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Transforming growth factor β1, a potent chemoattractant for human neutrophils, bypasses classic signal-transduction pathways*

(chemotaxis/guanine nucleotide-binding proteins/degranulation/superoxide anion)

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ABSTRACT **Transforming growth factor β1 (TGF-β1), a homodimeric polypeptide (M_r 25,000), derives from inflammatory cells and acts as a chemoattractant for monocytes and fibroblasts. We report here that TGF-β1 is also the most potent chemoattractant yet described for human peripheral blood neutrophils. Recombinant TGF-β1 elicited dose-dependent directed migration of neutrophils under agarose that was inhibited in the presence of a neutralizing antibody to TGF-β1. Maximal chemotaxis was evoked by TGF-β1 at femtomolar concentrations, whereas conventional chemoattractants act at nanomolar concentrations: on a molar basis, TGF-β1 was 150,000 times more potent than fMet-Leu-Phe. In contrast, TGF-β1 provoked neither exocytosis nor the production of superoxide by neutrophils. We further analyzed the mechanism by which TGF-β1 elicits chemotaxis (GTPase activity, [Ca^{2+}], and actin polymerization). In contrast to the conventional chemoattractant fMet-Leu-Phe, TGF-β neither activated classic heterotrimeric guanine nucleotide-binding proteins nor provoked global mobilization of intracellular Ca^{2+}. Chemoattraction by both fMet-Leu-Phe and TGF-β1 was inhibited by cycloheximide and actinomycin D. Moreover, chemotaxis in response to TGF-β1 was associated with the polymerization of actin. The selectivity and potency of TGF-β1 as a chemoattractant suggest that it elicits directed cell migration by means of a pathway that depends not on classic intracellular signals but on protein synthesis.**

Transforming growth factor β1 (TGF-β1), a homodimeric polypeptide (M_r 25,000) originally purified from human platelets and placenta and from bovine kidney, may also be derived from inflammatory cells (macrophages, T lymphocytes, and neutrophils) (1, 2). TGF-β1 is a member of a multigene family and, although initially defined by its ability to stimulate anchorage-independent growth of nonneoplastic rat kidney fibroblasts (3), it demonstrates a wide variety of effects. TGF-β1 elicits chemotaxis of fibroblasts in concert with the synthesis of matrix proteins (collagen, fibronectin) (4, 5) and is also a chemoattractant for peripheral blood monocytes at femtomolar concentrations (6).

Neutrophils are the initial cell type found at areas of tissue damage. After exposure to phlogistons such as complement components or bacterial chemoattractants, neutrophils adhere to vascular surfaces, undergo chemotaxis, degranulate, and generate toxic oxygen metabolites (7). Since latent TGF-β1 may be activated by the low pH at inflammatory sites or by tissue proteases or exoproteases found on inflammatory cells, we investigated whether TGF-β1 might in turn trigger neutrophil functions. We compared the effects of TGF-β1 with those of fMet-Leu-Phe (fMLP) on the recruitment (chemotaxis) and activation (degranulation and superoxide generation) of neutrophils. TGF-β1 was an exquisitely potent chemoattractant, yet selective in eliciting *only* chemotaxis by neutrophils. Moreover, TGF-β1 bypassed classic signaling mechanisms (GTPase activity, intracellular Ca^{2+}, actin polymerization) for chemotaxis and triggered protein synthesis.

MATERIALS AND METHODS

Materials. Hepes-buffered saline (150 mM Na^+/1.2 mM Mg^{2+}/1.3 mM Ca^{2+}/155 mM Cl^-/10 mM Hepes, pH 7.43) or phosphate-buffered saline with Ca^{2+} and Mg^{2+} (0.68 mM Ca^{2+}/0.5 mM Mg^{2+}, pH 7.43) was used for all cell suspensions. fMLP was stored at 10 mg/ml in dimethyl sulfoxide and diluted into buffer before use. Ficoll 400, horse ferricytochrome *c*, and bovine serum albumin were obtained from Sigma. [γ-^{32}P]GTP was purchased from Amersham. Recombinant human TGF-β1 and partially purified goat antibody to TGF-β1 were gifts of Michael Palladino (Genentech).

