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Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type α and type β transforming growth factors

(soft agar growth/epidermal growth factor/cell transformation/HPLC purification)

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ABSTRACT **Sarcoma growth factor (SGF) derived from conditioned medium of Moloney sarcoma virus-transformed cells and partially purified by gel filtration (crude SGF) has been characterized by its ability both to compete with epidermal growth factor (EGF) for binding to membrane receptors and to induce anchorage-independent growth of untransformed cells. We now show that further purification of crude SGF by reverse-phase HPLC on μBondapak C_{18} and CN columns at pH 2 resolves it into two distinctly different polypeptides, which we call types α and β transforming growth factors (TGFs). Type α TGF (TGF-α), but not type β TGF (TGF-β), competes for binding to the EGF receptor and induces the formation of small colonies (1,000–2,000 μm^2) of normal rat kidney cells in soft agar. Both TGF-β and EGF or TGF-α must be present in order to induce the formation of large colonies (7,000–15,000 μm^2). Based on EGF competing equivalents as determined from a radioreceptor assay with ^{125}I-labeled EGF in normal rat kidney cells, the relative ability of EGF and TGF-α to potentiate TGF-β-dependent colony formation is in the order conditioned-medium TGF-α > EGF > intracellular TGF-α. Suboptimal concentrations of the same polypeptides give additive potentiation of the TGF-β-dependent colony-forming response; saturating levels potentiate a similar maximum response whether used alone or in various combinations. The data indicate that the EGF-competing activity of crude SGF is due to its TGF-α component alone, whereas the soft-agar colony-forming activity is due to the combined action of two distinct polypeptides, TGF-α and TGF-β.**

Sarcoma growth factors (SGFs) are low molecular mass, 6- to 25-kilodalton (kDa), acid- and heat-stable polypeptides isolated from the conditioned medium of Moloney sarcoma virus (MSV)-infected mouse 3T3 cells (1). They compete with epidermal growth factor (EGF) for binding to membrane receptors and reversibly confer the transformed phenotype upon nonneoplastic cells in tissue culture (1, 2). Polypeptides similar to SGF have been reported in the culture media of other rodent sarcoma virus-transformed (3, 4) and leukemia virus-transformed (5) cells, certain chemically transformed cells (6, 7), and human tumor cell lines (8); intracellularly in virally and chemically transformed cells (9), embryonic mice (10, 11) and rats (12); and in fetal calf serum (13) and urine of normal pregnant or tumor-bearing humans (14). Other polypeptides from chemically transformed cells (6) and from nonneoplastic human, murine, and bovine tissues (15, 16) do not compete for binding to the EGF receptor yet induce anchorage-independent growth of indicator cells. These polypeptides all belong to a new family called transforming growth factors (TGFs) and are defined by their ability to confer the transformed phenotype on untransformed cells as evidenced by the loss of density-dependent inhibition of growth in monolayer, altered cell morphology, and acquisition of anchorage independence with the resultant ability to grow in soft agar (1, 9). Subsets of the TGF family have been classified as type α or β based on their interaction with the EGF receptor and their requirements for EGF for growth in soft agar (17, 18). Type α TGF (TGF-α) competes for the EGF receptor and induces the formation of small colonies of normal rat kidney (NRK) cells in soft agar; its colony-forming response is not potentiated by EGF. Amino acid analysis of TGF-α shows that it is distinct from both EGF (19) and type β TGF (TGF-β) (20, 21), and antibodies to EGF do not crossreact with TGF-α (1, 22). Unlike TGF-α, TGF-β does not compete for EGF receptors and requires EGF or TGF-α to induce formation of large colonies of NRK cells in soft agar. Neither TGF-α nor TGF-β can induce large colonies by themselves, suggesting a synergistic interaction (17).

In the present studies of SGF, we examined the biological activity of a crude preparation of SGF, similar to the one originally reported (1); we then purified this crude material by two steps of reverse-phase HPLC (RP-HPLC) and now provide evidence that the transforming activity of the crude SGF is the result of the presence of both TGF-α and TGF-β in this material.

