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Identification of a structural domain that distinguishes the actions of the type 1 and 2 isoforms of transforming growth factor β on endothelial cells

(growth factors/receptors/chimeras)

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ABSTRACT **A chimeric transforming growth factor β (TGF-β) molecule has been synthesized to map the amino acids responsible for the substantially greater activity of TGF-β_1 than TGF-β_2 on growth and migration of endothelial cells. This chimera consists of a dimer of a monomeric unit composed of amino acids 1–39 of TGF-β_2, 40–82 of TGF-β_1, and 83–112 of TGF-β_2. Structural identity of the purified recombinant protein has been confirmed by immunoblotting and NH_2-terminal sequencing. The biological potency of the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera was equal to that of TGF-β_1 in inhibition of growth of both fetal bovine heart endothelial cells and rat epididymal fat pad microvascular endothelial cells. Similarly, the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera was nearly equivalent to TGF-β_1 and at least 10-fold more active than TGF-β_2 in inhibiting migration of bovine aortic endothelial cells. These results identify the sequence between amino acids 40–82 as an important region within TGF-β that functions to specify a TGF-β_1- or TGF-β_2-like activity.**

The transforming growth factor βs (TGF-βs) are 25-kDa homodimeric proteins with potent effects on growth and differentiation (1–3). Although TGF-β_1 was first identified based on its ability to stimulate the growth of normal rat kidney cells in soft agar (4), it is now clear that the TGF-βs regulate the growth of virtually all cells. Consistent with this has been the identification of TGF-β receptors on virtually all cells (2, 5).

Three distinct isoforms of TGF-β have been identified in mammalian cells (for review, see ref. 3). All three isoforms are disulfide-linked dimers of two 112-amino acid chains. Each chain is synthesized as the COOH-terminal domain of a large precursor that is synthesized and secreted in a biologically latent form (1–3). The latent complex must be activated before TGF-β can exert its biological effects on cells. The three isoforms share ≈70% identical amino acids within the active TGF-β peptide. In addition, nine positionally invariant cysteine residues are found in each of the three isoforms.

Although many cultured cells respond equally to the different TGF-β isoforms, several differences in the biological potencies of the isoforms have been reported. Growth of endothelial cells in monolayer (6–8) and certain hematopoietic cells (9, 10) is readily inhibited by TGF-β_1 and TGF-β_3, whereas substantially greater concentrations of TGF-β_2 are required to achieve the same inhibition. For example, the ED_{50} concentration for inhibition of the growth of fetal bovine heart endothelial (FBHE) cells is 5 pM for TGF-β_1 and TGF-β_3 but is nearly 100 times higher for TGF-β_2 (7). In contrast, TGF-β_3 is 10 times more active than TGF-β_2, and TGF-β_1 is inactive in inducing the formation of mesoderm in *Xenopus laevis* (11, 12).

Currently, nothing is known about the structure/function relationships of TGF-β. In particular, the regions important in the binding of TGF-β to its receptors remain unmapped. To begin to investigate how TGF-β interacts with its receptors, we have designed a chimeric TGF-β and assayed it for its ability to inhibit the growth and migration of endothelial cells. Here we show that the sequence between amino acids 40 and 82 of the mature TGF-β molecule specifies TGF-β_1 potency on both the growth and migration of endothelial cells.

MATERIALS AND METHODS

Cell Culture. NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/10% calf serum/1% penicillin-streptomycin (GIBCO). Capillary endothelial cells derived from rat epididymal fat pads (RFCs) (13) and bovine aortic endothelial cells (BAECs) (14) were isolated and cultured as described. Mv1Lu (15) and FBHE (7) growth-inhibition assays were done by using published procedures. RFC proliferation assays and BAEC migration assays were done according to the methods of Merwin *et al.* (8).

Construction of Chimeric TGF-β Expression Plasmid. Expression plasmid pSQneo was created by inserting the neomycin marker gene [*Eco*RI fragment of pMMTneo (16)] into the *Eco*RI site of pEV142 (17). The cDNA coding for the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera (described below) was inserted into the unique *Bam*HI site, allowing expression of this cDNA from the metallothionein promoter. The partial cDNAs for TGF-β_1 and TGF-β_2 used to make the 2-1-2 construct were generated by the PCR from porcine TGF-β_1 cDNA (18) and simian TGF-β_2 cDNA (19), as the simian TGF-β_2 and porcine TGF-β_2 amino acid sequences are identical.

