STIMULATION OF THE CHEMOTACTIC MIGRATION OF HUMAN FIBROBLASTS BY TRANSFORMING GROWTH FACTOR β

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Transforming growth factors (TGFs) are polypeptides that have in certain nontumorigenic cells an ability to reversibly induce features that resemble those of malignant transformation (1, 2). There are two distinct types of TGFs, TGF-α and TGF-β (2, 3). TGF-α is related to epidermal growth factor (EGF) and shares the same cell surface receptors, while TGF-β is structurally and antigenically distinct (1, 2). TGF-β has been molecularly cloned (4). It is a homodimer with two disulfide-linked polypeptide chains of 12.5 kD (1, 4). Cell surface receptors for TGF-β are not related to the receptors of other known growth factors and are widely distributed in different cells and tissues (2, 5, 6).

TGF-β is present in a variety of cells including T cells, monocytes, and platelets (2, 7–9). TGF-β also is known to stimulate production of fibronectin and collagen by dermal fibroblasts in vitro (8, 10, 11). Roberts and coworkers (8) reported that the subcutaneous injection of TGF-β in newborn mice induced a rapid fibrosis and accumulation of fibroblasts. This observation suggested to us that a function of TGF-β might be to induce fibroblast chemotaxis. In this report we have observed that platelet-derived human TGF-β is a potent chemoattractant for fibroblasts in vitro.

Materials and Methods

TGF-β and EGF. TGF-β was purified from homogenates of outdated human platelets by procedures developed in our laboratory and previously published in detail (1, 5, 7). The preparations for TGF-β used in this study were tested for purity by polypeptide analysis using silver staining of SDS polyacrylamide gels (1). All preparations used contained only a 25-kD protein band on the gels which, after reduction with 2-ME, migrated as a single 12.5-kD band and were active at ng/ml concentrations in a soft agar colony formation assay using AKR-2B cells and growth inhibition assays using A549 indicator cells (1, 3). Antibodies were raised in rabbits to one of these preparations of

TGF-β and specifically blocked the binding of TGF-β to its receptor on AKR-2B cells. EGF was purified from male mouse submaxillary glands (5).

Preparation of Anti-TGF-β Antibodies.   Antibodies against homogeneously pure TGF-β were raised in rabbits, and the IgG fraction was purified using a protein A–Sepharose column (Keski-Oja, J., R. M. Lyons, and H. L. Moses, manuscript in preparation). Normal rabbit IgG was purified in a similar manner to be used as a control.

Fibroblast Chemotaxis.   Fibroblast chemotaxis was measured in modified blind-well chemotaxis chambers equipped with porous (8 μm) gelatin-treated polycarbonate filters as previously described (12). All samples were tested in triplicate or quadruplicate, and where indicated activity was expressed as the mean number of fibroblasts per 200:1 immersion fields (OIF) ± SEM of the replicates.

Other Fibroblast Chemoattractants.   Human plasma fibronectin, heat denatured type I calf skin collagen, and human platelet-derived growth factor (PDGF) were prepared as previously described (12–14).

Incubation of Fibroblast Chemoattractants with Anti-TGF-β Antibodies.   Purified IgG from control and TGF-β-immunized rabbits (14 and 140 μg/ml) were added (10 μl) to separate 200-μl aliquots of PBS (Grand Island Biological Co., Grand Island, NY) containing fibronectin (600 ng), denatured type I collagen (6 mg), TGF-β (3 pg), and PDGF (0.3 pg). All of the samples were incubated at 37° for 2 h with frequent mixing. Volumes were adjusted to 600 μl by addition of 390 μl serum-free Eagle's MEM (Grand Island Biological Co.) and were tested in triplicate for fibroblast chemotactic activity.

Reduction of TGF-β by 2-ME.   Two equal aliquots (1.98 ml) of TGF-β (500 ng/ml) were prepared, 2-ME (20 μl) was added to one aliquot while PBS (20 μl) was added to the other. Both TGF-β preparations were then kept at room temperature for 4 h. After extensive dialysis at 4°C against PBS, the preparations were tested for their chemotactic activity using the same assay at different doses.

Results

Chemotactic Property of TGF-β.   Purified human platelet-derived TGF-β stimulated migration of fibroblasts in modified Boyden chemotaxis chambers (Fig. 1). The dose-response curve was found to be bell-shaped, with maximal migration occurring at a concentration of 12.5 pg/ml in the experiment depicted in Fig. 1. This same general dose-response curve was repeatedly observed with different preparations of TGF-β, with maximal migration always being observed at doses ranging from 5–50 pg/ml (data not shown).

