Stimulation of Fibroblast Chemotaxis by Human Recombinant Tumor Necrosis Factor α (TNF-α) and a Synthetic TNF-α 31–68 Peptide

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Summary

Macrophages are a major source of fibrogenic factors that promote healing of injured tissue. The recruitment of fibroblasts to sites of tissue injury is a prerequisite for optimal repair of tissue damage. In the present study, human recombinant tumor necrosis factor α (hrTNF-α), a major macrophage-derived cytokine, was demonstrated to be a potent fibroblast chemoattractant, inducing migration at picomolar concentrations. Anti-hrTNF-α monoclonal antibody neutralized most of the fibroblast chemotactic activity generated during short-term culture of human peripheral blood monocytes stimulated with bacterial lipopolysaccharide, suggesting that TNF-α is a major monocyte-derived fibroblast chemoattractant. The portion of the human TNF-α molecule responsible for its chemotactic stimulation of fibroblasts appears to reside in residues 31–68. This region is highly conserved between TNF-α and lymphotoxin. This peptide is not only itself chemotactic but is also able to block the chemotactic response of fibroblasts to hrTNF-α and vice versa, suggesting that they each mediate fibroblast migration through similar mechanisms. These data further underscore the potential importance of TNF-α in modulating a variety of fibroblast functions, including chemotaxis and synthesis of collagen, glycosaminoglycans, interleukin 1α (IL-1α) and -β, human histocompatibility leukocyte antigen A and B antigens, collagenase, prostaglandin E_2, and IL-6.

Macrophages are a major source of fibrogenic factors that promote healing of damaged connective tissue resulting from injury inflicted by various types of trauma, inflammation, and immune reactions. An early event in fibrogenesis is the recruitment of neighboring fibroblasts to the site of injury to synthesize and remodel new connective tissue matrix (1). Several macrophage-derived fibroblast chemoattractants (e.g., TGF-β, fibronectin, and platelet-derived growth factor) have been previously described that could direct this fibroblast recruitment (2–4). In the present study, it has been demonstrated that an additional macrophage product, TNF-α, possesses fibroblast chemotactic activity. Structure-function studies using synthetic TNF-α peptides indicate that fibroblast chemotactic activity may be mediated through amino acid sequences residing between residues 31 and 68.

Materials and Methods

Fibroblast Chemotaxis. Fibroblasts grown in monolayer cultures from infant foreskin explants were used as indicator cells in chemotaxis studies. Fibroblasts were cultured in maintenance medium (Eagle's MEM supplemented with 9% FCS, nonessential amino acids, 50 μg/ml ascorbic acid, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml amphotericin B). Fibroblast migration was quantitated as previously described using blind-well Boyden-type chambers equipped with gelatin-treated polycarbonate filters (8-μm pore size; Nucleopore Corp., Pleasanton, CA) (5).

Chemoattractants. Human recombinant TNF-α (HrTNF-α)[1] was purchased from Genzyme Corp. (Boston, MA) and had a specific activity of 2×10^7 neutralizing U/mg of protein. Porcine platelet-derived TGF-β1 was purchased from R & D Systems (Minneapolis, MN). FMLP was purchased from Sigma Chemical Co. (St. Louis, MO).

Anti-TNF-α and Anti-TGF-β1 Antibodies. Specific rabbit anti-porcine TGF-β1 IgG was purchased from R & D Systems. Rabbit anti-hrTNFα antiserum was purchased from Genzyme Corp. Anti-hrTNF-α mAb was a generous gift from Genentech Corp. (San Francisco, CA). Aliquots of these antibody preparations diluted in PBS (Gibco Laboratories, Grand Island, NY) or PBS alone were incubated with TGF-β1, hrTNF-α, or supernatants from monocytes cultured with LPS (described below) at 4°C for 12 h with continuous mixing on a rocker platform.