A two-step PCR was used to mutate Cys-33 of the TGF-β_1 pre-pro sequence. This Cys → Ser mutation allows enhanced expression of active TGF-β dimers by reducing aberrant disulfide-bond formation (20). In the first step, the oligonucleotides 5′-GAATTCAGATCTGAGATGGCGCCTTC-GGGGCTGC-3′ (oligonucleotide A) and 5′-TGGTCTTCGA-GGTGGACAG-3′ (oligonucleotide B) were used in PCR to amplify the cDNA for amino acids 1–35, and the oligonucleotides 5′-CTGTCCACCTCGAAGACCA-3′ (oligonucleotide C) and 5′-GGTATCCAGTGCACGGCGGTGCCG-3′ (oligonucleotide D) were used in an additional PCR to amplify the

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Abbreviations: RFC, capillary endothelial cell derived from rat epididymal fat pad; BAEC, bovine aortic endothelial cell; FBHE, fetal bovine heart endothelial.

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cDNA for amino acids 30–278. Because the cysteine codon at position 33 was changed to a serine codon (underlined nucleotides) in oligonucleotides B and C and oligonucleotide A (underlined base) was changed to the Kozak consensus sequence, the resulting PCR products contained the desired modifications. In the second step, a full-length cDNA for the complete pre-pro region was generated. In this procedure, the previously amplified cDNAs were mixed together and incubated through five PCR cycles (denaturing at 94°C, 1.5 min; annealing at 55°C, 2 min; elongation at 72°C, 3 min) in the absence of oligonucleotide primers. During the annealing step, the two added cDNAs (previously denatured) hybridized with each other through the complementary 19-base-pair region of overlap, forming a full-length, partially double-stranded product. Complete double-stranded DNA was produced during the elongation step as a result of the *Taq* polymerase activity. Amplification of the full-length double-stranded DNA was accomplished by adding oligonucleotides A and D to the reaction mixture beginning with the sixth PCR cycle and continuing for an additional 25 cycles. Because oligonucleotides A and D are from the ends of the pre-pro sequence, only the full-length, mutated cDNA was obtained.

The cDNA for the mature region of TGF-β was generated by PCR with oligonucleotide primers centered around amino acids 1, 32, 84, and 112 of the mature TGF-β. These primers had the sequences 5′-CGGAAGAAGCGTGCACTGGATGCG-3′ and 5′-TTTGGGTTCGTGGATCCATTTCCA-3′ (TGF-β_2, amino acids 1–32), 5′-TGGAAGTGGATCCATGAACCCAAG-3′ and 5′-GATGGGCAGTGGCTCGAGCGCCTG-3′ (TGF-β_1, amino acids 32–84), and 5′-CAAGACCTCGAGCCTCTAACCATT-3′ and 5′-GAATTCAGATCTTGCCACTTTTCCAAGAAGT-3′ (TGF-β_2, amino acids 84–112). Primers were designed such that these three PCR fragments and the pre-pro-region cDNA could be ligated together by using unique restriction sites at the junctions (see Fig. 1). The PCR product coding for the pre-pro-region of TGF-β_1 had a *Bgl* II restriction site at its 5′ end and a *Apa*LI site at its 3′ end. The cDNA for amino acids 1–32 of TGF-β_2 had a 5′ *Apa*LI and 3′ *Bam*HI restriction site. The cDNA for amino acids 33–84 of TGF-β_1 had a 5′ *Bam*HI site and a 3′ *Xho* I site. The cDNA for amino acids 85–112 of TGF-β_2 had a 5′ *Xho* I site and a 3′ *Bgl* II site. Thus, the chimeric TGF-β was constructed by the sequential ligation of the pre-pro-region to the 1–32 PCR product, to the 32–84 PCR product, and to the 84–112 PCR product. Importantly, the junction regions were designed to code for amino acids common to both TGF-β_1 and TGF-β_2 and did not create any additional amino acids. Thus, the amino acids specific to TGF-β_1 were between positions 40 and 82 of the mature TGF-β protein. The plasmid pSQ1 was generated by cloning