MATERIALS AND METHODS

Cell Culture. NRK cells, clone 49F, were grown in Dulbecco's modified Eagle's medium (GIBCO) containing 10% calf serum (GIBCO) supplemented with penicillin (50 units/ml) and streptomycin (50 μg/ml) in humidified 5% CO_2/95% air at 37°C.

Source of Growth Factors. SGF was obtained from serum-free conditioned medium from MSV-transformed 3T3 cell line 3B11-IC; collection of sarcoma conditioned medium and subsequent chromatography on Bio-Gel P-60 in 1 M acetic acid were done as described (1), and the peak of soft-agar colony-forming activity migrating with an apparent molecular mass of 10 kDa was pooled and used for further purification on HPLC. Intracellular (IC) TGF-α and TGF-β were isolated from the same MSV-transformed 3T3 cells as described previously (9, 23), except that Bio-Gel P-30 pool I was used for HPLC purification.

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Abbreviations: SGF, sarcoma growth factor; EGF, epidermal growth factor; CM or IC TGF, conditioned-medium or intracellular transforming growth factor; kDa, kilodaltons; MSV, Moloney sarcoma virus; NRK, normal rat kidney; RP-HPLC, reverse-phase high-performance liquid chromatography; TGF-α and TGF-β, type α and type β TGFs; CN column, cyanopropylsilica column.

Soft-Agar Assay. Soft-agar colony-forming activity was determined in NRK clone 49F cells grown for 7 days in 0.3% agar in the presence or absence of added growth factors as described (1, 9). One unit of soft-agar activity is defined as the ED_{50} determined from the dose–response curve for formation of colonies larger than 3,100 μm^2. Colonies were counted by using an Omnicon Image analysis system programmed to count a 5-cm^2 area of a 35-mm-diameter plate used in the assay.

EGF Radioreceptor Assay. EGF was purified from mouse submaxillary glands by acid-ethanol extraction (9) and was labeled with ^{125}I by the method of Greenwood *et al.* (24) as described (22). To prepare cells for binding studies, 7–8 × 10^4 NRK cells in 1 ml of Dulbecco's modified Eagle's medium were plated on 24-well 16-mm-diameter dishes (Costar, Cambridge, MA). After 36–48 hr, binding competition was measured by the simultaneous addition of the competing substance and ^{125}I-labeled EGF (^{125}I-EGF; specific activity, 760 mCi/μmol; 1 Ci = 37 GBq). Activity of TGF-α is calculated as ng EGF equivalents obtained from the radioreceptor assay.

HPLC Purification. RP-HPLC of the Bio-Gel P-60 pool of conditioned-medium SGF was carried out as described on a μBondapak C_{18} column (0.78 × 30 cm, Waters Associates) with acetonitrile containing 0.1% CF_3COOH as the counterion and subsequently on a μBondapak cyanopropylsilica (CN) column (0.78 × 30 cm, Waters Associates) with *n*-propanol containing 0.1% CF_3COOH (23). Solvents were HPLC grade whenever available. Lyophilized SGF from the Bio-Gel P-60 column (14.5 mg) was dissolved in 4 ml 0.1% CF_3COOH and was loaded on a μBondapak C_{18} column equilibrated in 20% acetonitrile/0.1% CF_3COOH. The gradient, at a flow rate of 0.8 ml/min, consisted of 20–40% acetonitrile. Aliquots (80 μl) of 1.6-ml fractions were used for determination of colony-forming activity in soft agar and EGF binding competition. The regions of TGF activity were combined, and the activity was lyophilized, redissolved in 0.1% CF_3COOH, and loaded on a μBondapak CN column (10-μm particle size) equilibrated with the starting *n*-propanol concentration containing 0.1% CF_3COOH as indicated in the figure legends. Aliquots (60 μl) of 1.6-ml fractions were used for bioassay. HPLC fractions were stored at 4°C up to 4 wk without loss of activity.

