Possible Involvement of Interleukin-1 in the Pathogenesis of Dermatofibroma

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Dermatofibroma (DF) is histologically characterized by proliferation of fibroblasts in the dermis. Multiple DFs occasionally develop in patients with autoimmune disorders under immunosuppressive therapy; however, the pathogenesis of DF is still unclear. To elucidate immunological involvement in the mechanism of the fibrosis in DF, we studied the role of interleukin-1 (IL-1), which has a number of biological functions, including proliferation and collagen production of fibroblasts, on DF-derived fibroblasts. ^{3}H-thymidine incorporation was used to examine the effects of IL-1α and IL-1β in 4 cultured fibroblast strains derived from DF and 5 fibroblast strains from normal skin. Expression of mRNA of IL-1 was also analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). Basal ^{3}H-TdR incorporation without stimulant of DF-derived fibroblasts showed a significantly greater growth activity than normal skin-derived fibroblasts (2, 632±525 vs. 762±144 dpm, $p < 0.01$). Both IL-1α and IL-1β showed a stronger growth-stimulatory activity on DF-derived fibroblasts in a dose-dependent manner than normal fibroblasts, and the percent ^{3}H-TdR uptake of DF was 1.4-fold (IL-1α; 1,000 U/ml) and 1.3-fold (IL-1β; 1,000 U/ml) as compared with normal fibroblasts; however, the differences did not reach any significance. When increasing concentrations of IL-1 receptor antagonist (IL-1ra) were added to culture medium stimulated with IL-1α, the proliferative response of fibroblasts was significantly reduced. Expression of IL-1β mRNA was detected on both DF-derived and normal skin-derived fibroblasts, while that of IL-1α mRNA was detected only on DF-derived fibroblasts. Our results suggest that IL-1 may be involved in the fibrotic process in DF at the transcriptional level and play a role in the fibroblast proliferation in an autocrine manner. *Key words: fibroblast; mRNA.*

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Dermatofibroma (DF) is a benign tumor, showing localized fibroblast proliferation. Although DF has been considered to be a reactive hyperplasia rather than a true neoplasm, the issue remains unsettled (1). The effects of the fibrogenic cytokine network on proliferation of fibroblasts in wound healing have been investigated (2–5), and various effects of cytokines on fibroblasts derived from lesional skin of systemic sclerosis (SSc) or keloids have also been analyzed (6, 7). However, little is known about the involvement of cytokines and growth factors in DF. We have previously found that the mast cell number is increased in the layer between the DF lesion and the overlying epidermis and that some growth factors derived from a patient's serum promote the proliferation of fibroblasts in the case of multiple DFs associated with systemic lupus erythematosus (SLE) (8, 9).

Interleukin-1 (IL-1) is a cytokine with a number of biological functions, including proliferation of keratinocytes and fibroblasts, induction of synthesis of matrix proteins, and increase of collagen production (10–13). It is suggested that IL-1 may regulate the balance between matrix deposition and degradation (14–17). IL-1 is involved in the process of wound healing; however, the mitogenic effect on fibroblasts is reported to be indirectly mediated by induction of platelet-derived growth factor (PDGF) A-chain synthesis and its secretion (18). Normal fibroblasts stimulated by IL-1 are also known to produce several cytokines and hormones, such as IL-6 (19), IL-8 (20), colony-stimulating factor (21), PDGF (18) and prostaglandins (21). It is supposed that several fibrogenic cytokines are also involved in the pathogenesis of DF. In this report, to address the question of the possible role of IL-1 in DF fibroblasts, we examined the expression of IL-1 mRNA and the mitogenic effects of IL-1 on DF-derived fibroblasts.

MATERIALS AND METHODS

Cells and culture

Four solitary DFs, present for 2–6 years, were obtained by surgery from the lower legs, shoulder and upper limb of 2 female and 2 male patients, aged 38–51 years. Each section was cut into 1 × 1-mm pieces, which were placed beneath sterile glass coverslips in tissue culture dishes. Fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM), containing 100 μg/ml streptomycin, 62.5 μg/ml penicillin and 0.58 g L-glutamine, supplemented with 7% fetal calf serum (FCS) at 37°C in humidified 95% air-5% CO_2. As control, 5 normal skin-derived fibroblasts were obtained from uninvolved skin of excised pigmented nevi localized on the thigh or upper limb in age-matched, otherwise healthy subjects. Fibroblasts from the fourth or fifth passages were used in the proliferation assays.