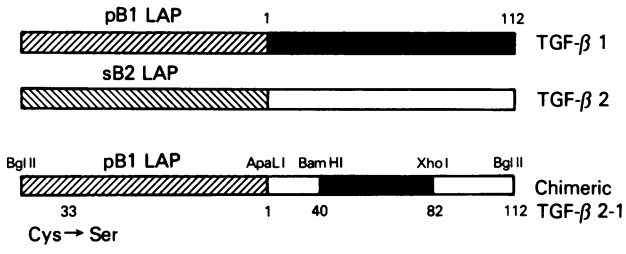

FIG. 1. Structure of chimeric TGF-$\beta_{2\text{-}1\text{-}2}$. Chimeric TGF-$\beta_{2\text{-}1\text{-}2}$ cDNA was constructed by first swapping a region of porcine TGF-β_1 cDNA (dark bars) with the corresponding region of simian TGF-β_2 cDNA (open bars). The cDNA for the porcine TGF-β_1 latency-associated peptide (LAP, shaded bars), which contains a Cys-33 → Ser mutation, was ligated to the chimeric cDNA. The resulting product was then ligated into the unique *Bam*HI site of plasmid pSQneo, generating expression plasmid pSQ1; pSQ1 was used to express the chimeric cDNA in NIH 3T3 cells.

the chimeric cDNA PCR product into the *Bam*HI site of pSQneo. The TGF-β cDNA insert in pSQ1 was sequenced and shown to contain the correct sequence. pSQ1 was then used for the over-expression of TGF-$\beta_{2\text{-}1\text{-}2}$.

Expression of Recombinant TGF-$\beta_{2\text{-}1\text{-}2}$. The plasmid pSQ1 was introduced by the calcium phosphate-transfection method into NIH 3T3 cells. After 24 hr, these cells were switched to Dulbecco's modified Eagle's medium/10% calf serum/1% penicillin-streptomycin/G418 at 800 μg/ml and grown until resistant colonies became visible. Twenty of these resistant colonies were isolated, expanded, and then assayed for TGF-β expression levels after induction of the metallothionein promoter with 5 μM $CdCl_2$. The clone with the highest expression level was further expanded and used to generate large quantities of expression medium.

Purification of Recombinant TGF-$\beta_{2\text{-}1\text{-}2}$. NIH 3T3 cells having a high level of expression from the plasmid pSQ1 were plated on 500-cm^2 dishes (Sumitomo Bakelite, Tokyo) and grown until confluent in 80 ml of Dulbecco's modified Eagle's medium/10% calf serum/1% penicillin-streptomycin/G418 at 250 μg/ml. The cells were then washed two times with phosphate-buffered saline and changed to 60 ml of Dulbecco's modified Eagle's medium/0.2% calf serum/1% penicillin-streptomycin/G418 at 250 μg/ml/5 μM $CdCl_2$ to induce expression of the metallothionein promoter. After 72 hr the medium was collected, assayed for TGF-β concentration, and then used to purify the chimeric TGF-β.

Expression medium (1.5 liter) was dialyzed in 3500 M_r cutoff tubing (Spectrum Medical Industries) against three changes of 1 M acetic acid and then partially lyophilized to reduce the volume to ≈10% of the original. Acetonitrile and trifluoroacetic acid were added to the thawed concentrate to a final concentration of 10% and 0.05%, respectively. Insoluble material was removed by centrifuging at 2000 × g for 10 min.

The supernatant was loaded at a flow rate of 1 ml/min onto a semi-preparative Synchropak RP-P, C_{18} column (SynChrome, Lafayette, IN), which was previously equilibrated in 10% acetonitrile/0.05% trifluoroacetic acid (buffer A). After extensive washing with buffer A, protein was eluted from the column by using a gradient of 60% (vol/vol) acetonitrile/0.045% trifluoroacetic acid (buffer B) at a flow rate of 0.6 ml/min (10–20% acetonitrile over 10 min; 20–45% acetonitrile over 125 min). Three-minute fractions were collected. The peak of TGF-β activity was determined by using the Mv1Lu growth-inhibition assay and typically eluted between fractions 29 and 36.