Synthesis of Human TNF-α Peptides. Peptides of various lengths representing the COOH-terminal 148 residues of the deduced human TNF-α sequence (6) were synthesized by the solid-phase

[1] *Abbreviations used in this paper:* hrTNF-α, human recombinant TNF-α; MNL, mononuclear leukocytes; OIF, oil immersion field; PDGF, platelet-derived growth factor.

method of Merrifield (7) with the aid of an automated peptide synthesizer (990; Beckman Instruments, Inc., Palo Alto, CA). The peptides were purified by gel filtration and reverse-phase HPLC (8). Amino acid composition of each peptide was confirmed by use of an automatic amino acid analyzer (121 MB; Beckman Instruments, Inc.) (9).

Isolation and Culture of Peripheral Blood Monocytes. Blood taken by venipuncture from healthy human volunteers was collected in heparinized syringes and centrifuged on Ficoll-Hypaque cushions to obtain a population of mononuclear leukocytes (MNL), as previously described (10). After washing in RPMI 1640 containing fresh L-glutamine, penicillin (100 U/ML), and streptomycin (100 μg/ml) (Gibco Laboratories), a monocyte-enriched population was obtained by subjecting the MNL to centrifugal elutriation, as previously described (11). To obtain highly purified monocyte populations of cells, elutriated MNL were labeled with M02-R01 (monocyte-specific PE conjugate) and KC56-FITC (T 200 Ag) (pan leukocyte marker) (Coulter Cyto-Stat; Coulter Immunology, Hialeah, FL) and sorted as previously described (12).

To generate supernatants containing products from activated monocytes, elutriated monocytes (4×10^6 cells) and sorted monocytes (6×10^5) were placed in separate wells of 24-well 3424 Mark II Cluster Dishes (Costar, Cambridge, MA), each in 1 ml of serum-free RPMI 1640 containing streptomycin (100 μg/ml), penicillin (100 U/ml), fresh L-glutamine (300 μg/ml), and 100 ng/ml LPS W from *Escherichia coli* 055:BB (Difco Laboratories, Inc., Detroit, MI). Monocytes were cultured for 48 h at 37°C in an incubator with a humidified atmosphere containing 5% CO_2. Culture supernatants were harvested, clarified by centrifugation, and stored at −70°C for up to 4 d before study.

Results

Chemotaxis of Fibroblasts to hrTNF-α. On a total of three occasions when wide ranges of concentrations of hrTNF-α were studied, maximal migration of fibroblasts was consistently induced by concentrations of hrTNF-α ranging from 50 to 900 pg/ml (1.39–25 pM). A representative dose-response curve is presented in Fig. 1. The response curve was always dose dependent and bell shaped. Similar bell-shaped response curves have been previously reported with other fibroblast chemoattractants (2, 3). To assess whether TNF-α was inducing migration of fibroblasts by acting as a chemokinetic or chemotactic agent, a Zigmond-Hirsch checkerboard analysis was performed. TNF-α caused migration of fibroblasts only when it was present in excess in the lower compartment of the chemotaxis chambers, indicating that it acts as a chemotactic agent (Table 1). Similar results were obtained when this experiment was repeated (data not shown).

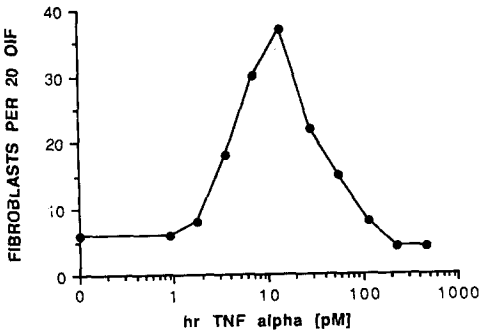

Figure 1. HrTNF-α was added to the lower compartment of chemotaxis chambers at the concentrations indicated, and migration of fibroblasts was quantitated.