Other Procedures. Protein determination was done by using the dye-binding method of Bradford (25) or fluorometrically (26) by using fluorescamine reagent (Sigma) with bovine gamma globulin (Bio-Rad) as a standard.

RESULTS

Because previous experiments had shown that crude TGF (Bio-Gel P-30 pool) from the cellular extract of MSV-transformed 3T3 cells (3B11-IC) could be separated into TGF-α and TGF-β components by further purification on HPLC (17, 23), the crude SGF (Bio-Gel P-60 pool) from the conditioned medium of these same cells was subjected to the identical procedures. RP-HPLC of this crude SGF on a μBondapak C_{18} column with an acetonitrile gradient containing 0.1% CF_3COOH resolved two peaks of activity in soft agar (Fig. 1). TGF-α, which competes for binding to the EGF receptor and induces the formation of small colonies ranging in size from 1,500 to 2,000 μm^2 in the soft-agar assay, was eluted at 31–32% acetonitrile (fractions 25–30). TGF-β, which does not compete with EGF in a radioreceptor assay, was eluted at 38–40% acetonitrile (fractions 45–47); it did not induce colonies in soft agar by itself, but, in the presence of EGF at 5 ng/ml, induced the formation of large colonies in soft agar ranging from 7,000 to 15,000 μm^2. Separation of TGF-α and TGF-β from other contaminating proteins resulted in 4- to 6-fold purification and 70% recovery of activity.

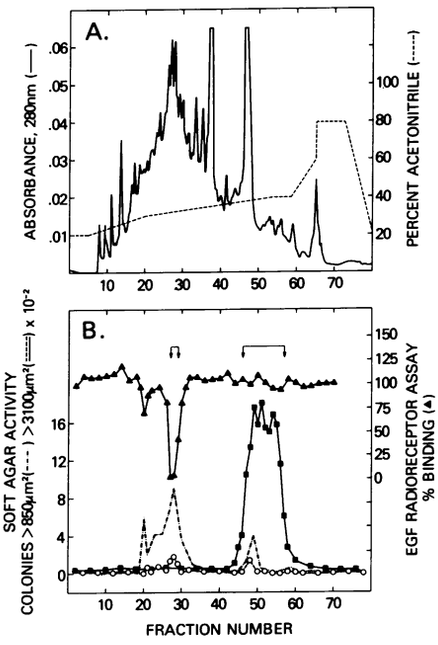

FIG. 1. RP-HPLC of SGF on a μBondapak C_{18} column. SGF isolated from conditioned medium of MSV-transformed cells after Bio-Gel P-60 chromatography (14.5 mg) was dissolved in 4 ml of 0.1% CF_3COOH, and 2 ml was loaded onto a Waters μBondapak C_{18} column equilibrated with 20% acetonitrile/0.1% CF_3COOH. The gradient (----) consisted of 20–40% acetonitrile for 100 min, followed by a 15-min linear gradient to 60% and a final step to 80% at a flow rate of 0.8 ml per min. Aliquots (80 μl) of 1.6-ml fractions were used for radioreceptor EGF competition and soft-agar assay. (*A*) Absorbance at 280 nm. (*B*) EGF radioreceptor assay (▲) and soft-agar colony-forming activity profile in the absence and presence of EGF. EGF absent: –·–, colonies larger than 850 μm^2; ○, colonies larger than 3,100 μm^2. EGF present at 5 ng/ml: ■, colonies larger than 3,100 μm^2. Arrows indicate the fractions pooled. Profiles given are representative of two separate chromatographic runs.