The pooled fractions from the C_{18} column were diluted with 2 vol of 1.5 M acetic acid, resulting in a final concentration of 10% acetonitrile/1 M acetic acid. This sample was loaded at a flow rate of 1 ml/min onto a TSK-Gel CM-2SW column (TosoHaas, Philadelphia) previously equilibrated with 10% acetonitrile/1 M acetic acid (buffer C). After being washed, protein was eluted by using a linear gradient of 10% acetonitrile/1 M acetic acid/1.5 M ammonium acetate (buffer D), so that after 170 min the composition of eluant was 20% C/80% D, corresponding to 1.2 M ammonium acetate. During the gradient, the flow rate was 0.6 ml/min, and 3-min fractions were collected. Column fractions were analyzed by loading 5 μl of each fraction onto a Tricine gel (Novel Experimental Technology, San Diego); after electrophoresis, the proteins were identified by silver-staining (Bio-Rad). Typically, TGF-β elutes in fractions 26–29.

The pooled fractions from the TSK-Gel CM-2SW column were applied directly to a Brownlee Aquapore C_4 guard column previously equilibrated in 10% acetonitrile/0.05% trifluoroacetic acid. After being washed, proteins were eluted by using a linear gradient of 60% acetonitrile/0.045% trifluoroacetic acid over a 60-min period. Samples (1 μl) of each fraction (200 μl) were subjected to electrophoresis, and the

proteins were stained with silver. Typically, pure TGF-β elutes into fractions 14–17.

RESULTS

A chimeric TGF-β molecule (Fig. 1) was constructed to identify a region of TGF-β_1 responsible for the greater biological activity of TGF-β_1 compared with TGF-β_2 in endothelial cell growth and migration assays. This chimera contained amino acids 1–39 of the mature region of TGF-β_2 linked to amino acids 40–82 of mature TGF-β_1 and amino acids 83–112 of mature TGF-β_2. Importantly, this chimera was designed so that the junction sites between cassettes did not create any alterations in the TGF-β_1 or TGF-β_2 sequences and so that the final chimeric TGF-β would remain a total of 112 amino acids. Because mature, active TGF-β does not fold correctly when expressed alone, the chimera was expressed as part of the larger latency complex with the porcine TGF-β_1 pre-pro sequence. Expression of this chimera in NIH 3T3 cells was driven by the metallothionein promoter in vector pSQ1.

The concentration of chimeric TGF-β in the cell culture medium, after induction with 5 μM $CdCl_2$, was determined (data not shown). In these experiments, TGF-β was activated by heating the medium to 80°C for 8 min, and then the medium was assayed for its ability to inhibit growth of Mv1Lu cells. Expression of the chimeric TGF-β was >100 ng/ml, whereas NIH 3T3 cells transfected with the control plasmid produced only 0.5 ng of endogenous TGF-β per ml (data not shown). Therefore, the concentration of the chimeric TGF-β represents ≈200 times more than the background TGF-β level.

A three-step purification of the chimeric molecule was achieved by using reverse-phase chromatography, cation-exchange chromatography, and C_4 narrow-bore chromatography. Analysis of the purified material, after silver-staining of the products separated on a tricine gel, shows that the chimeric TGF-β is essentially homogeneous (Fig. 2). Thus, results obtained during the assay of this recombinant molecule cannot be complicated by the presence of other mitogens. Furthermore, this material comigrates with homogeneous porcine TGF-β_1 (R&D Systems, Minneapolis) under nonreducing conditions. Quantitation of the purified molecule was done by silver-staining serial dilutions of the protein and comparing the band intensities to those of TGF-β_1 standards (data not shown).

To ensure that the purified TGF-β corresponds to the expected chimeric product and does not represent endogenous TGF-β produced by NIH 3T3 cells, both immunoblotting and NH_2-terminal sequencing were done. For immunoblotting, 100 ng each of TGF-β_1, TGF-β_2, and the purified protein were electrophoresed in a 12.5% gel, transferred to nitrocellulose, and treated with antibodies specific for amino acids 48–77 of TGF-β_1, which had been shown (21) to react with TGF-β_1 but not with TGF-β_2. As expected, this antibody reacted with TGF-β_1 and the purified chimeric TGF-$\beta_{2\text{-}1\text{-}2}$ protein but did not react with the control TGF-β_2 (Fig. 3). The slightly faster migration of TGF-$\beta_{2\text{-}1\text{-}2}$ as compared with TGF-β_1 is consistent with the faster migration of TGF-β_2 than TGF-β_1 under reducing conditions, a result that has been reported (22). Importantly, the positive reaction with this antibody confirms that the purified TGF-β contains the sequence of TGF-β_1 from amino acids 40–82.