Inhibition of TNF-α-induced Chemotaxis by Anti-TNF-α Antibodies. To assess the effect of anti-hrTNF-α antibodies on the ability of hrTNF-α to induce fibroblast chemotaxis, hrTNF-α or TGF-β1 (an unrelated chemotactic cytokine) were incubated for 12 h at 4°C with polyclonal rabbit anti-hrTNF-α or anti-TGF-β1 antibodies, or with anti-hrTNF-α mAb. There was marked inhibition of the chemotactic response of fibroblasts to TNF-α when it was preincubated with polyclonal anti-hrTNF-α antiserum or with anti-hrTNF-α mAb, but no inhibition of chemotaxis was observed when TNF-α was treated with anti-TGF-β1 IgG (Table 2). The anti-hrTNF-α antiserum and anti-hrTNF-α mAb did not reduce the chemotactic response of fibroblasts to TGF-β1, indicating that their effect was not through a general suppression of the chemotactic responsiveness of fibroblasts (Table 2).

Chemotaxis of Fibroblasts to FMLP. It has been suggested that the chemotactic activity for neutrophils and monocytes reported with some preparations of hrTNF-α derived from *E. coli* might be due to small amounts of contaminating *N*-formylated methionyl peptides or *N*-formylated methionyl-hrTNF-α (13). Therefore, it was of interest to explore the possible role of *N*-formylated compounds in mediating the fibroblast chemotactic response to hrTNF-α. FMLP is a prototype of bacteria-derived *N*-formylmethionyl chemotactic peptides that have been studied extensively in neutrophils and monocytes (14, 15). Therefore, the ability of FMLP to induced fibroblast migration was assessed. In a total of three dose-response experiments performed, FMLP induced fibroblast migration at 10 fM to 10 pM. A representative dose-

Table 1. *Effect of Varying Concentration Gradients of hrTNF-α on Fibroblast Migration*

hrTNF-α concentration in upper compartment	hrTNF-α contentration in lower compartment				
	14.4 pm	72. pm	3.6 pm	1.8 pm	0 pm
pm					
14.4	8 ± 1*	7 ± 1	7 ± 1	8 ± 2	8 ± 1
7.2	17 ± 3	8 ± 1*	7 ± 1	7 ± 1	9 ± 2
3.6	52 ± 6	32 ± 4	12 ± 3*	10 ± 1	15 ± 2
1.8	44 ± 2	32 ± 5	34 ± 4	11 ± 1*	8 ± 1
0	72 ± 11	45 ± 2	36 ± 5	15 ± 2	13 ± 2*

Different concentrations of hrTNF-α were added to the upper and/or lower compartments of chemotaxis chambers, and the number of fibroblasts migrating to the lower surface of each filter was quantitated. Each value represents the mean of four replicates ± SEM of fibroblasts per 20 oil immersion field (OIF).

* The number of fibroblasts migrating when hrTNF-α was present in a given chemotaxis chamber at the same concentration in the upper and lower compartments.

Table 2. *Inhibition of hrTNF-α–induced Chemotaxis by Anti-hrTNF-α Antibodies*

Exp.	Condition*	Chemotactic activity	*p* value‡
1	hrTNF-α (29.4 pM) + PBS	33 ± 4§	
	hrTNF-α (29.4 pM) + anti-hrTNF-α	4 ± 1	<0.0025
	hrTNF-α (29.4 pM) + anti-TGF-β1	42 ± 6	NS
	TGF-β1 (1.6 pM) + PBS	35 ± 5	
	TGF-β1 (1.6 pM) + anti-hrTNF-α	39 ± 4	NS
	TGF-β1 (1.6 pM) + anti-TGF-β1	10 ± 2	<0.0025
	PBS	9 ± 1	
2	hrTNF-α (29.4 pM) + PBS	103 ± 15	
	hrTNF-α (29.4 pM) + anti-hrTNF-α mAb	9 ± 2	<0.0001
	TGF-β1 (4 pM) + PBS	76 ± 10	
	TGF-β1 (4 pM) + anti-hrTNF-α mAb	77 ± 13	NS
	PBS	12 ± 1	