Fibroblast proliferation assay

Fibroblasts in DMEM with 7% FCS were seeded into 96-well tissue culture plates (Falcon) at a density of 5×10^3 cells/well. Culture medium was discarded after 24 h and replaced with DMEM supplemented with 0.1% FCS. Twenty-four hours later, IL-1α and IL-1β were added at the concentration of 10-1,000 U/ml for 24 h. In a separate experiment, IL-1 receptor antagonist (IL-1ra) (PeproTeck, Ltd., London, UK) was simultaneously added to cultured fibroblasts stimulated with IL-1α (1, 000 U/ml) for 24 h. Then 0.2 μCi/well of ^{3}H-thymidine was added to the culture medium for the last 8 h before harvesting. Fibroblasts were treated with 0.1 ml of 0.125% trypsine-0.01% EDTA per well at 37°C for 15–20 min and then harvested on a glass filter sheet by using a semiautomatic cell harvester (Labomash LM 101; Laboscience, USA). The radioactivity of the glass filter was performed in triplicate and counted using a liquid scintillation counter.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from 10^6 fibroblasts of fourth to fifth passages using RNA zol (Biotecx CS 101) and then reversely transcribed to cDNA by RAV-2 reverse transcriptase (Takara 2610B, Kyoto, Japan). PCR analysis was accompanied by using oligonucleotide primers specific for IL-1α (5′ GTCTCTGAATCAGAAATCCTTCTATC 3′

and 5′ CATGTCAAATTTCACTGCTTCATCC 3′) and IL-1β (5′ AAACAGATGAAGTGCTCCTTCCAGG 3′ and 5′ TGGAGAA CACCACTTGTTGCTCCA 3′). We used the following conditions: 35 cycles each of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min and 5 s. PCR products were electrophoresed in 1.7% agarose gel. The gel was stained with 1% ethidium bromide and visualized under ultraviolet light.

Statistical analysis

Results were expressed as mean $\pm$ SD. Significance was assessed by Student's *t*-test. A *p*-value <0.05 was considered significant.

RESULTS

The value for ^{3}H-TdR incorporation (DPM $\pm$ SD) in control wells that had no growth factors added was set at 100%. There was a significant difference in basal ^{3}H-TdR incorporation between DF fibroblasts and normal fibroblasts (2, 632 $\pm$ 525 vs. 762 $\pm$ 144 dpm, $p<0.01$). The mitogenic responses to both IL-1α and IL-1β (10-1,000 U/ml) increased in a concentration-dependent manner similarly in both cell strains. ^{3}H-TdR incorporation of DF-derived fibroblasts showed a greater response by stimulation of both IL-1α (1.4-fold at the dose of 1,000 U/ml) and IL-1β (1.3-fold at the dose of 1,000 U/ml) rather than that of normal fibroblasts, but the differences did not reach any significance (Fig. 1). IL-1ra significantly inhibited the DF fibroblast proliferation induced by IL-1α in a dose-dependent manner (Fig. 2), as well as in normal fibroblasts (data not shown). mRNA expressions of both IL-1α and IL-1β were detected in DF-derived fibroblasts, while IL-1α mRNA was not detected in normal fibroblasts (Fig. 3).
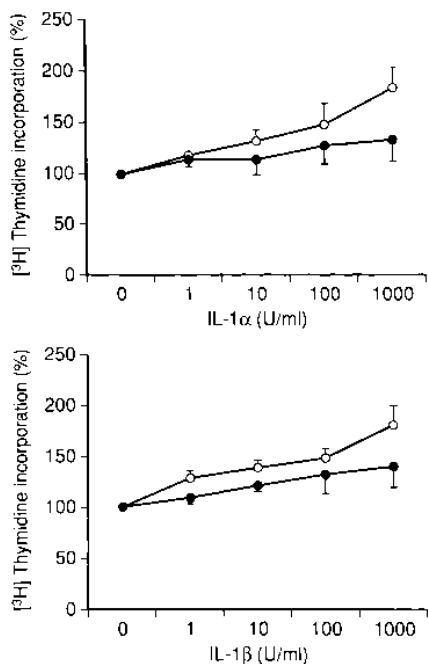
Fig. 2. Effects of IL-1ra on the proliferative response of DF-derived cultured fibroblasts initiated by IL-1α. Cells (5×10^3/well) were cultured with IL-1α (1, 000 U/ml) and increasing concentrations of IL-1ra for 24 h. Results are expressed as the mean $\pm$ SD of triplicate experiments. *$p<0.05$.

Fig. 3. RT-PCR assay for IL-1α and IL-1β. *Lanes 1–4*: DF-derived fibroblasts. *Lanes 5–6*: normal skin-derived fibroblasts.