To show that the purified protein contained the expected TGF-β_2 sequence at its NH_2 terminus, the purified protein was sequenced by using the Edman degradation reaction. As predicted, the NH_2-terminal sequence (20 amino acids) exactly matched the sequence of TGF-β_2 but did not match the sequence of TGF-β_1 (data not shown). Positions 4, 5, 9–13, 17, and 19 of the first 20 amino acids are different in TGF-β_1 and TGF-β_2. As expected, assignment of the cysteines at positions 7, 15, and 16 was ambiguous because these residues were not protected before sequencing. Taken together, the immunoblot and sequencing results confirm that the purified protein is the chimeric TGF-$\beta_{2\text{-}1\text{-}2}$.

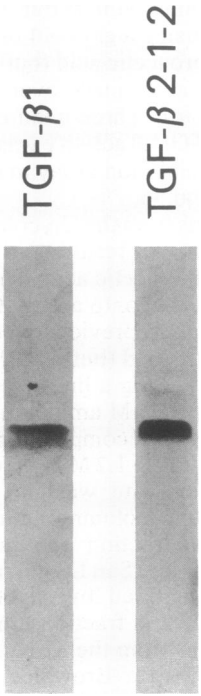

FIG. 2. Silver-stain of purified TGF-$\beta_{2\text{-}1\text{-}2}$. Protein was electrophoresed on a 10% Tricine gel and then silver stained. Lanes correspond to 50 ng of TGF-β_1 and 150 ng of purified chimeric TGF-$\beta_{2\text{-}1\text{-}2}$.

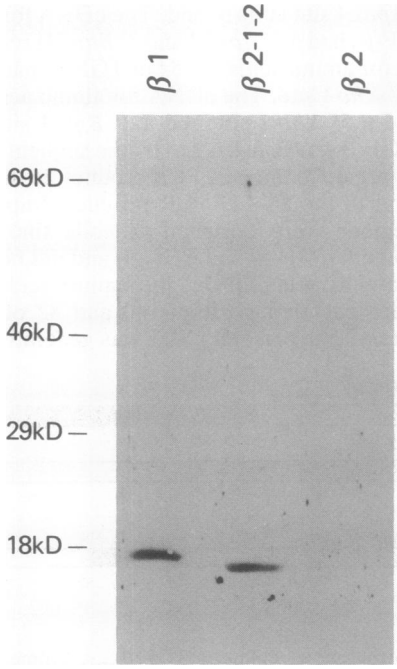

FIG. 3. Immunoblot of TGF-$\beta_{2\text{-}1\text{-}2}$. Protein was electrophoresed on a 12.5% acrylamide gel under reducing conditions, transferred to nitrocellulose, and immunoblotted by using an antibody specific for amino acids 48–77 of TGF-β_1 (21). Lanes correspond to 100 ng of homogeneous TGF-β_1, 100 ng of purified chimeric TGF-$\beta_{2\text{-}1\text{-}2}$, and 100 ng of homogeneous TGF-β_2. Positions of molecular-size markers are shown at left in kDa (kD).

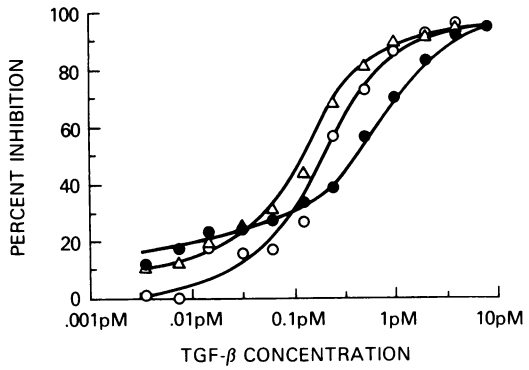

FIG. 4. Inhibition of Mv1Lu growth by TGF-$\beta_{2\text{-}1\text{-}2}$. Mv1Lu cells were assayed for growth with TGF-β_1 (●), TGF-β_2 (○), or the purified chimeric TGF-$\beta_{2\text{-}1\text{-}2}$ (△). DNA synthesis was measured as described, and percent inhibition was calculated as percent difference between counts of ^{3}H (dpm) measured in cells treated with TGF-β compared with nontreated cells.

