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Interleukin 1 receptor on fibroblasts from systemic sclerosis patients induces excessive functional responses to interleukin 1β

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Summary: To determine if SSc fibroblasts are more sensitive to exogenous IL-1 than are normal fibroblasts, we studied the inductions of IL-6, prostaglandin E_2 (PGE_2), IL-1β mRNA production by IL-1-stimulated SSc and normal fibroblasts. The minimal IL-1β concentrations for stimulation of IL-6 and PGE_2 production by SSc fibroblasts were 10-fold and 100-fold lower, respectively, than those for normal fibroblasts. The minimal IL-1β concentration for stimulation of IL-1β mRNA expression by SSc fibroblasts was also 10-fold lower than that for normal fibroblasts. These results suggest that the IL-1 signal transduction through IL-1R could be induced excessively in SSc fibroblasts. It is suggested that the IL-1 signal transduction plays an important role in the abnormal cytokine networks observed in the fibrosis of SSc.

Systemic sclerosis (Scleroderma, SSc) is a connective tissue disorder characterized by fibrosis in systemic organs and especially in skin. Although the mechanisms underlying the skin fibrosis are not completely known, it has been reported that fibroblasts cultured from SSc patients show higher levels of collagen synthesis at the protein and messenger RNA (mRNA) levels than do fibroblasts from normal individuals (1,2). Mononuclear cell (MNC) infiltration is also reported to be prominent in sclerodermatous skin (3), and cultured peripheral blood mononuclear cells from SSc patients have been shown to produce high levels of interleukin 1 (IL-1) (4,5). These reports suggest that IL-1 may be involved in the overproduction of collagen by SSc fibroblasts, but the quantitative aspects IL-1 effects on SSc remain to be discovered.

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IL-1 (α and β) is a polypeptide hormone that affects proliferation and collagen synthesis of human fibroblasts (6). In recent studies, the action of IL-1 is shown to be mediated through IL-1 receptors (IL-1R), of which at least two distinct forms (type I and II) are known to exist (7,8). In an earlier study, we found an increased expression of IL-1R (type I) by SSc fibroblasts and hypothesized that SSc fibroblasts are highly responsive to exogenous IL-1 (9).

The purpose of the present study is to test our hypothesis by examining the effects of exogenous IL-1β on the production of IL-6 and prostaglandin E_2 and on the expression of IL-1β mRNA by SSc and normal fibroblasts.

MATERIALS AND METHODS

Study cells: Dermal fibroblasts were explanted from 3 patients with SSc and from 3 normal individuals. The SSc patients conformed to American Rheumatism Association criteria (10), and the normal individuals were approximately matched for sex and age with the patient group. Primary cell cultures were established according to procedures described earlier (11). Fibroblasts from cultures of the 2nd to 3rd passages were transferred into 24-well culture plates (Linbro, Flow Laboratories, Inc., McLean, VA) containing Dulbecco's modified essential medium (DMEM, Flow Laboratories, Inc.) supplemented with 10% fetal calf serum (FCS, Flow Laboratories, Inc.) in an atmosphere of 5% CO_2-95% air at 37°C. The medium was changed at confluence to serum-free medium (ASF-301, Ajinomoto Co., Ltd., Tokyo, Japan) (12).