Further chromatography on a μBondapak CN column (Fig. 2*A*) of TGF-α pooled as in Fig. 1 increased the purification to 54-fold, with a total recovery of 40% relative to the Bio-Gel P-60 pool starting material. TGF-α was eluted at 36–39% *n*-propanol (fractions 42–47). The stippled area in Fig. 2*A* shows where the activity that competes with EGF was eluted in the column profile; this region coincided with the small colony-forming activity characteristic of TGF-α. Fig. 2*B* shows further chromatography of the TGF-β pool from Fig. 1 on a μBondapak CN column. TGF-β was retarded on the column and was eluted at 50–52% *n*-propanol (fractions 34–41); contaminating proteins were less retarded, suggesting that TGF-β has a more hydrophobic nature. This step resulted in a 290-fold purification of TGF-β relative to Bio-Gel P-60-stage material, with a 37% total recovery of biological activity.

A comparison of both activities, competition for the EGF receptor and soft-agar colony-formation, of TGF-α and TGF-β at various steps of purification is shown in Fig. 3. In a radioreceptor assay with ^{125}I-EGF and NRK cells, crude SGF after Bio-Gel P-60 chromatography showed 50% competition with EGF at 6 μg/ml (Fig. 3*A*). In the soft-agar assay, crude SGF induced formation of large colonies (>3,100 μm^2) both in the absence and presence of 5 ng of EGF per ml (ED_{50} of 450 ng/ml and 200 ng/ml, respectively; Fig. 3*C*). After μBondapak C_{18} and CN purification, TGF-α still showed strong EGF binding competition but no longer had the ability to induce the formation of large colonies in soft agar (Fig. 3*B*); however, it could induce ≈10% of the NRK cells to form small colonies (850–3,100 μm^2). This property was shared by EGF (Fig. 3*B*). TGF-β did

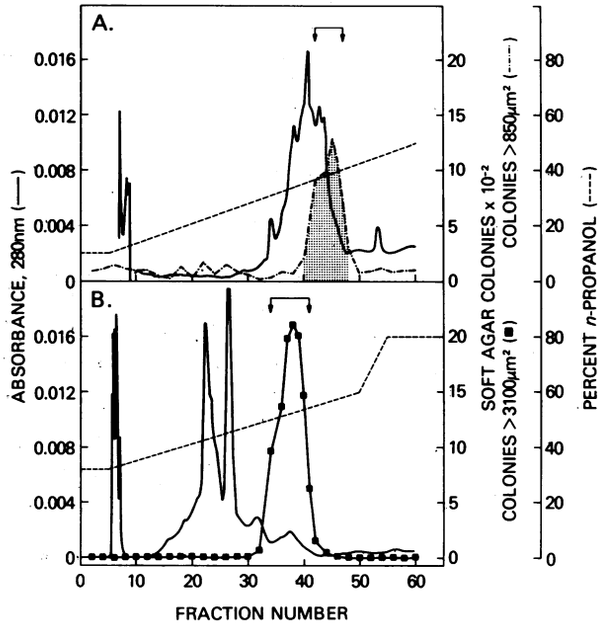

FIG. 2. RP-HPLC of TGF-α and TGF-β on a μBondapak CN column. (*A*) TGF-α pooled fractions from the C_{18} column (fractions 27–29 in Fig. 1) were lyophilized and dissolved in 4 ml of 0.1% CF_3COOH, and 2 ml was loaded onto the CN column. A 110-min linear gradient (– – –) from 10% to 50% *n*-propanol containing 0.1% CF_3COOH at a flow rate of 0.8 ml/min was used and 60-μl aliquots of 1.6-ml fractions were used for radioreceptor EGF competition and soft-agar assay. ——, Absorbance at 280 nm; ▨, area where the radioreceptor activity was eluted (shaded area); –·–, soft-agar colony-forming activity profile (colonies larger than 850 μm^2) in the absence of EGF. (*B*) TGF-β pooled fractions from the C_{18} column (fractions 46–57 in Fig. 1) were lyophilized and dissolved in 5 ml of 0.1% CF_3COOH, and 2 ml was loaded onto the CN column. The gradient (– – –) consisted of 32–60% *n*-propanol containing 0.1% CF_3COOH for 90 min, followed by a 10-min linear gradient to 80% as indicated at a flow rate of 0.8 ml/min. Aliquots (60 μl) of 1.6-ml fractions were used for soft-agar assay. ——, Absorbance at 280 nm; ■, soft-agar colony-forming activity profile in the presence of EGF at 5 ng/ml (colonies larger than 3,100 μm^2). Arrows indicate the fractions pooled. Profiles given are representative of two separate chromatographic runs.