When assayed for growth-inhibitory activity on Mv1Lu cells, TGF-β_1, TGF-β_2, and the chimeric TGF-$\beta_{2\text{-}1\text{-}2}$ have approximately equal activities (Fig. 4). This result is consistent with previous ones that showed TGF-β_1, -β_2, and -β_3 each to have very similar biological potencies on these cells (7, 15). Importantly, this result shows that proper folding and full biological activity of the chimeric TGF-β were achieved in this recombinant system.

The TGF-$\beta_{2\text{-}1\text{-}2}$ chimera was also assayed for its ability to inhibit growth of both FBHE cells (Fig. 5*A*) and RFCs (Fig. 5*B*) as well as for its ability to inhibit the migration of BAECs (Fig. 5*C*). In all of these assays, TGF-β_1 is substantially more potent than TGF-β_2. Importantly, the chimeric protein was almost as active as TGF-β_1 in each of these assays. TGF-β_1 and the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera were equivalent in inhibiting growth of FBHE cells, whereas TGF-β_2 was 100-fold less potent as a growth inhibitor (Fig. 5*A*). Similarly, TGF-β_1 and the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera inhibited the growth of RFCs by 35% at 0.5 ng/ml, whereas at this same concentration, only 15% inhibition was obtained with TGF-β_2 (Fig. 5*B*). In addition, migration of BAECs was ≈20% less than the control when the cells were treated with TGF-β_1 or the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera, whereas only a 3% decrease was seen with TGF-β_2 (Fig. 5*C*). The different forms of TGF-β were assayed at equal concentrations for Fig. 5 *B* and *C*. The response to the different TGF-β forms was dose dependent in both the RFC growth assay and the BAEC migration assay (data not shown). From these results, it can be concluded that there exists within the region from amino acids 40–82 a specific amino acid or amino acids responsible for the different potencies of TGF-β_1 and -β_2 in these assays.

DISCUSSION

To begin to investigate the structural regions of TGF-β required for its biological functions, we have created a chimeric TGF-β containing amino acids 1–39 of TGF-β_2, 40–82 of TGF-β_1, and 83–112 of TGF-β_2. This recombinant chimeric protein was purified to homogeneity from conditioned medium and then assayed for its ability to inhibit the growth or migration of endothelial cells. The growth of FBHE cells is readily inhibited by TGF-β_1, whereas nearly 100 times more TGF-β_2 is required to achieve the same inhibition. Importantly, the biological potency of the TGF-$\beta_{2\text{-}1\text{-}2}$ chimera was equal to that of TGF-β_1 in inhibiting growth of FBHE cells. Similarly, the chimeric TGF-β was as potent as TGF-β_1 in inhibiting growth of RFCs. In addition, the chimera was nearly equivalent to TGF-β_1 and was much more active than TGF-β_2 at inhibiting migration of BAECs. Thus, the chimera was as potent as TGF-β_1 when assayed in three different assays that clearly distinguish the biological potencies of TGF-β_1 and TGF-β_2. Therefore, the region from amino acids 40–82 of TGF-β_1 contains the information sufficient for specifying the greater biological potency of TGF-β_1 as compared with TGF-β_2. Within this 42-amino acid region, only 14 differences are found when TGF-β_1 and TGF-β_2 sequences are compared.