Interleukin-6 (IL-6) and Prostaglandin E_2 (PGE_2) assays: Monolayer confluent cells were cultured with ASF-301 in 24-well culture plates. Different concentrations of IL-1β, ranging form none to 10^{-15} M through 10^{-9} M, were added to each well. After culturing for up to 24 h, IL-6 and PGE_2 were measured in the supernatants by ELISA. IL-6 and PGE_2 proteins were quantified by enzyme-immunoassay kits (Intertest 6 , Genzyme Corporation, Cambridge, MA, and ACE Enzyme Immunoassay kit, Cayman Chemical Co., Ann Arbor, MI, respectively). The assays were capable of detecting concentrations of IL-6 and PGE_2 as low as 160 pg/ml and 3.9 pg/ml, respectively.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of fibroblast mRNA: For isolation of total RNA, fibroblast cultures were carried to confluency in 100 mm plastic culture dishes containing ASF-301. After 3 days, the medium was changed and the various concentrations (0 to 10^{-15} M through 10^{-9} M) of IL-1β were added for 6 h. Fibroblasts were washed twice by PBS and then collected by a rubber policeman. Total RNA was isolated by using RNA Zol^{TM} (Biotecx Laboratories, Inc., Houston, TX). Detection and analysis of gene expression at the RNA level was performed using a GeneAmp RNA PCR kit (Perkin Elmer Cetus, Norwalk, CT) (13). Briefly, a 20 μl reverse transcription reaction mixture containing 100 ng of total cellular RNA was incubated at 42°C for 15 min, using 50 U of Moloney murine leukemia virus reverse transcriptase. After the RT reaction, the products were held at 95°C for 5 min. PCR amplification was performed using 20 μl of the product in PCR buffer containing 0.15 μM primer A (sense) and B (antisense) and 2.5 U AmpliTaq DNA Polymerase. The sequences of the sense and antisense primers for IL-1β are 5'-GCA GAA GTA CCT GAG CTC GC-3' and 5'-ACC AGA CAT CAC CAA GCT TT-3', respectively. Amplification of the same RNA with β-actin primers confirmed that

equal amounts of RNA were reverse-transcribed. The sequences of primers for β-actin are 5'-TAC ATG GCT GGG GTG TTG AA-3' and 5'-AAG AGA GGC ATC CTC ACC CT-3', respectively. The mixture was amplified with the Perkin-Elmer Cetus thermal cycler. The amplification profile was 30 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec, and extention at 72°C for 1 min. At the end of 30 cycles the reaction was continued with an extension incubation at 72°C for 7 min. The PCR products were electrophoresed in 3% NuSieve/1% SeaKem agarose gel stained with ethidium bromide and visualized under ultraviolet light.

RESULTS

IL-1β induction of IL-6 in SSc and normal fibroblasts

As expected, IL-1β induced the production of IL-6 by fibroblasts. However, fibroblasts from SSc patients responded to IL-1β concentrations of 10^{-14} M, whereas fibroblasts from normal individuals did not respond to concentrations less than 10^{-13} M, a 10-fold difference. Dose-response curves for IL-1β-induced IL-6 production from the 3 SSc and 3 normal fibroblast cultures for 24 h (Fig. 1A) reveal that IL-6 levels peaked at 10^{-11} M in SSc cultures and at 10^{-10} M in cultures from normals. The mean ± SD of maximal

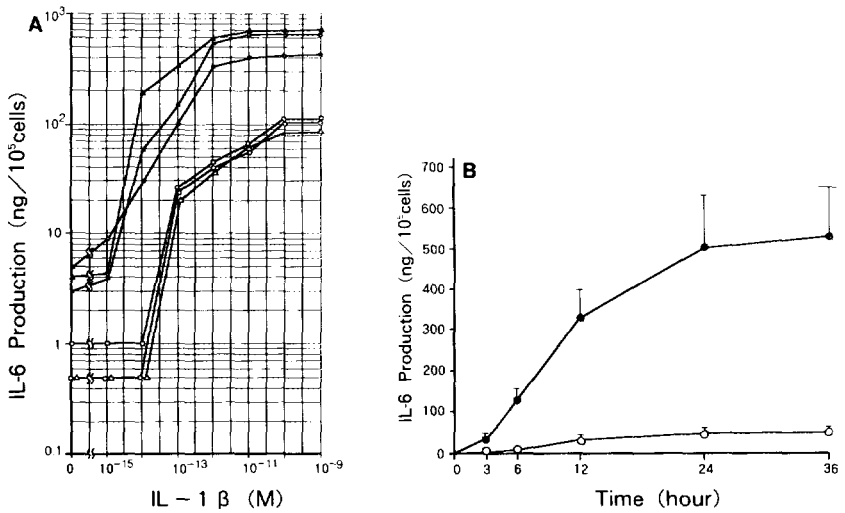

Figure 1. Effect of IL-1β on IL-6 production in SSc and normal fibroblasts.

A, Dose-response curves of IL-6 production by fibroblasts from 3 SSc (solid symbols) and 3 normal (open symbols) subjects cultured for 24 h with varying concentrations (>10^{-15} M through 10^{-9} M) of IL-1β. Supernatants were collected and assayed by ELISA, and values were corrected for total cell numbers. Each symbol represents a different subject.