Zigmond-Hirsch checkerboard analysis revealed that TGF-β effected migration of fibroblasts only when present in higher concentration in the lower compartment of the chemotaxis chambers (Table I), suggesting that it induces chemotactic migration and not chemokinesis of fibroblasts (15).

Denaturation of TGF-β by reduction of its disulfide bonds with 2-ME resulted in a marked decrease in the ability of TGF-β to act as a chemoattractant for fibroblasts (e.g., chemotactic activity of TGF-β, 5 pg/ml, was diminished from 54 ± 5 fibroblasts per 20 OIF to a value of 14 ± 3 after reduction by 2-ME [migration to PBS control was 5 ± 1]).

EGF has recently been shown (8, 16) to be capable of inhibiting TGF-β-induced collagen synthesis by fibroblasts and to reverse TGF-β-mediated growth inhibition of A549 human lung carcinoma cells. In light of these observations, we wanted to determine whether EGF might alter the chemotactic response of fibroblasts to TGF-β. To address this issue, fibroblasts used in a chemotaxis assay were incubated with different doses of EGF before and while they were present in the upper compartment of chemotaxis chambers whose lower compartments
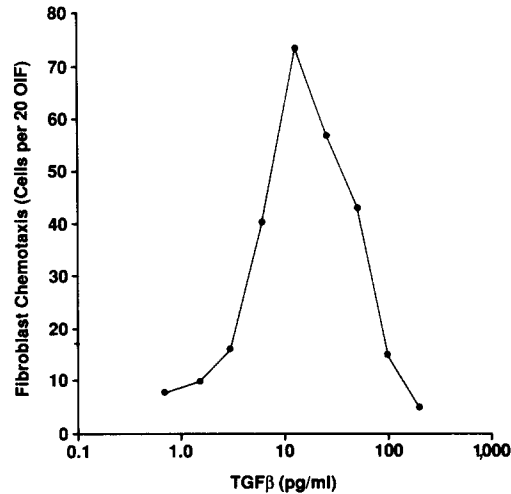
FIGURE 1.   TGF-β was tested in quadruplicate at the concentrations indicated for its ability to induce fibroblast migration in modified blind-well chemotaxis chambers (see Materials and Methods). The SE for each point on the graph were >15% of each mean value. Migration to PBS control was 5 ± 1.

TABLE I

Effect of Varying Concentration Gradients of TGF-β on Fibroblast Migration

TGF-β concentration in upper compartment	Fibroblasts per 20 OIF at TGF-β concentration (pg/ml) in lower compartment of:				
	50.0	25.0	12.5	6.3	0
pg/ml					
50.0	8 ± 1	7 ± 1	5 ± 1	5 ± 1	6 ± 1
25.0	20 ± 2	8 ± 1	7 ± 1	6 ± 1	5 ± 1
12.5	24 ± 2	16 ± 2	8 ± 1	6 ± 1	5 ± 1
6.3	38 ± 3	26 ± 2	18 ± 1	5 ± 1	5 ± 1
0	52 ± 9	42 ± 3	27 ± 3	17 ± 1	5 ± 1

Different concentrations of TGF-β were added to the upper and lower compartments of the chemotaxis chamber and the number of fibroblasts migrating to the lower surface of each filter was quantitated. Each value represents the mean of four replicates ± SEM. The values between the parallel lines were obtained when TGF-β was present in a given chemotaxis chamber at the same concentration in the uppr and lower compartments.

contained TGF-β or denatured type I collagen. EGF did not alter the chemotactic response of fibroblasts to either chemoattractant (Table II). These results indicate that the chemotactic responsiveness of fibroblasts to TGF-β is probably not modulated by EGF.

Inhibition of TGF-β-induced Chemotaxis by Anti-TGF-β Antibodies.   Specific anti-TGF-β antibodies have been shown by others (8) to inhibit TGF-β-induced stimulation of collagen production by fibroblasts. To determine whether the chemotactic activity of TGF-β was susceptible to inhibition by specific anti-TGF-β antibodies, TGF-β and other chemoattractants were incubated with IgG from a rabbit immunized with TGF-β (Table III). Normal rabbit IgG served as a control. Anti-TGF-β antibodies totally inhibited the ability of TGF-β to act as a

TABLE II

Chemotactic Response of EGF-treated Fibroblasts to TGF and Denatured Collagen

EGF concentrations in upper compartment	Fibroblast per 20 OIF with lower compartment contents of:	
	TGF (5 pg/ml)	Denatured type I collagen (10 mg/ml)
ng/ml		
50.0	55 ± 4*	33 ± 2*
5.00	58 ± 5*	37 ± 2*
0.50	54 ± 6*	35 ± 4*
0.05	47 ± 6*	32 ± 3*
0	57 ± 3	38 ± 6

Different concentrations of EGF were preincubated with fibroblasts for 30 min at 37°C before they were added to prepared chemotaxis chambers containing either TGF or denature type I calf skin collagen. The EGF was present with the fibroblasts in the upper compartments of the chemotaxis chambers during their incubation in the chemotaxis assay. The values represent the mean of four replicates ± SEM.
* p values are not significant.