The mechanisms governing the different biological potencies of the TGF-β isoforms are beginning to be understood. Considerable evidence has accumulated showing that the

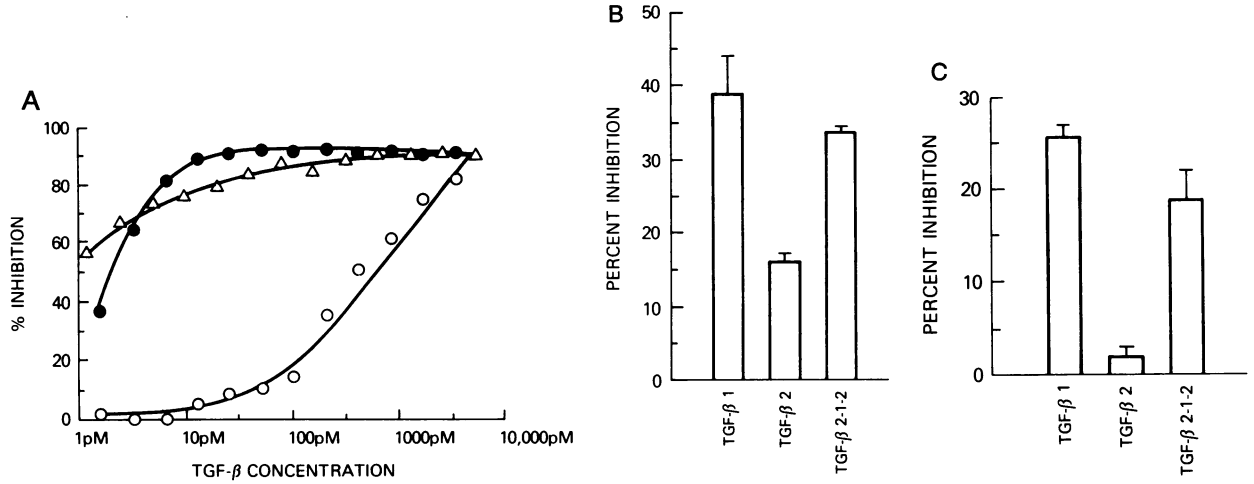

FIG. 5. Inhibition of endothelial cell growth and migration by TGF-$\beta_{2\text{-}1\text{-}2}$. (*A*) Inhibition of FBHE cell growth. FBHE cells were assayed for growth with porcine TGF-β_1 (●), porcine TGF-β_2 (○), and the purified chimeric TGF-$\beta_{2\text{-}1\text{-}2}$ (△). Incorporation of [^{3}H]thymidine into DNA was measured and expressed as percent inhibition relative to that of untreated cells. (*B*) Inhibition of RFC growth. RFCs were assayed for growth without added growth factors or with TGF-β_1, TGF-β_2, or purified TGF-$\beta_{2\text{-}1\text{-}2}$ at 0.5 ng/ml. The total number of cells in each tissue culture dish was determined by removing the cells from the plate with trypsin/EDTA and counting in a Coulter Counter. Values represent percent decrease in cell number in dishes treated with growth factors relative to cell number in control dishes. (*C*) Inhibition of BAEC migration. BAECs were assayed for inhibition of migration in response to added growth factors as described. After fixing with neutral buffered formalin and staining with Harris hematoxylin, the net increase in surface area covered by migrating cells was determined by using an overhead projector. Percent inhibition of migration was determined by using a computerized graphics tablet and MACMEASURE, EXCEL, and STATWORKS programs. T bars represent SDs.

TGF-β isoforms have different affinities for their receptors (23–26) and different cell-surface-binding proteins (27, 28). For example, although TGF-β_1 and β_3 are much more potent than TGF-β_2 in binding to the overall population of receptors on most cell lines, a subset of the type 1 and 2 receptors has been detected on Mv1Lu cells that has a higher affinity for TGF-β_2 than for TGF-β_1 or TGF-β_3 (7). This subset of receptors was not detected in FBHE cells. In contrast, four additional TGF-β binding proteins have been identified on FBHE cells and MG-63 human osteosarcoma cells (28); two of these binding proteins preferentially bound TGF-β_1, whereas the remaining two proteins preferentially bound TGF-β_2.

Although α_2-macroglobulin, an abundant serum protein, binds and inactivates TGF-β_2 with 10-fold greater affinity than TGF-β_1 (29, 30), it has been shown that this affinity is not the basis of the greater biological potency of TGF-β_1 than TGF-β_2 in the FBHE cell growth-inhibition assay (7). Importantly, results from growth-inhibition assays with excess α_2-macroglobulin or in serum depleted of α_2-macroglobulin indicate that the different response to the TGF-β isoforms is dictated by cell-specific determinants, such as those described above.