* For exp. 1, aliquots of hrTNF-α (1 ng in 100 μl PBS) and TGF-β1 (80 pg in 100 μl PBS) were separately incubated at 4°C overnight with 10 μl rabbit anti-hrTNF-α antiserum plus 15 μl PBS, 25 μl rabbit anti-TGF-β1 IgG (5 μg), or 25 μl PBS. The samples were then brought to a volume of 2 ml in serum-free maintenance medium and assayed for fibroblast chemotactic activity. For Exp. 2, aliquots of hrTNF-α (1 ng in 100 μl PBS and TGF-β1 [200 pg in 100 ml PBS]) were separately incubated at 4°C overnight with 25 μl PBS or anti-hrTNF-α monoclonal IgG1 (20 μg/25 μl PBS). The samples were then brought up to a value of 2 ml in serum-free maintence medium and assayed for fibroblast chemotactic activity.
‡ Chemotactic values obtained with treatment of each chemoattractant with anti-hrTNF-α or anti-TGF-β1 were compared with values obtained after similar treatment with PBS and analyzed by student's *t* test.
§ Fibroblasts per 20 OIF.

response curve is plotted in Fig. 2. Zigmond-Hirsch checkerboard analysis indicated that this migration was predominantly chemotactic in nature since most migration occurred when FMLP was present in excess in the lower compartment of the chemotaxis chambers (Table 3). Similar results were observed when this experiment was repeated (data not shown). On neutrophils, there appears to be a single class of receptor that recognizes most formylated methionyl peptides, and FMLP can compete for binding of synthetic and bacteria-derived chemotactic formylated methionyl peptides to this class of receptor (14, 15). If there were formylated methionyl peptides in the hrTNF-α preparation, these neutrophil-related data would imply that it should be possible to block their chemotactic effect on fibroblasts by allowing FMLP to be present in the upper cell compartment of the chemotaxis chambers when the chemotaxis assay is performed. When FMLP was added to the upper compartment of chemotaxis chambers containing fibroblasts, it could not block the migration to hrTNF-α, but it did block the response to FMLP (Table 4). These findings, although indirect, suggest that the chemotactic response of fibroblasts to hrTNF-α is not mediated through a receptor recognized by or a mechanism related to FMLP.

Chemotaxis of Fibroblasts to Synthetic TNF-α 31–68. In an effort to localize the region of the TNF-α molecule involved in the fibroblast chemotactic response, synthetic peptides were made representing all but the first nine amino acids of human TNF-α, and tested for their ability to induce fibroblast migration (Fig. 3). Peptides were repeatedly tested over a wide concentration range for their ability to induce fibroblast migration. Only one peptide, TNF-α 31–68, induced fibroblast migration (Fig. 4). The response curve, like that of hrTNF-α, was bell shaped (Fig. 4). Zigmond-Hirsch checkerboard analysis indicated that this active peptide induced mostly chemotactic migration of fibroblasts (Table 5). A small amount of chemokinetic activity was repeatedly observed at intermediate concentrations of the peptide (Table 5). To assess the rela-

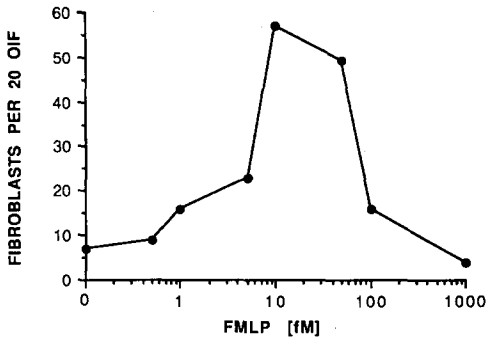

Figure 2. A stock solution of FMLP (1 mM) was prepared by slowly adding $NaHCO_3$ to a suspension of FMLP in water with frequent vortexing until the solution was clear. Various concentrations of FMLP prepared from this stock solution were tested for their ability to induce fibroblast migration.