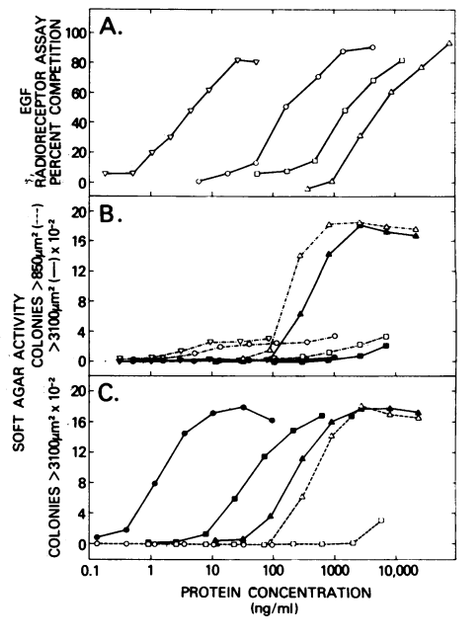

FIG. 3. Biological activity of EGF and TGFs at various steps of purification. (*A*) ^{125}I-EGF binding competition of various concentrations of EGF (▽); Bio-Gel P-60 SGF (△); C_{18} TGF-α, fractions 27–29 in Fig. 1 (□); or CN TGF-α, fractions 42–47 in Fig. 2*A* (○). Specific binding averaged 90% of total binding, which in the absence of unlabeled EGF was 1,000–1,300 cpm and in the presence of EGF at 1 μg/ml was 90 cpm. (*B*) Soft-agar colony-forming activity of EGF, and the same TGF-αs at various steps of purification as described in *A* were assayed in the absence of added EGF for colonies larger than 850 μm^2 (–·–) or colonies larger than 3,100 μm^2 (——). Symbols used are described in *A*. (*C*) Soft-agar colony-forming activity of various concentrations of Bio-Gel P-60 SGF (△, ▲); C_{18} TGF-β, fractions 46–57 in Fig. 1 (□, ■); CN TGF-β, fractions 34–41 in Fig. 2*B* (○, ●) assayed either alone (– – –) or in the presence of EGF at 5 ng/ml (——) for colonies larger than 3,100 μm^2. Values are representative patterns from three separate experiments.

not compete with radiolabeled EGF for binding to the EGF receptor (Fig. 1*B*) and, once separated from TGF-α, required EGF or TGF-α for colony-forming activity. Assays of its soft-agar colony-forming activity in the presence of EGF at 5 ng/ml showed an ED_{50} (900 colonies) for stimulation of large colonies (>3,100 μm^2) in soft agar at 200, 40, and 1.5 ng/ml after Bio-Gel P-60, μBondapak C_{18}, and μBondapak CN chromatography, respectively. After purification on the C_{18} column, the highest concentration of TGF-β tested (6 μg/ml), gave a colony-forming response of only 350 colonies larger than 3,100 μm^2 when assayed in the absence of EGF; assayed in the presence of EGF at 5 ng/ml, only 12 ng of TGF-β per ml was required to induce the formation of 350 colonies. After purification on the CN column, TGF-β, assayed at 100 ng/ml in the absence of EGF, lost its ability to induce NRK cells to form colonies in soft agar, whereas 1.5 ng of this same TGF-β per ml assayed in the presence of EGF at 5 ng/ml induced the formation of 900 colonies larger than 3,100 μm^2 (1 unit of activity). Background colonies due to EGF ranged from 30 to 80 colonies larger than 3,100 μm^2.