Cell Suspension. Heparinized (10 units/ml) venous blood was obtained from healthy donors and polymorphonuclear leukocytes were prepared as described (8).

Chemotaxis. Chemotaxis of neutrophils and mononuclear cells under agarose was measured in triplicate by the modified method of Nelson *et al.* (9). Neutrophils (3×10^5 per ml) or mononuclear cells (6×10^5 per ml) were placed in a center well. Control buffer was placed in an inner well, and either buffer or a chemotactic stimulus was placed in the outer flanking well. Plates were incubated (3–4 hr for neutrophils, 18 hr for mononuclear cells) at 37°C in humidified 5% CO_2/95% air. The reaction was terminated with formaldehyde (3.7% by volume) and the cells were stained with Weigert's hematoxylin. The linear distance migrated by cells from the margin of the well was measured using an inverted microscope. Spontaneous migration (SM) was determined as the movement of cells toward the control well. Net migration (NM) was determined after subtracting SM from the distance traveled by the cells toward the chemoattractant. Chemotaxis in a Boyden chamber was measured as described (10).

Granule Enzyme Release and Superoxide Generation. The extracellular release of the granule-associated enzymes lysozyme and β-glucuronidase was measured as described (11). The generation of superoxide was measured in the presence of cytochalasin B (5 μg/ml) as the superoxide dismutase-inhibitable reduction of cytochrome *c* over 5 min (12).

Abbreviations: TGF-β1, transforming growth factor β1; fMLP, fMet-Leu-Phe; PT, pertussis toxin; SM, spontaneous migration; NM, net migration; $[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration.

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GTPase Activity of Neutrophil Membranes. Neutrophil membranes were isolated by nitrogen cavitation and sucrose gradient centrifugation (13). GTPase activity was assayed according to Wilde *et al.* (14).

Cytosolic Ca^{2+} Concentration ($[Ca^{2+}]_i$). Neutrophils (10^8 per ml) were incubated with 10 μM fura-2 acetoxymethyl ester in Hepes-buffered saline at 37°C for 5 min. They were then diluted 10-fold with Hepes-buffered saline, incubated at 37°C for 20 min, and washed twice. Fluorescence changes were monitored at 37°C with excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm in a Perkin–Elmer 650-10S spectrofluorimeter. $[Ca^{2+}]_i$ was calculated according to the equations of Grynkiewicz *et al.* (15).

Fluorescence Labeling of F-Actin. Cells were stained with rhodamine-labeled phalloidin by the modified method of Amato *et al.* (16). Glass coverslips were coated with agarose (containing human serum albumin) in Falcon Petri dishes and cells were plated in wells under the same conditions as for the chemotaxis assays. After a specified time, the agarose was removed and the cells were fixed with formaldehyde (3.7%), permeabilized with lysophosphatidylcholine (0.1 mg/ml, 5 min), and stained with rhodamine-labeled phalloidin (0.16 μM, 37°C, 15 min in the dark). Cells were viewed in a Leitz Orthoplan microscope (×63 oil-immersion objective) equipped with a mercury lamp and a Wild camera.

RESULTS

TGF-β1 Is Chemotactic for Neutrophils. TGF-β1 provoked directed migration of neutrophils in a dose-dependent fashion (Fig. 1). Maximal chemotaxis (NM = 316.7 ± 38.9 μm, mean ± SEM, n = 9) was found at an exceedingly low concentration (40 fM) of growth factor (EC_{50} = 10 fM). This response was 60% of that generated by the chemotactic peptide fMLP at 10 nM (NM = 525.8 ± 49.5 μm). As noted with most other chemoattractants, the chemotactic effect of TGF-β1 diminished at higher concentrations. TGF-β1 elicited directed migration as opposed to chemokinesis, since (*i*) leading-front determinations were made after the subtraction of SM of cells toward the opposite well containing control medium and (*ii*) SM of cells did not differ between the sets exposed to chemoattractant and those exposed to buffer alone.