Table 3. *Effect of Varying Concentration Gradient of FMLP on Fibroblast Migration*

FMLP concentration in upper compartment	FMLP concentration in lower compartment			
	5 fM	1 fM	0.5 fM	0 fM
fM				
5	24 ± 3*	14 ± 1	13 ± 2	9 ± 1
1	30 ± 5	18 ± 2*	17 ± 2	14 ± 2
0.5	42 ± 2	31 ± 2	13 ± 3*	17 ± 2
0	55 ± 9	35 ± 5	27 ± 6	14 ± 3*

FMLP was added to upper and lower compartments of modified blind-well chemotaxis chambers at the concentrations indicated, and fibroblast migration was quantitated. Each value represents the mean of four replicates ± SEM.

* The number of fibroblasts migrating when FMLP was present in a given chemotaxis chamber at the same concentration in the upper and lower compartments.

Table 4. *Effect of incubation of Fibroblasts with FMLP on the Chemotactic Response to hrTNF-α*

Condition*	Chemotactic activity	*p* value‡
Fibroblasts incubated with media		
hrTNF-α (29.4 pM)	37 ± 6§	
FMLP (10 fM)	45 ± 6	
Media	5 ± 1	
Fibroblasts incubated with FMLP (100 pM)		
hrTNF-α (29.4 pM)	36 ± 4	NS
FMLP (10 fM)	7 ± 1	<0.0004
Media	3 ± 1	NS

* Fibroblasts were suspended in complete serum-free maintenance media with or without 100 pM FMLP and added to the upper compartment of chemotaxis chambers. Migration of fibroblasts to FMLP and complete serum-free media was then measured. Values represent the mean ± SEM of quadruplicate values.

‡ The chemotactic activities obtained when lower compartments of chemotaxis chambers contained hrTNF-α, FMLP, or media, and the upper compartment contained fibroblasts suspended in FMLP (100 pM) were each compared by the two-sample student's *t* test with the values determined in chambers containing fibroblasts suspended in media.

§ Fibroblasts per 20 OIF.

tionship of the chemotactic response of fibroblasts to TNF-α 31–68 and hrTNF-α, we repeatedly performed experiments in which the fibroblasts in the upper cell compartment of the chemotaxis chambers were suspended in either media, hrTNF-α, or TNF-α 31–68, and compared the migration of variously treated fibroblasts to hrTNF-α, TNF-α 31–68, TGF-β1, and media. Results of a typical experiment are shown in Table 6. HrTNF-α and TNF-α 31–68 were equally effective in blocking the migration of fibroblasts to each other, but neither blocked the response to TGF-β1 (Table 6). These data suggest that hrTNF-α and TNF-α 31–68 induce fibroblast migration through either a common receptor or mechanism.

Neutralization by Anti-TNF-α mAb of Fibroblast Chemotactic Activity in Supernatants from Cultures of Human Monocytes Stimulated by LPS. Since monocytes/macrophages are potential sources of several fibroblast chemoattractants (e.g., TGFβ-1, fibronectin, leukotriene B_4, platelet-derived growth factor [PDGF]), it was important to determine the relative portion of fibroblasts chemotactic activity in cultures of stimulated human peripheral blood monocytes attributable to TNF-α. To assess this, peripheral blood monocytes purified by centrifugal elutriation or centrifugal elutriation and FACS were stimulated for 24–48 h by LPS, and the harvested supernatants, after treatment with anti-TNF-α mAb or PBS as a control, were tested at various dilutions for fibroblast chemotactic activity. Supernatant from cultures of elutriated monocytes stimulated with LPS elicited maximal chemotactic response of fibroblasts at a 1:80 dilution (Fig. 5 *A*). The chemotactic activity at this and other dilutions of the supernatant was almost totally neutralized by anti-hrTNF-α mAb (Fig. 5 *A*). Supernatants from elutriated and sorted monocytes stimulated with LPS induced fibroblast migration at 1:20, 1:40, and 1:80 dilutions (Fig. 5 *B*). Anti-hrTNF-α mAb neutralized most of the fibroblast chemotactic activity in the supernatant at these dilutions (Fig. 5 *B*). These data suggest that