The ability of EGF to potentiate TGF-β-dependent colony formation in soft agar also was shared by TGF-α, whether it was derived from conditioned medium (CM TGF) or intracellularly (IC TGF) by extracting the cells directly. This was demonstrated in two different ways. Fig. 4 *A* and *B* show the colony-forming response of a constant amount of various TGF-αs with various concentrations of CM TGF-β or of a constant amount of CM TGF-β with various concentrations of the TGF-αs, respectively. Assayed at a concentration that supported competition in the EGF radioreceptor assay equivalent to 5 ng/ml, CM TGF-α and EGF provided a similar maximum response in the colony-forming assay, whereas IC TGF-α provided a response that was only 15% of maximum (Fig. 4*A*). TGF-β was devoid of any colony-forming activity when assayed in the absence of TGF-α or EGF (Fig. 4*A*). Conversely, dose–response curves of EGF, CM TGF-α, or IC TGF-α assayed for potentiation of colony formation in the presence of a constant amount (2 units/ml) of CM TGF-β showed that the ED_{50} for CM TGF-α, EGF, or IC TGF-α was 0.5, 2, or 20 ng/ml of EGF or EGF binding equivalents, respectively (Fig. 4*B*). Assayed in the absence of TGF-β, the maximum colony-forming response of EGF and the TGF-αs was only 100–200 colonies larger than 3,100 μm^2 compared to 2,000–2,200 colonies when the same polypeptides were assayed in the presence of CM TGF-β at 2 units/ml. Similar results were obtained with IC TGF-β instead of CM TGF-β.

To determine whether or not the promoting effects of EGF and TGF-α on TGF-β-dependent colony formation were additive or synergistic, various concentrations of EGF, CM TGF-α, or IC TGF-α were assayed alone or in combination with each other in the presence of constant amounts (2 units/ml) of IC or CM TGF-β (Fig. 5). Assay of EGF and CM TGF-α at levels below the ED_{50} value gave an additive response, whereas assay

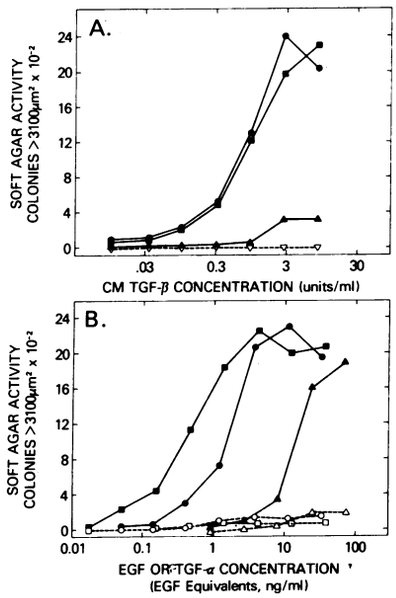

FIG. 4. Synergistic interaction of TGF-β with EGF or TGF-α to induce formation of large colonies (larger than 3,100 μm^2) of NRK cells in soft agar. (*A*) Soft-agar colony-forming activity of various concentrations of CM TGF-β assayed either alone (∇) or in the presence of a constant amount (5 ng/ml) of EGF equivalents of either EGF ($\bullet$), CM TGF-α ($\blacksquare$), or IC TGF-α ($\blacktriangle$). (*B*) Soft-agar colony-forming activity of various concentrations of EGF ($\circ$, $\bullet$), CM TGF-α ($\square$, $\blacksquare$), and IC TGF-α ($\triangle$, $\blacktriangle$) assayed either alone ($\circ$, $\square$, $\triangle$) or in the presence of a constant amount (2 units/ml) of CM TGF-β ($\bullet$, $\blacksquare$, $\blacktriangle$). CM or IC TGF-α and TGF-β were purified through the μBondapak CN step. One unit of CM TGF-β contains 1.3 ng of protein, and one ng of EGF binding equivalent of CM TGF-α or IC TGF-α contains 28 ng and 212 ng of protein, respectively. Values are representative patterns from three separate experiments.

at optimum concentration gave a similar maximum response. Other combinations such as EGF and IC TGF-α or CM TGF-α and IC TGF-α showed a similar response pattern. These data strongly suggest that EGF, CM TGF-α, or IC TGF-α are potentiating the colony-forming activity of TGF-β through similar mechanisms.