Because of the exceedingly low concentration of TGF-β1 required for chemotaxis, we confirmed the mass of our original sample of TGF-β1 by amino acid analysis (Pico·Tag system, Waters). To confirm the specificity of the chemotactic response, we evaluated the effect of a neutralizing anti-TGF-β1 antibody on chemotaxis. Pretreatment of TGF-β1 with an antibody directed against TGF-β1 (4 μg/ml) inhibited chemotaxis elicited by TGF-β1 (87.0 ± 9.0% inhibition, n = 5, $P \leq 0.03$); the antibody neither elicited chemotaxis on its own (NM = 43.6 ± 30.2 μm, n = 3) nor had any effect on that elicited by fMLP (data not shown). Nonimmune serum did not affect chemotaxis induced by TGF-β1.

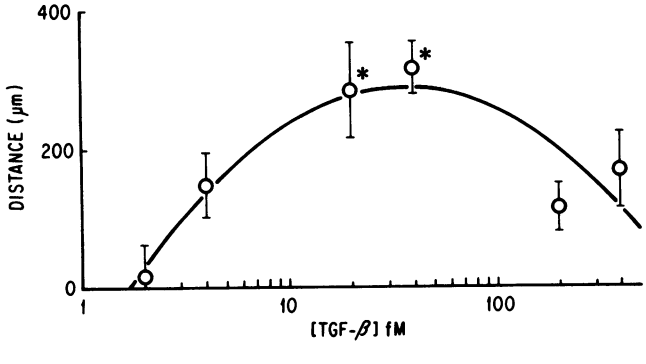

FIG. 1. Chemotaxis of human peripheral blood neutrophils in response to recombinant TGF-β. Chemotaxis of neutrophils under agarose was measured by the modified method of Nelson *et al.* (9). Data are the means of five or more experiments. Asterisks indicate $P < 0.003$.

Recombinant TGF-β1 contains small but measurable amounts of endotoxin (1 pg/μg of TGF-β1; M. Palladino, personal communication). To exclude contaminating endotoxin as the chemoattractant, we examined the effect of lipopolysaccharide on chemotaxis of neutrophils under agarose. Endotoxin (*Escherichia coli*, 10 pg/ml) did not significantly elicit chemotaxis under agarose (NM = 94.5 μm, n = 2 in triplicate).

To confirm that chemotaxis under agarose was comparable to that in the Boyden chamber, we examined the effect of TGF-β1 on neutrophil migration in a Boyden chamber. In this system, neutrophils migrated to TGF-β at peak concentrations (400 fM, n = 3) minimally greater than that seen under agarose.

TGF-β1 Does Not Activate Neutrophils. Having shown that TGF-β1 was capable of recruiting neutrophils to a site of injury (chemotaxis), we determined whether it provoked activation of neutrophils (degranulation and superoxide generation). TGF-β1 (37°C, 60 min) did not elicit significant release of azurophil granules (β-glucuronidase, lysozyme) or specific granules (lysozyme) at concentrations that elicited chemotaxis (femtomolar) or at concentrations that elicit cytokine production in other cells (picomolar) (Table 1). Prolonged incubation of cells with TGF-β1 (5 min to 4 hr) did not change this response (data not shown). Furthermore, pretreatment of cells with TGF-β1 (37°C, 60 min, 40–4000 fM) neither enhanced nor inhibited degranulation elicited by 100 nM fMLP (Table 1). Thus TGF-β1 neither stimulated degranulation on its own nor primed cells to respond to other stimuli.

The generation of superoxide by neutrophils proceeds after assembly of the multicomponent respiratory burst oxidase. We evaluated the effect of TGF-β1 on the generation of superoxide by human peripheral blood neutrophils. Incubation of neutrophils with TGF-β1 over a wide range of doses and times (37°C, 5 min to 4 hr, 40–4000 fM) did not provoke superoxide generation (Table 1). Furthermore, preincubation of neutrophils with these concentrations of TGF-β1 neither inhibited nor enhanced the ability of cells to generate superoxide in response to 100 nM fMLP (Table 1).