10-36‡	Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Ile Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
31-68	Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
69-100	Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Cys Val Asn Leu Leu Ser Ala Ile Cys Ser Pro
101-135	Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu
136-157	Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu

Figure 3. Peptides representing the deduced human TNF-α sequence were synthesized by the solid-phase method of Merrifield (7) on an automated peptide synthesizer (990; Beckman Instruments, Inc.) (8). (‡)This peptide was synthesized without residues 1–9. It has been previously demonstrated that NH_2-terminal residues are not essential for TNF-α activity (19).

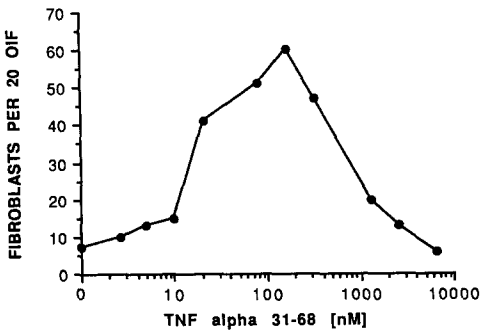

Figure 4. Synthetic TNF-α 31–68 was added to the lower compartment of chemotaxis chambers at the concentrations indicated, and migration of fibroblasts was quantitated.

TNF-α is the major fibroblast chemoattractant produced by peripheral blood monocytes under these experimental conditions.

Discussion

HrTNF-α is a potent chemotactic agent for fibroblasts in vitro inducing their migration at picomolar concentrations. The TNF-α fibroblast chemotactic dose-response curve is bell shaped. This type dose-response curve has been previously observed with several different fibroblast chemotactic agents and with FMLP in this present study (2–4). A synthetic peptide representing the TNF-α amino acid sequence 31–68 also induced chemotaxis of fibroblasts. TNF-α 31–68 was much less potent than hrTNF-α in inducing chemotaxis. A similar reduction of potency has been reported with synthetic peptides of growth hormone and of human IL-1β (16–18). The reduced potency of TNF-α 31–68 relative to hrTNF-α is likely

Table 6. *Effect of Incubation of Fibroblasts with hrTNF-α or TNF-α 31–68*

Condition*	Chemotactic activity	*p* Value‡
Fibroblasts suspended with media		
hrTNF-α (29.4 pM)	118 ± 10§	
TNF-α 31–68 (164 nM)	144 ± 5	
TGF-β (1.6 pM)	137 ± 10	
Media	16 ± 1	
Fibroblasts suspended in hrTNF-α (29.4 pM)		
hrTNF-α (29.4 pM)	12 ± 3	<0.0001
TNF-α 31–68 (164 nM)	11 ± 3	<0.0001
TGF-β (1.6 pM)	123 ± 10	NS
Media	17 ± 3	NS
Fibroblasts suspended in TNF-α 31–68 (164 nM)		
hrTNF-α (29.4 pM)	16 ± 2	<0.0001
TNF-α 31–68 (164 nM)	17 ± 3	<0.0001
TGF-β1 (1.6 pM)	135 ± 16	NS
Media	12 ± 1	NS

* Fibroblasts were suspended in complete serum-free maintenance media with or without hrTNF-α (29.4 pM) or TNF-α 31–68 (164 nM) and added to the upper compartment of chemotaxis chambers. Migration of fibroblasts to hrTNF-α, TNF-α 31–68, TGF-β1, or media was then quantitated. Values represent the mean ± SEM of quadruplicate values.
‡ The chemotactic activities obtained when the lower compartment of chemotaxis chambers contained each chemoattractant or media and the upper compartment of the chambers contained fibroblasts suspended in hrTNF-α or TNF-α 31–68 were each compared by the two-sample student's *t* test with the values generated in chambers containing fibroblasts suspended in media.
§ Fibroblasts per 20 OIF.