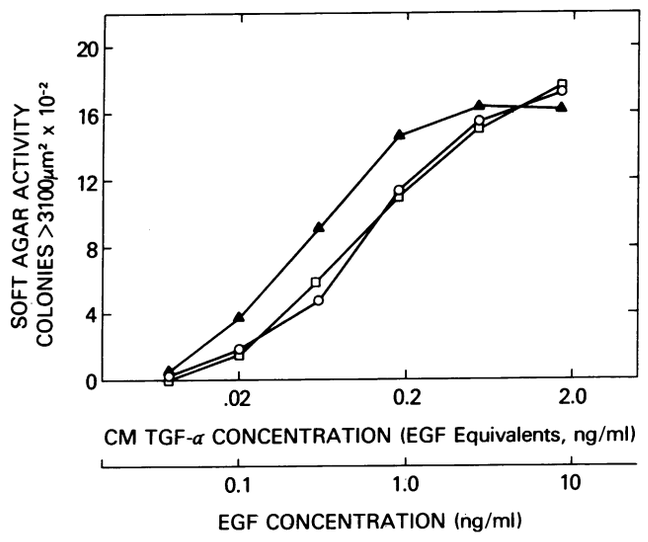

FIG. 5. Potentiation of the constant amount of CM TGF-β with various concentrations of EGF or CM TGF-α alone and of the combination of the two. A constant amount of CM TGF-β at 2 units/ml (1.3 ng of protein/ml) was titrated with various concentrations of EGF ($\circ$) or CM TGF-α ($\square$) separately and in the presence of both EGF and CM-TGF-α ($\blacktriangle$). Values are representative patterns from four separate experiments.

DISCUSSION

Crude SGF competes strongly for EGF membrane receptors in NRK cells and induces large colonies in soft agar either in the presence or absence of EGF. We have demonstrated that further purification of crude SGF by sequential HPLC steps resolves it into two distinct classes of polypeptides, which we have called TGF-α and TGF-β. The activity of crude SGF to compete for EGF receptors is due to the TGF-α component alone, whereas the colony-forming activity in soft agar is due to the combined action of TGF-α and TGF-β, neither of which induce the formation of large colonies in soft agar by themselves.

The finding that both crude SGF from conditioned medium and the comparable TGF activity extracted directly from the transformed cells (IC TGF) are a mixture of two different subtypes of TGFs suggests that caution must be exercised in any attempts to characterize the properties of crude TGFs. Despite differences in molecular mass, TGF-α and TGF-β comigrate when chromatographed on Bio-Gel P-60 in 1 M acetic acid. Other studies of TGFs isolated from conditioned media (i.e., CM TGFs) (7) or from rat fetuses (12) have shown the absence of a potentiating effect of EGF on colony formation; however, our data demonstrate that the marginal effect of exogenous EGF on colony formation by crude SGF (Fig. 3*B*) is due to the presence of intrinsic TGF-α in the preparation. In tissue extracts, the presence of intrinsic EGF similarly can lead to misconceptions regarding the properties of the isolated TGFs (16). In addition, we have frequently observed that TGFs extracted directly from cells with acid-ethanol may not demonstrate EGF receptor competition at the Bio-Gel stage of purification, even though the presence of a TGF-α component can be demonstrated after subsequent purification on HPLC (unpublished data). Clearly, further purification of TGF preparations is required before the existence of novel TGF subsets, distinct in properties from those of either TGF-α or TGF-β, can be verified.