Role of G Proteins in Chemotaxis Provoked by TGF-β1. Exposure of neutrophils to the chemoattractant fMLP results in the activation of plasmalemmal heterotrimeric guanine nucleotide-binding proteins (G proteins) and hydrolysis of GTP (17). Since other chemoattractant-induced responses are mediated by pertussis toxin (PT)-sensitive GTP-binding proteins (7), we examined whether chemotaxis provoked by

Table 1. Effect of TGF-β on activation of human peripheral blood neutrophils

Stimulus	β-Glucuronidase	Lysozyme	Superoxide
TGF-β (40 fM)	3.1 ± 0.9	0.7 ± 0.4	0.0 ± 0.3
TGF-β (40 pM)	2.1 ± 0.8	1.0 ± 1.0	0.0 ± 0.5
fMLP (100 nM)	26.1 ± 1.5	53.1 ± 7.2	32.2 ± 3.3
fMLP + TGF-β (40 fM)	21.2 ± 2.6	48.5 ± 8.8	32.7 ± 2.5
fMLP + TGF-β (40 pM)	24.1 ± 0.9	42.7 ± 7.0	31.6 ± 2.4

The release of β-glucuronidase and lysozyme is expressed as percent of total cell enzyme released by 0.2% Triton X-100. Background release was subtracted. The generation of superoxide is expressed as nmol of cytochrome *c* reduced per 10^6 cells (mean ± SEM of three experiments). Neutrophils (1.24×10^6 per ml) were treated with TGF-β1 and the reaction was terminated after 5 min, or neutrophils were pretreated with TGF-β1 for 60 min before stimulation with 100 nM fMLP for 5 min.

TGF-β1 was also PT-inhibitable. Exposure of neutrophils to *Bordetella pertussis* toxin (1 μg/ml) throughout the migration period inhibited chemotaxis under agarose in response to fMLP (52.5 ± 10.8% of control, $n = 3$, $P < 0.02$). In contrast, PT did not inhibit chemotaxis elicited by TGF-β1 (104.4 ± 5.5% of control, $n = 3$). Thus chemotaxis in response to TGF-β1 was not mediated by PT-sensitive G protein(s).

Since fMLP triggers PT-insensitive as well as PT-sensitive GTPases in neutrophils (18), we determined the ability of TGF-β1 to activate plasmalemmal GTPases. Plasmalemmal GTPase activity was determined by the release of P_i from [γ-^{32}P]GTP. Basal levels of 56.4 ± 5.7 pmol of P_i per mg of protein per min ($n = 7$) were obtained after a 5-min incubation. fMLP elicited a dose-dependent hydrolysis of membrane-bound GTP in neutrophils, with significant release elicited by concentrations in the chemoattractant range (1–100 nM) (Fig. 2). In contrast neither femtomolar nor picomolar concentrations of TGF-β1 provoked an increase in GTPase activity (Fig. 2).

TGF-β1 Does Not Affect $[Ca^{2+}]_i$. Rapid and transient (within seconds) increments in $[Ca^{2+}]_i$ are associated with activation and chemotaxis of neutrophils (7, 19). Using the fluorescent probe fura-2 (15), we examined whether TGF-β1 also elicited a rapid increase in $[Ca^{2+}]_i$. Treatment of neutrophils with 100 nM fMLP generated a rapid increase in $[Ca^{2+}]_i$ from resting levels of 48.1 ± 4.6 nM to 332.9 ± 18.0 nM (mean ± SEM, $n = 5$; Fig. 3*A*). Even 1 nM fMLP elicited a rapid increase in $[Ca^{2+}]_i$. Exposure of cells to 40 fM TGF-β1 for up to 5 min did not significantly mobilize cytosolic Ca^{2+} (Fig. 3*A*). Moreover, pretreatment of neutrophils with 40 fM TGF-β1 for 5 min did not alter the peak increase in $[Ca^{2+}]_i$ elicited in response to 100 nM fMLP (fMLP, 332.9 ± 40.3 nM Ca^{2+}; fMLP plus TGF-β1, 282.1 ± 43.1 nM Ca^{2+}, $n = 5$; Fig. 3*B*). Increasing the concentration of TGF-β1 to 400 fM did not alter these results (data not shown). Thus TGF-β1 neither mobilized $[Ca^{2+}]_i$ on its own nor altered that induced by fMLP.