Fig. 1. Effects of IL-1α (*upper*) and IL-1β (*lower*) on mitogenic response in normal (*closed circles*) and DF fibroblasts (*open circles*). Cells (5×10^3/well) were cultured with various concentrations of IL-1 for 24 h. Results are expressed as the mean $\pm$ SD of triplicate experiments.
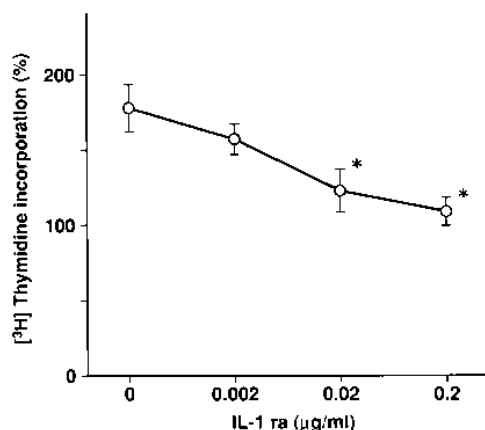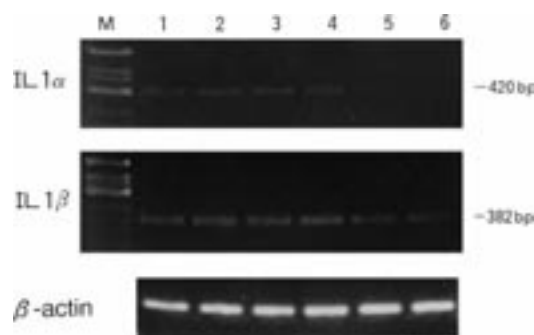
DISCUSSION

Although it is still uncertain whether DF is a truly neoplastic disease or a reactive process, an immunoreactive origin has been emphasized (1). Nestle et al. (22) have recently shown that MHC class II molecules and co-stimulatory molecules such as B7-1 and B7-2 are expressed on lesional cells in DF. These molecules are almost exclusively expressed on professional antigen-presenting cells (APCs) and therefore support the notion that potent APCs may be present in the lesions of DF. DF occasionally occurs in association with immunosuppressive conditions (23–28). Mast cell number is increased both in solitary and multiple DF, especially in the layer between the DF lesion and the overlying epidermis (8). Histologically, the overlying epidermis of DF shows acanthosis, which is supposed to be due to stimulation of several cytokines derived from mast cells. Mast cells contain numerous potent mediators, including histamine, heparin, proteinases, leukotrienes, prostaglandin D2, and several cytokines or growth factors including stem cell factor, basic fibroblast growth factor (bFGF), transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), IL-3 and IL-4.

In DFs, infiltrating mononuclear cells and even the formation of abortive lymphoid follicles at the periphery of the DF lesion are occasionally seen. Thus, DF fibroblasts may be exposed to certain cytokines derived from these infiltrating cells.

IL-1 is a proinflammatory cytokine and possesses multiple biological functions. Fibroblast proliferation, collagen, fibronectin, proteoglycan synthesis and collagenase secretion are all increased by IL-1. The effects of IL-1 are mediated by specific cell membrane receptors. In SSc, an increased spontaneous production of IL-1 is seen in unstimulated peripheral blood mononuclear cells in patients' sera (29), and expression of IL-1 receptor in SSc fibroblasts is constitutive and augmented at the mRNA and protein levels (30). These findings prompted us to address the question whether a similar cytokine network is involved in the local fibrotic process of DF.

Our results demonstrated that DF-derived fibroblasts showed a stronger response to both IL-1α and IL-1β than normal skin-derived fibroblasts. IL-1ra completely inhibited the IL-1α-induced component of the proliferative response in a concentration-dependent manner, which indicates that IL-1 α-augmented proliferative responses in fibroblasts are mediated by type I IL-1 receptor rather than type II receptor in both DF and normal fibroblasts. Both IL-1α and IL-1β mRNA were expressed in DF fibroblasts, while IL-1β was also expressed in normal fibroblasts. These data suggest that IL-1 may be involved in the fibrotic process at the transcriptional level and may play a role in the fibroblast proliferation in an autocrine manner. Although the mechanism whereby enhanced expression of IL-1α mRNA is achieved in only DF fibroblasts is obscure, it is speculated that DF fibroblasts are transformed by unknown agents. Keratinocytes synthesize and store IL-1, which acts as a reservoir of readily available active cytokines. The possibility that IL-1 is derived from activated keratinocytes of the overlying epidermis of DF may be considered. DF fibroblasts are not unresponsive to IL-1, owing to constant exposure to several fibrogenic cytokines derived from mast cells or infiltrating lymphocytes or macrophages. These findings are different from those of scleroderma, in which fibroblasts derived from sclerotic lesional skin are unresponsive to various growth factors in vitro (6), which may be explained by the fact that constant effects of fibrogenic cytokines or growth factors in DF are weak compared with SSc. Further studies are necessary to define the agents inducing DF.

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