The presence of TGF-α and TGF-β in conditioned medium of MSV-transformed cells is consistent with our previous reports on the isolation of TGF-α and TGF-β by direct extraction of the same cells. The chromatographic behavior of CM TGF-α and TGF-β in RP-HPLC is similar to IC TGF-α and TGF-β extracted from the same MSV-transformed cells (17, 23). IC and CM TGF-α are both eluted at 31% acetonitrile on μBondapak C_{18} columns and at 39% *n*-propanol on μBondapak CN columns with the gradients shown in Figs. 1 and 2*A*; IC and CM TGF-β are both eluted at 38–39% acetonitrile on μBondapak C_{18} columns and at 48–50% *n*-propanol on μBondapak CN columns with the gradients described in Figs. 1 and 2*B*. In addition, analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis of HPLC (CN column)-purified CM or IC TGF-β shows a silver-staining protein band migrating at 23–25 kDa under nonreducing conditions; the colony-forming activity of TGF-β coeluted with this band (data not shown). Other TGF-βs obtained from human platelets (20, 27), human placenta (21, 27), and bovine kidney (27) each have an apparent molecular mass of 23–25 kDa under nonreducing conditions. Under reducing conditions, each of these proteins migrate on a gel at 13 kDa, suggesting that TGF-β is composed of two subunits of similar molecular size.

Although both CM and IC TGF-α as well as EGF share the properties of competing for EGF receptor binding and potentiating TGF-β-dependent colony formation in soft agar, quantitative differences in response are apparent. When concentrations of these polypeptides are normalized based on EGF equivalents derived from a radioreceptor assay, we observed

that CM TGF-α is 5 times and 40 times more potent than EGF and IC TGF-α, respectively, in inducing large colonies in the presence of either CM or IC TGF-β. Whether this is a reflection of intrinsic differences in binding ability or whether binding abilities of these TGFs are equivalent and the results reflect different potentiating activities cannot be answered without homogeneous preparations of TGF-α. One possibility is that the binding site of TGF-α active in potentiating the TGF-β-dependent colony formation is different from the EGF binding site. Massague *et al.* (28) have presented evidence for a specific 60-kDa receptor on the NRK cells that displays high affinity for SGF but not for EGF and have suggested that expression of the transformed phenotype may require occupation of this receptor in addition to the 140- to 170-kDa EGF receptor with which both SGF and EGF can interact to mediate normal cellular growth. Alternatively, these differences in potentiation of TGF-β-dependent colony formation by EGF or TGF-α could be due to differences in binding affinity to the EGF receptor. We have shown that EGF and TGF-α are interchangeable in potentiating TGF-β in the soft-agar assay, and it has been reported recently that an anti-EGF receptor antibody blocks the soft-agar colony-forming activity of SGF (29). Regardless, our observation of greater potentiating ability of CM TGF-α relative to IC TGF-α derived from the same MSV-transformed cells suggests that IC TGF-α is converted into a more potent form during the process of secretion into the conditioned medium.

The observations of Ozanne *et al.* (3) and De Larco *et al.* (30) that cells transformed by a temperature-sensitive sarcoma virus secrete high levels of SGF at temperatures permissive for transformation but only negligible amounts at the nonpermissive temperature, as well as the studies on various human tumor cells lines (8) showing that the ability of tumor cells to grow in soft agar correlates with the quantity of polypeptides in competition with EGF binding that are released into the medium, suggests that TGF-αs are important in inducing phenotypic transformation. Whether expression of TGF-β at permissive or nonpermissive temperatures is altered in a similar manner remains to be investigated.

In conclusion, the demonstration that the intrinsic "transforming" activity of crude SGF is due to the concerted action of TGF-α and TGF-β on untransformed cells suggests that study of the regulation of the expression of genes for both TGF-α and TGF-β and for their receptors and study of the mode of interaction of these two polypeptides in inducing expression of the transformed phenotype will broaden our understanding of the mechanisms of carcinogenesis and of control of normal cell growth.

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