TGF-β1 Elicits Polymerization of Actin. Resting neutrophils are in a steady state with regard to concentrations of G-actin (monomer) and F-actin (polymer). Upon exposure to fMLP, F-actin increases rapidly and is concentrated chiefly within lamellipodia. We examined the effect of TGF-β1 on the distribution of polymerized actin in adherent cells with the use of rhodamine-labeled phalloidin (16). After 15 min, cells exposed to buffer alone were slightly flattened and displayed

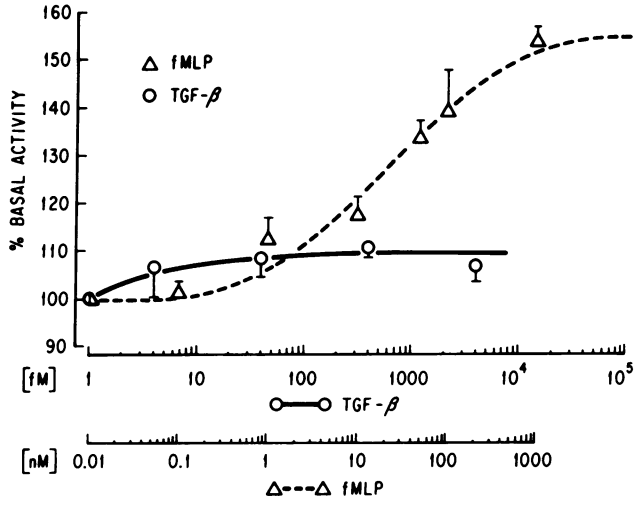

FIG. 2. Alterations in GTPase activity of neutrophil plasma membranes exposed to fMLP (Δ) or TGF-β1 (O). Membranes were prepared after nitrogen cavitation and GTPase activity was assayed according to the method of Wilde *et al.* (14). GTPase activity was calculated as pmol of P_i per mg of protein per min ($n = 3$).

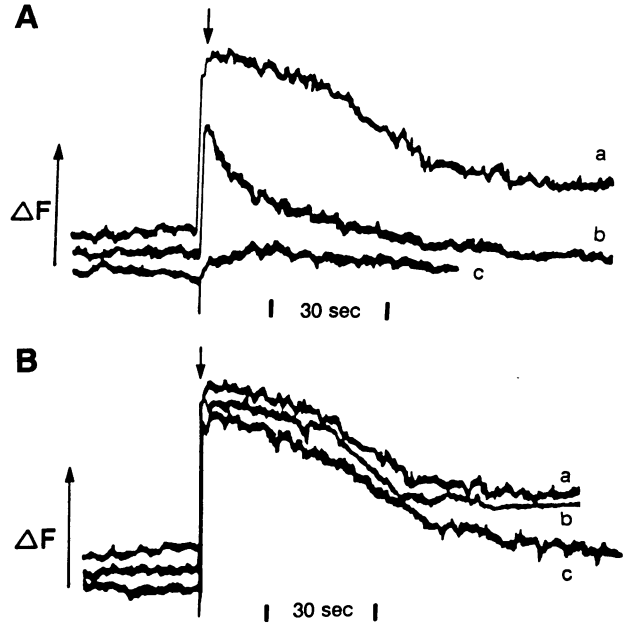

FIG. 3. Effect of TGF-β1 on $[Ca^{2+}]_i$ in human peripheral blood neutrophils. Neutrophils (10^8 per ml) were loaded with fura-2 acetoxymethyl ester and fluorescence changes (ΔF) were monitored. (*A*) Downward arrow denotes the addition of stimulus: 100 nM fMLP (trace a), 1 nM fMLP (trace b), or 40 fM TGF-β1 (trace c). This is a representative experiment of three. (*B*) Cells were preincubated with buffer (trace a), 40 fM TGF-β1 (trace b), or 400 fM TGF-β1 (trace c) for 5 min at 37°C, before injection of 100 nM fMLP (arrow).