Table 5. *Effect of Varying Concentration Gradients of TNF-α Peptide 31–68 on Fibroblast Migration*

TNF-α peptide 31–68 concentration in upper compartment	TNFα peptide 31–68 concentration in lower compartment				
	329 nM	164 nM	41 nM	21 nM	0 nM
nM					
329	8 ± 1*	12 ± 2	9 ± 1	10 ± 1	16 ± 3
164	43 ± 3	22 ± 4*	16 ± 3	11 ± 1	14 ± 2
41	57 ± 6	47 ± 5	26 ± 4*	28 ± 5	31 ± 2
21	67 ± 2	46 ± 2	49 ± 7	9 ± 1*	20 ± 6
0	86 ± 13	73 ± 4	57 ± 3	31 ± 4	17 ± 3*

Different concentrations of synthetic TNF-α 31–68 were added to the upper and/or lower compartments of chemotaxis chambers, and the number of fibroblasts miograting to the lower surface of each filter was quantitated. Each value represents the mean of four replicates ± SEM of fibroblasts per 20 OIF.
* The number of fibroblasts migrating when the peptide was present in a given chemotaxis chamber at the same concentration in the upper and lower compartments.

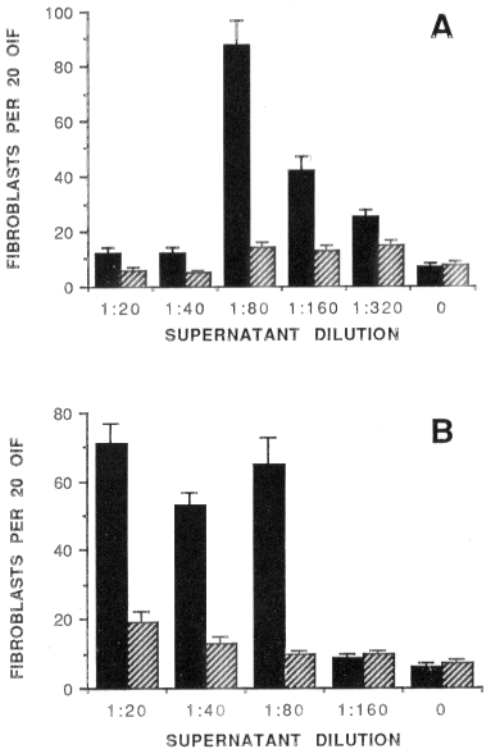

Figure 5. (*A*) Monocytes were purified by centrifugal elutriation (>85% pure as assessed by FACS analysis). (*B*) Monocytes from a different elutriator run were purified further by sorting (by >98% monocytes as assessed by FACS anlaysis). Elutriated and sorted monocytes were cultured for 48 h with LPS as described in Materials and Methods. Aliquots of supernatants (100 μl) were incubated overnight with 5 μl PBS (as a control) or with 5 μl (4 μg) anti-TNF-α mAb (Genentech) at 4°C. The supernatants were then tested at dilutions indicated for their ability to induce fibroblast migration. Results in *A* and *B* are from two different fibroblast chemotaxis assays. (■) PBS-treated supernatants; (▨) anti-TNF-α mAb-treated supernatant.

due to its small size and lack of other amino acid sequences necessary for binding to TNF-α receptors or for conferring secondary, tertiary, and quaternary structural features for optimal interaction with fibroblasts.

The finding that a synthetic peptide of TNF-α composed of residues 31–68 has a biologic activity of the parent TNF-α molecule is of special interest since a particularly conserved region of human TNF-α occurs in amino acids 35–66. This region shares 50% amino acid homology with human lymphotoxin (6). X-ray crystallographic analyses indicate that TNF-α forms a trimer with residues near the COOH and NH_2 termini, being located at the base of the trimer and likely involved in receptor binding (19). A pocket lined by highly conserved (between human TNF-α and lymphotoxin) sequences 11–13, 37–42, 49–57, and 155–157 may be critically involved in binding of TNF-α and lymphotoxin to its receptor (19). We have not yet assessed the ability of TNF-α 31–68 or the remaining TNF-α peptides used in this study to compete with hrTNF-α binding to its receptor.