B, Time course of IL-1β induction of IL-6. Confluent fibroblast cultures were stimulated with 10^{-12} M IL-1β for the indicated times.

IL-6 production was 576 ± 127 ng/10^5 cells in SSc fibroblasts and 100 ± 13.2 ng/10^5 cells in normal fibroblasts.

IL-6 production reached a plateau at 24 h of incubation, as illustrated by the kinetics of IL-6 production by SSc and normal fibroblasts in response to a concentration of 10^{-12} M IL-1β (Fig. 1B).

IL-1β induction of PGE_2 in SSc and normal fibroblasts

As with IL-6, IL-1β also induced fibroblasts to produce PGE_2. Again, there was a difference with respect to the concentrations at which the cells would respond: SSc fibroblasts responded to IL-1β concentrations of 10^{-14} M whereas fibroblasts from normal individuals did not respond to concentrations less than 10^{-12} M, a 100-fold difference. Dose-response curves for IL-1β-induced PGE_2 production from the 3 SSc and 3 normal fibroblast cultures for 24

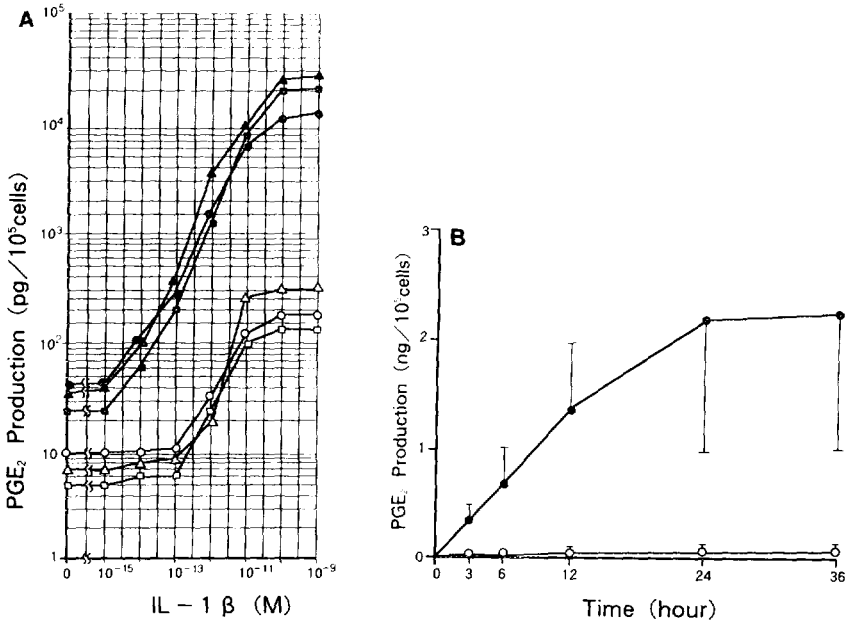

Figure 2. Effect of IL-1β on PGE_2 production in SSc and normal fibroblasts.

A, Dose-response curves of PGE_2 production by fibroblasts from 3 SSc (solid symbols) and 3 normal (open symbols) subjects cultured for 24 h with varying concentrations (>10^{-15} M through 10^{-9} M) of IL-1β. Supernatants were collected and assayed by ELISA, and values were corrected for total cell numbers. Each symbol represents a different subject.

B, Time course of IL-1β induction of PGE_2. Confluent fibroblast cultures were stimulated with 10^{-12} M IL-1β for the indicated times.

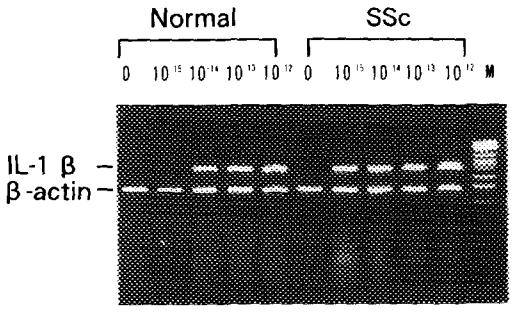

Figure 3. Induction of IL-1β mRNA in SSc and normal fibroblasts.
Fibroblasts from 3 SSc and normal subjects were cultured with various concentrations (0-10^{-9} M) of IL-1β for 6 h. Total RNAs were extracted by the RNA Zol, and a RT-PCR analysis was performed as described in Materials and Methods. Lane M is the DNA molecular weight marker (BglI-digested pBR328 and HinfI-digested pBR328).

h (Fig. 2A) reveal that PGE_2 levels were approximately maximal at 10^{-10} M in both SSc and normal cultures. The mean ± SD of maximal PGE_2 production was 19300 ± 7000 pg/10^5 cells in SSc fibroblasts and 200 ± 89 pg/10^5 cells in normal fibroblasts.