faint fluorescence predominantly around the periphery. After 60 min, cells had spread and displayed staining in some localized areas (Fig. 4*A*). After a 15-min exposure to a gradient of fMLP, cells were ruffled and polarized, and they displayed bright staining around the periphery as well as some punctate areas. After 60 min, cells remained polarized with nexi of actin staining brightly (Fig. 4*B*). After 15 min, cells exposed to TGF-β1 remained predominantly rounded and displayed some areas of localized staining. By 60 min, although not consistently polarized, neutrophils had clearly spread and demonstrated an abundance of brightly stained nexi (Fig. 4*C*). Thus TGF-β1 provoked the polymerization and redistribution of F-actin over a prolonged time course.

Role of Protein Synthesis in Chemotaxis. Since chemotaxis elicited by TGF-β1 was not mediated by classic signals, it remained possible that chemotaxis required the synthesis of unidentified proteins. To examine this possibility, we evaluated the effect of cycloheximide (10 μg/ml) and actinomycin D (5 μg/ml) on chemotaxis elicited by 10 nM fMLP or 40 fM TGF-β1. Cycloheximide inhibited chemotaxis elicited by fMLP (82.5 ± 8.3% inhibition, mean ± SEM, $n = 4$) or by TGF-β1 (86.0 ± 5.0% inhibition). Chemotaxis provoked by fMLP or TGF-β1 was also inhibited by actinomycin D (46.6 ± 8.8% and 61.1 ± 20.3% inhibition, respectively). The inhibition of chemotaxis by cycloheximide was not due to cell death, as release of lactate dehydrogenase did not differ from control cells. The prolonged incubation with cycloheximide (10 μg/ml, 4 hr, 37°C) neither altered the resting generation of superoxide nor inhibited superoxide generation elicited by fMLP (102.6% of value for fMLP-treated cells, $n = 2$ in duplicate). Prolonged incubation with actinomycin D did inhibit fMLP-stimulated superoxide generation (34.6% inhibition, $n = 2$ in duplicate).

DISCUSSION

These data demonstrate that the polypeptide growth factor TGF-β1 is the most potent chemoattractant yet described for