TABLE III

Inhibition of TGF-β-induced Chemotaxis by Anti-TGF-β Antibodies

Condition*	Chemotactic activity of fibroblasts per 20 OIF	P values[‡]
TGF-β + PBS	88 ± 6	
TGF-β + anti-TGF-β (140 μg/ml)	9 ± 1	<0.0025
TGF-β + anti-TGF-β (14 μg/ml)	9 ± 1	<0.0025
TGF-β + IgG (140 μg/ml)	83 ± 8	NS
TGF-β + IgG (14 μg/ml)	88 ± 7	NS
Denatured collagen + PBS	70 ± 3	
Denatured collagen + anti-TGF-β (140 μg/ml)	70 ± 6	NS
Denatured collagen + anti-TGF-β (14 μg/ml)	71 ± 7	NS
Fibronectin + PBS	91 ± 4	
Fibronectin + anti-TGF-β (140 μg/ml)	90 ± 6	NS
Fibronectin + anti-TGF-β (14 μg/ml)	92 ± 8	NS
PDGF + PBS	76 ± 6	
PDGF + anti-TGF-β (140 μg/ml)	78 ± 7	NS
PDGF + anti-TGF-β (14 μg/ml	75 ± 9	NS
PBS	12 ± 1	

* Chemoattractants were incubated with anti-TGF-β or control IgG as described in Materials and Methods.
[‡] Chemotactic values obtained with treatment of each chemoattractant with anti-TGF-β or IgG were compared with values for that chemoattractant treated with PBS and analyzed by Student's t test.

fibroblast chemoattractant but did not inhibit the chemotactic activity of human plasma fibronectin, human PDGF, or denatured type I calf skin collagen (Table III).

Discussion

TGF-β purified from human platelets is a very potent chemoattractant for fibroblasts in vitro. Dissociation by reduction of chains of TGF-β causes a marked loss of its chemotactic potency, suggesting that the native structure of TGF-β is essential for its ability to induce fibroblast chemotaxis. Polyvalent antibodies raised in rabbits effectively block the chemotactic activity of TGF-β. These same

antibodies have been demonstrated to block the growth inhibitory effects of TGF-β in fibroblasts (Keski-Oja, J., R. M. Lyons, and H. L. Moses, manuscript in preparation) and is probably exerting its effect in this study by preventing TGF-β from binding to receptors involved in the chemotactic response. The fact that the anti-TGF-β antibodies did not inhibit the chemotactic activity of PDGF, fibronectin, or collagen further demonstrates specificity of the antibodies for TGF-β.

A number of neoplastic and normal cells synthesize TGF-β (1, 2, 9, 11). The fact that TGF-β is produced by a variety of cells and can modulate several important fibroblast functions (i.e., chemotaxis, collagen, and fibronectin synthesis) may have far-reaching implications. For example, certain tumors (e.g., schirrous carcinoma of the breast and stomach and fibrosarcomas) are characterized by an excessive fibrotic reaction which could be caused by their releasing TGF-β (17). During embryogenesis, TGF-β may play a critical role in directing the migration of fibroblasts that synthesize the matrix scaffolding necessary for organogenesis. The release of TGF-β from platelets, lymphocytes, and monocytes/macrophages at sites of immune and nonimmune tissue injury may play a critical role in effecting the migration of neighboring connective tissue fibroblasts. It could then act as a stimulus for fibroblasts to produce increased quantities of matrix components (collagens and fibronectin).

Summary

Transforming growth factor β (TGF-β) is a potent chemoattractant in vitro for human dermal fibroblasts. Intact disulfide and perhaps the dimeric structure of TGF-β is essential for its ability to stimulate chemotactic migration of fibroblasts, since reduction with 2-ME results in a marked loss of its potency as a chemoattractant. Although epidermal growth factor (EGF) appears to be capable of modulating some effects of TGF-β, it does not alter the chemotactic response of fibroblasts to TGF-β. Specific polyvalent rabbit antibodies to homogenously pure TGF-β block its chemotactic activity but has no effect on the other chemoattractants tested (platelet-derived growth factor, fibronectin, and denatured type I collagen). Since TGF-β is secreted by a variety of neoplastic and normal cells including platelets, monocytes/macrophages, and lymphocytes, it may play a critical role in vivo in embryogenesis, host response to tumors, and the repair response that follows damage to tissues by immune and nonimmune reactions.

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