Although FMLP was also found to be a potent fibroblast chemoattractant in the present study, several different lines of evidence suggest that trace or low concentrations of *N*-formylmethionyl peptides are not involved in the chemotactic response of fibroblasts to the hrTNF-α preparations tested. First, mAb to hrTNF-α selectively blocked the chemotactic response of fibroblasts to hrTNF-α but not to TGF-β1. This argues against trace contaminants of *N*-formylmethionyl peptides in the hrTNF-α preparations being responsible for the fibroblast chemotactic response. Second, a synthetic peptide consisting of TFN-α residues 31–68 is itself chemotactic for fibroblasts and can selectively inhibit the migration of fibroblasts to hrTNF-α and vice versa, suggesting both the peptide and hrTNF-α induce fibroblast chemotaxis through a similar mechanism(s). Finally, FMLP, a prototype peptide for *N*-formylmethionyl chemoattractants that should block the chemotactic response of fibroblasts to *N*-formylmethionyl peptides, does not block the chemotactic response of fibroblasts to hrTNF-α.

Other fibroblast functions have been reported to be affected by TNF-α in vitro, including stimulation of growth and synthesis of collagenase, hyaluronic acid, prostaglandin E_2, and IL-6 (20–23). TNF inhibits collagen gene transcription and collagen synthesis by fibroblasts (24). Synthesis of IL-1α and -1β, and HLA-A,B mRNAs, are increased by TNF-α, as is surface expression of HLA A,B antigens by fibroblasts (25, 26). Studies are being performed to assess the effect of the synthetic TNF-α peptides used in this study on these fibroblast functions as well as their ability to lyse L cell targets.

Under certain culture conditions, monocytes/macrophages have been shown to produce several fibroblast chemoattractants (i.e., TGF-β1, fibronectin, PDGF, and LTB_4). Our data suggest that TNF-α is the major biologically active fibroblast chemoattractant produced during short-term culture of peripheral blood monocytes stimulated by LPS. The fact that most of the fibroblast chemotactic activity produced by LPS-stimulated monocytes in short-term culture is TNF-α may be due to the following. (*a*) TGF-β1 is secreted by monocytes/macrophages as a latent molecule bound to an acid-labile binding protein that blocks its biologic activity (27, 28). (*b*) Fibronectin is synthesized by macrophages in long-term culture (4–10 d) and not during short-term culture (29). (*c*) Most (up to 80%) of PDGF secreted by macrophages is bound to acid labile proteins (mostly α_2-macroglobulin) and, like TGF-β1, require acidification for expression of biologic and receptor binding activity (30). (*d*) Macrophages do not synthesize LTB_4 in response to LPS stimulation (31).

TNF-α is synthesized by a variety of different macrophages, including those of pulmonary, hepatic, bone marrow, and peritoneal origins, and perhaps by T lymphocytes, mast cells, smooth muscle cells, and NK cells under certain circumstances (see reference 32 for review). Macrophages produce TNF-α after exposure to many different stimuli, including LPS, some Gram-positive organisms, lysates of certain parasites, and Sendai and influenza viruses (see reference 32 for review). When hrTNF-α is daily injected subcutaneously into laboratory animals, it induces an acute inflammatory response that is followed by chronic inflammation, accumulation of fibroblasts, and fibrosis (33). This present study suggests that TNF-α is a major monocyte/macrophage–derived product that may

function to direct the recruitment of fibroblasts, as do other growth factors and products from activated macrophages, and may be critically involved in the fibrogenic response that typically follows tissue inflammation in vivo that accompanies tissue injury and many different types of infections and certain tumors.

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