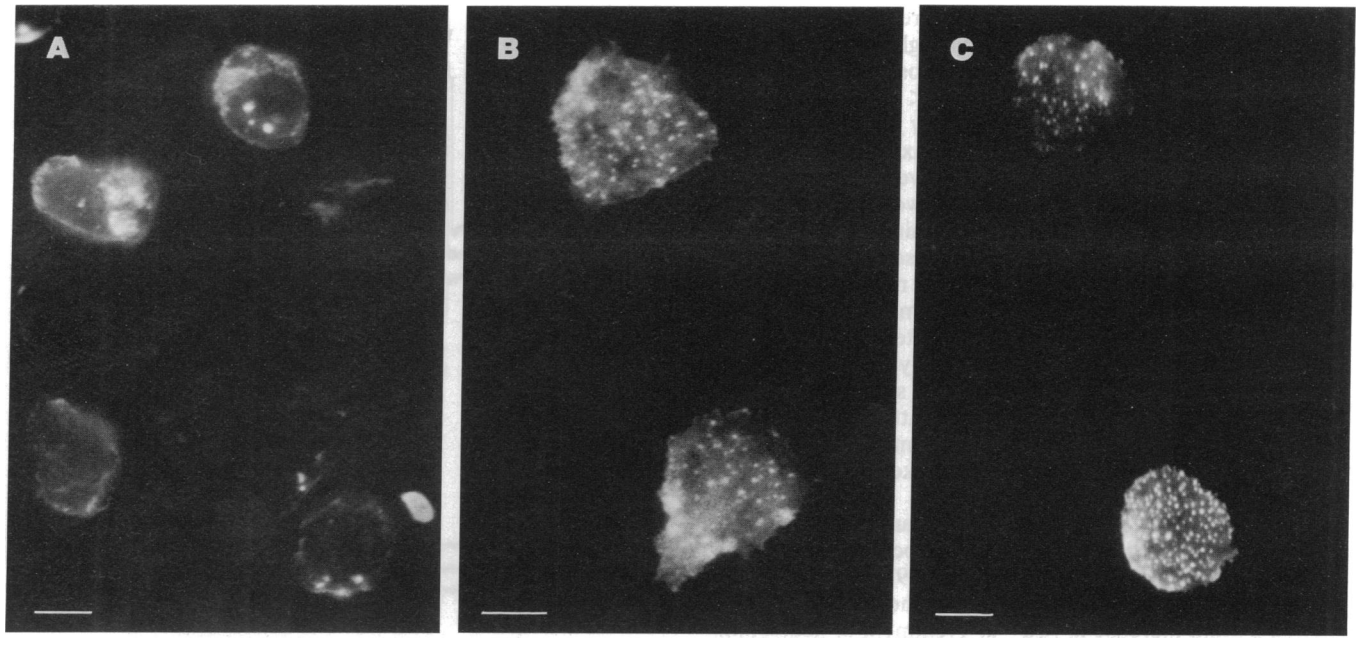

FIG. 4. Fluorescence images of adherent neutrophils in the absence or presence of chemoattractant. Cells were stained with rhodamine-labeled phalloidin by the modified method of Amato *et al.* (16). After 60 min, cells exposed to RPMI 1640 medium or appropriate solvent (*A*) were round and displayed only faint fluorescent staining. Cells exposed to fMLP for 60 min (*B*) were polarized and flattened with brightly staining punctate regions. Cells exposed to TGF-β for 60 min (*C*) demonstrated spreading and an abundance of bright punctate staining. (Bar = 10 μm.)

neutrophils. Other chemoattractants, including complement components (C5a), polypeptide growth factors (nerve growth factor, platelet-derived growth factor), cytokines (interleukin 8, tumor necrosis factor α), and lipid mediators (leukotriene B_4, platelet-activating factor) are all active in the nanomolar as opposed to femtomolar range. TGF-β1 at 40 fM generated fully 60% of chemotaxis provoked by the chemotactic peptide fMLP at 10 nM; thus TGF-β1 was $\approx$150,000 times more potent on a mol/mol basis than the conventional chemoattractant. The chemotactic response to femtomolar concentrations of TGF-β1 is not without precedent; both monocytes and fibroblasts migrate to this polypeptide (4, 6). The similarity of the response of these three cell types suggests that their chemotaxis may involve a similar signal-transduction pathway.

Receptors for TGF-β1 have not yet been demonstrated on neutrophils. As high-affinity receptors for this molecule are ubiquitous (20), it would be expected that neutrophils display a glycoprotein (type I, II, or IV) or β-glycan receptor as well. The affinity of receptors for TGF-β is extremely high, with a K_d as low as 5 pM (21, 22). Thus even at the femtomolar concentrations used in these studies, a significant portion of the receptors should be occupied. Furthermore, in most cells only 10–20% of receptors for TGF-β1 need to be occupied to provoke biological responses (23). A parallel situation exists for the classic stimulus fMLP: <3% receptor occupancy is required to initiate the generation of intracellular signals and <0.1% occupancy is required for actin polymerization (24, 25). Thus it is not surprising that the exceedingly low concentrations of TGF-β1 employed in our studies are capable of activating neutrophils.

