNA levels and are presented as -fold induction over control levels for parallel cultures incubated without IL-10.

contrast, decorin mRNA expression was reduced by TGF-β. IL-10 partially recovered this reduction (Fig. 4A). Quantification by densitometry was shown in Fig. 4B.

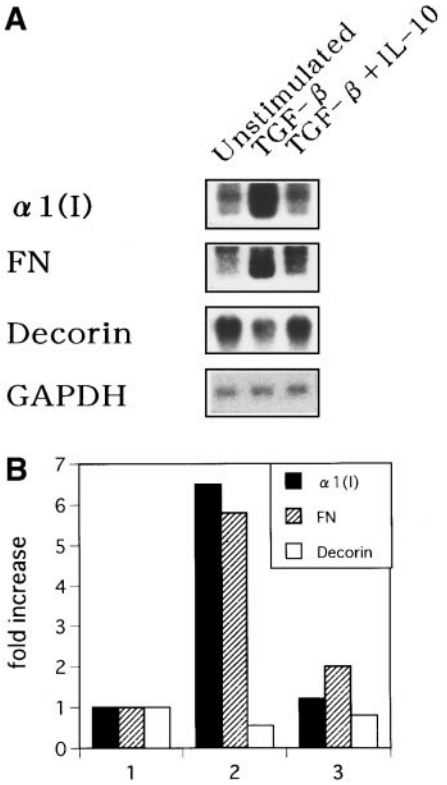

FIG. 4. Effect of IL-10 on TGF-β-elicited α1(I) collagen, fibronectin, and decorin mRNA levels. Normal skin fibroblasts were incubated with TGF-β (5 ng/ml) and IL-10 (10 ng/ml) concomitantly for 24 h in monolayers. Total RNA (5 μg/lane) was analyzed by Northern blot hybridization (A). Quantification was performed by densitometric scanning (B). The data were normalized with corresponding GAPDH mRNA levels and are presented as -fold increase over control levels.

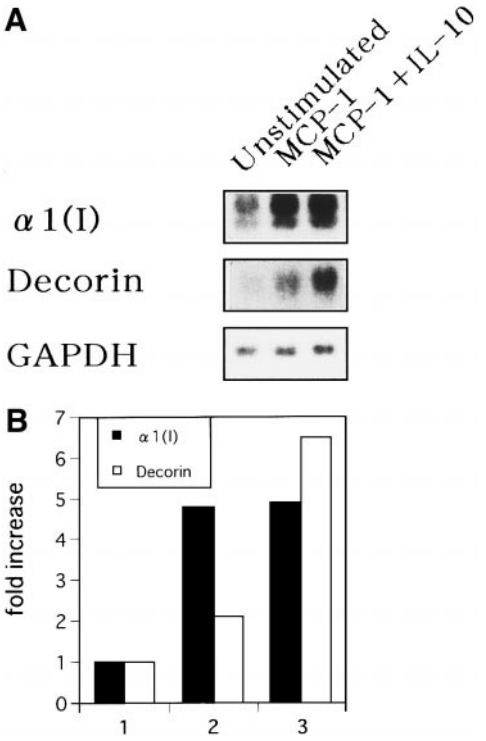

FIG. 5. Effect of IL-10 on MCP-1-elicited α1(I) collagen and decorin mRNA levels. Normal skin fibroblasts were incubated with MCP-1 (10 ng/ml) and IL-10 (10 ng/ml) concomitantly for 24 h in monolayers. Total RNA (5 μg/lane) was analyzed by Northern blot hybridization (A). Quantification was performed by densitometric scanning (B). The data were normalized with corresponding GAPDH mRNA levels and are presented as -fold increase over control levels.

IL-10 Does Not Affect MCP-1-Induced Increase of α1(I) Collagen, but Further Upregulates Decorin mRNA Expression in Fibroblasts

MCP-1 upregulated α1(I) collagen mRNA expression in normal dermal fibroblasts, which, however, was not affected by concomitant addition of IL-10 (10 ng/ml) for 24 h (Fig. 5A). By contrast, decorin mRNA was upregulated by MCP-1, which was synergistically increased by concomitant addition of IL-10 for 24 h (Fig. 5A). Quantification by densitometry was shown in Fig. 5B.

DISCUSSION

A number of inflammatory and fibrogenic cytokines are suggested to play an important role in the induction of fibrosis. However, little is known about the potential role of IL-10. IL-10 is well known to exert an inhibitory effect mainly on T cells or monocytes. In addition, a recent report has shown that IL-10 regulates collagen and collagenase gene expression in human dermal fibroblasts (15). Also, IL-10 suppresses collagen synthesis in scar fibroblasts (25). Since IL-10 is suggested to be involved in the regulation of type I

collagen synthesis and degradation (15), we examined the direct effect of IL-10 on the expression of selected ECM genes in this study. In agreement with previous results (15), our study also showed the inhibitory effect of IL-10 on α1(I) collagen mRNA expression. Furthermore, IL-10 downregulated mRNA expression of fibronectin, but upregulated decorin in a time- and dose-dependent manner in human skin fibroblasts. Fibroblasts from scleroderma responded to IL-10 similarly to normal dermal fibroblasts, and demonstrated inhibition of type I collagen and fibronectin and upregulation of decorin gene expression following incubation with IL-10 for 24 h. The effects of IL-10 are mediated by specific receptors (IL-10R) on the target cells. Murine mast cells, B cells and fibroblasts as well as human peripheral blood leukocytes and epidermal cells express the IL-10R gene (26–29), although further work is needed on the expression of IL-10R in human fibroblasts.

TGF-β can upregulate collagen and fibronectin synthesis (4–6), and is an important candidate responsible for scleroderma. In fact, a number of previous reports have shown the crucial role of TGF-β in scleroderma (30, 31). Furthermore, a recent study shows that MCP-1 is also capable of upregulating type I collagen mRNA levels in cultured fibroblasts (17). We have also observed that MCP-1 can upregulate collagen mRNA expression in human dermal fibroblasts. Thus, we next investigated the effect of IL-10 on the ECM mediated by TGF-β and MCP-1 *in vitro*. We found that IL-10 inhibited the basal and TGF-β-elicited increase of α1(I) collagen and fibronectin expression. Decorin mRNA expression was reduced by TGF-β in dermal fibroblasts, in keeping with previous results (32). IL-10 partially recovered this TGF-β-mediated reduction in decorin mRNA expression. IL-10 is shown to suppress TGF-β synthesis in mouse bone marrow cultures (33). On the contrary, IL-10 did not exert any effect on MCP-1-elicited increase of α1(I) collagen gene expression. However, IL-10 synergistically upregulated the MCP-1-mediated increase of decorin gene expression. These results may suggest a different mechanism of the influence of IL-10 on TGF-β and MCP-1 in the regulation of ECM.

Very recently, an *in vivo* effect of IL-10 on bleomycin-induced lung injury was reported (34). They showed that human IL-10-HVJ treatment significantly inhibited lung fibrosis induced by bleomycin, suggesting that exogenous IL-10 may be useful in the therapeutic approach of pulmonary fibrosis.

In conclusion, we demonstrate here that IL-10 directly influences the ECM mRNA expression, suggesting that IL-10 can modify the development of tissue fibrosis. Our data suggest that exogenous IL-10 can modify the tissue remodeling and raised the possibility of a therapeutic role of IL-10 in scleroderma.

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