As with IL-6, PGE_2 production reached a plateau at 24 h of incubation, as illustrated by the kinetics of PGE2 production by SSc and normal fibroblasts in response to a concentration of 10^{-12} M IL-1β (Fig. 2B).

IL-1β induction of IL-1β mRNA expression

As with IL-6 and PGE_2 production, SSc fibroblasts were more sensitive to IL-1β stimulation than were normal fibroblasts with respect to the expression of IL-1β mRNA. Total RNA was extracted from the 3 SSc and 3 normal fibroblasts, and RT-PCR analysis was performed; again, SSc fibroblasts responded at a concentration of 10^{-15} M, whereas normal fibroblasts required a concentration of at least 10^{-14} M (a 10-fold difference) before responding. Representative data from 1 SSc and 1 normal fibroblast culture are shown in Figure 3; results in the other 2 SSc and normal fibroblast cultures are similar. PCR amplified products were quantified relative to constitutively expressed β-actin mRNA and the equivalent amount of β-actin mRNA was detected in each lane (Fig. 3).

DISCUSSION

The results of the present study clearly demonstrate that SSc fibroblasts are more highly responsive to IL-1β than are normal fibroblasts. IL-1β is well recognized as an inducer of IL-1 (14), IL-6 (15), PDGF (16) and cyclooxygenase (17) production by human fibroblasts. To test IL-1β signal transduction through an IL-1 receptor (IL-1R) in SSc fibroblasts, we selected IL-6 production, PGE_2 production and the expression of IL-1β mRNA as different physiological responses to IL-1. The concentration of IL-1β that was capable of inducing of IL-6, PGE_2 and IL-1β mRNA production by SSc fibroblasts was 10 (Fig. 1A), 100 (Fig. 2A), and 10 (Fig. 3) times lower than that needed in normal subjects. This is the first demonstration that SSc fibroblasts are highly responsive to IL-1β, although it has been reported that mononuclear cells are accumulated in SSc skin (1, 2) and that the production of IL-1 by mononuclear cells from SSc patients is significantly higher than that from normal subjects (4, 5). These observations suggest the possibility that excessive IL-1 action may be induced in SSc fibroblasts in vivo. Since IL-1 is described as a potent stimulator for proliferation and collagen synthesis in fibroblasts, the increased signal transduction of IL-1 might participate in the pathogenesis of SSc through fibroblast activation.

In an earlier study, we reported an increase in the number of IL-1R on SSc fibroblasts (9). The results from the earlier and present studies indicate that the IL-1 signal transduction in fibroblasts is regulated at the level of IL-1R expression. This conclusion is consistent with previously reported values for Chinese hamster ovary cells and rabbit chondrocytes (18, 19). It is inferred from this fact that the down-regulation of IL-1R expression can block the IL-1 action.

Not only did fibroblasts from SSc patients respond to lower concentrations of IL-1β than did normal fibroblasts, but the responses were constitutively higher. SSc fibroblasts produced as much as 6 times more immunoreactive IL-6 (Fig. 1A) and 4.6 times more PGE_2 (Fig. 2A) than was produced by normal fibroblasts. Enhanced IL-6 production has been reported earlier (20),

but the mechanism remains obscure. To our knowledge, the present study is the first to report that PGE_2 production by SSc fibroblasts is also constitutively excessive. PGE_2 is recognized as a potent inflammatory modulator that is induced by IL-1 and several other cytokines.

One possible explanation for the overproduction of IL-6 and PGE_2 observed in this study might be that unknown factors produced by SSc fibroblasts themselves may spontaneously induce IL-6 and PGE_2 production in SSc fibroblast cultures; another possibility might be that SSc fibroblasts in vivo are transformed to activated states that constitutively produce an excessive amount of IL-6 and PGE_2. It remains to be seen which of these possibilities, if not some other, will eventually prevail.

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