Exposure of neutrophils to the chemoattractant fMLP results in the rapid activation of one or more PT-sensitive G proteins, the generation of phospholipid products (phosphatidic acid, 1,2-diacyl-*sn*-glycerol, inositol trisphosphate, inositol tetrakisphosphate), a rapid increase in $[Ca^{2+}]_i$, and the activation of protein kinases (7). Chemotaxis elicited by fMLP requires a G protein; functional uncoupling of this protein by PT results in inhibition of movement (26). The intracellular signals launched by TGF-β1 for growth inhibition or induction are unknown. TGF-β1 inhibits proliferation of hamster lung fibroblasts but does not alter breakdown of inositolphospholipids, activation of protein kinase C, or elevations in intracellular pH (27). In contrast, TGF-β1 stimulates transformation of AKR-2b fibroblasts and Rat-1 cells, a response that is sensitive to PT and is associated with an increase in GTPase activity in plasma membranes (28, 29). Furthermore, incubation of these cells with TGF-β1 (4 hr) elicits an increase in inositol trisphosphate and tetrakisphosphate (30). We have shown that in contrast to classic chemoattractants, chemotaxis provoked by TGF-β1 is not associated with the activation of a PT-sensitive G protein. Signal transduction in neutrophils also involves PT-insensitive G proteins (18). However, since TGF-β1 did not alter GTPase activity, it is unlikely that TGF-β1 acts via either PT-sensitive or PT-insensitive membrane-associated G proteins.

Classic chemoattractants elicit a global rise in $[Ca^{2+}]_i$ (7, 19) that may be both PT-sensitive and PT-insensitive (31, 32). Nevertheless, the importance of intracellular Ca^{2+} fluxes in chemotaxis remains unclear; depletion of this ion does not completely inhibit locomotion (33, 34). We demonstrate that whereas fMLP generated a rapid increase in $[Ca^{2+}]_i$, TGF-β1 did not; the data confirm that chemotaxis can proceed in the absence of global increments of $[Ca^{2+}]_i$.

Chemotaxis of neutrophils in response to classic chemoattractants is accompanied by an increase in F-actin (35). Chemotaxis in response to TGF-β1 was also accompanied by an increase in actin polymerization; neutrophils displayed spreading and an abundance of brightly stained clusters of actin. The mechanism whereby TGF-β1 promotes actin polymerization remains obscure. In neutrophils, actin polymerization is triggered by PT-sensitive as well as -insensitive G proteins (36). However, our data suggest that neither PT-sensitive nor PT-insensitive plasmalemmal G proteins participated in actin polymerization. Moreover, since TGF-β1 did not alter $[Ca^{2+}]_i$, the data confirm that actin polymerization can proceed in the absence of either activated PT-sensitive G protein(s) or global increments of $[Ca^{2+}]_i$.

Many of the effects of TGF-β1 depend on the synthesis of proteins (actin, collagen, and fibronectin) (5, 37, 38). Whereas the inflammatory responses of neutrophils (degranulation and the generation of superoxide) are rapid (seconds) and thus most likely do not involve the synthesis of new proteins, chemotaxis of neutrophils under agarose is measured over hours. Since chemotaxis by neutrophils toward TGF-β1 was not mediated by the rapid generation of known intracellular signals, it remained possible that chemotaxis required the synthesis of unidentified proteins. We have demonstrated that inhibition of translation or transcription (cycloheximide, actinomycin D) in neutrophils diminished chemotaxis in response to both TGF-β1 and fMLP. The role of protein synthesis in chemotaxis of human neutrophils has been controversial (39–41). We suspect that the discrepancy between our results and other studies may be explained by differences in incubation times, chemotactic chambers, and concentrations of chemoattractant. Our data demonstrate inhibition of chemotaxis by cycloheximide and actinomycin D with both chemoattractants (fMLP, TGF-β1), and we postulate that multiple mechanisms may be involved in chemotaxis, some of which are dependent on newly synthesized proteins. The proteins induced by the respective chemoattractants may differ and remain to be defined. We speculate that the exceedingly low concentrations of TGF-β1 required for chemotaxis may act by provoking the synthesis of unidentified proteins that serve to amplify the chemoattractant signal.

In summary, these data suggest that TGF-β1 is the most potent chemoattractant for neutrophils described to date, yet unlike most chemoattractants, it neither acts via a pertussis toxin-sensitive G protein nor elicits a global increase in $[Ca^{2+}]_i$. The novelty of TGF-β1 as a chemoattractant for neutrophils lies in its extreme potency in the absence of the generation of classic intracellular signals. The effects of TGF-β1 on chemotaxis and F-actin formation may be mediated by alterations in the synthesis of as yet unknown proteins.

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