Although many cells in culture respond equally to the TGF-β isoforms, substantial *in vivo* differences in the biological potencies of the TGF-β isoforms will probably be discovered. In fact, complex systems, such as the induction of mesoderm in *Xenopus* embryos, respond selectively to the various TGF-β isoforms (11, 12), suggesting that part of the process of adapting a single cell to sustained tissue culture conditions may be an alteration in expression of the TGF-β receptors (31). As we continue to learn more about the complex *in vivo* actions of TGF-β, we will likely find additional important consequences of isoform switching.

The use of chimeric molecules, as described here, is a practical approach to investigating the structure/function relationships in closely related proteins. Although one could not be certain that a chimeric TGF-β molecule would fold correctly, this approach has been successful in understanding the structure of other dimeric, disulfide-linked molecules, such as platelet-derived growth factor (16, 32). Because this approach produces a recombinant protein of the same size as the endogenous molecule, it was likely that the chimeric TGF-β would maintain biological activity, in contrast to recombinant proteins made by using other mutagenesis techniques (33).

Further investigations into the structure/function relationships of TGF-β are warranted because of the broad clinical applications of TGF-β (34). Chimeric molecules, such as the one described here, may prove important *in vivo* as activators of specific TGF-β responses separating the growth effect of TGF-β from its gene-activation effects (35). In addition, because certain disease processes, particularly those involving fibrosis, may be characterized by excessive and inappropriate actions of TGF-β, there are also clear indications for the development of TGF-β antagonists. Analyses such as the one described here will be important to use in designing further therapeutically important analogs of TGF-β.

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1. Sporn, M. B. & Roberts, A. B. (1990) *Cell Regul.* **1**, 875–882.
2. Massagué, J. (1990) *Annu. Rev. Cell. Biol.* **6**, 597–641.
3. Roberts, A. B. & Sporn, M. B. (1990) in *Peptide Growth Factors and Their Receptors*, eds. Sporn, M. B. & Roberts, A. B. (Springer, Berlin), Vol. 1, pp. 419–472.
4. Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M. & Sporn, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5339–5343.
5. Massagué, J., Cheifetz, S., Boyd, F. T. & Andres, J. L. (1990) *Ann. N.Y. Acad. Sci.* **593**, 59–72.
6. Jennings, C. J., Mohan, S., Linkhart, T. A., Widstrom, R. & Baylink, D. J. (1988) *J. Cell Physiol.* **137**, 167–172.
7. Cheifetz, S. C., Hernandez, H., Laiho, M., ten Dijke, P., Iwata, K. K. & Massagué, J. (1990) *J. Biol. Chem.* **265**, 20533–20538.
8. Merwin, J. R., Newman, W., Beall, L. D., Tucker, A. & Madri, J. A. (1991) *Am. J. Pathol.* **138**, 37–51.
9. Ottmann, O. G. & Pelus, L. M. (1989) *J. Immunol.* **140**, 2661–2665.
10. Ohta, M., Greenberger, J. S., Anklesaria, P., Bassols, A. & Massagué, J. (1987) *Nature (London)* **329**, 539–541.
11. Rosa, F., Roberts, A. B., Danielpour, D., Dart, L. L., Sporn, M. B. & Dawid, I. B. (1988) *Science* **239**, 783–786.
12. Roberts, A. B., Kondaiah, P., Rosa, F., Watanabe, S., Good, P., Danielpour, D., Roche, N. S., Rebbert, M. L., Dawid, I. B. & Sporn, M. B. (1990) *Growth Factors* **3**, 277–286.
13. Madri, J. A. & Williams, S. K. (1983) *J. Cell Biol.* **97**, 153–165.
14. Madri, J. A., Dreyer, F. A., Pitlick, F. A. & Furthmayr, H. (1980) *Lab. Invest.* **43**, 305–315.
15. Danielpour, D., Dart, L. L., Flanders, K. C., Roberts, A. B. & Sporn, M. B. (1989) *J. Cell. Physiol.* **138**, 79–86.
16. LaRochelle, W. J., Giese, N., May-Siroff, M., Robbins, K. C. & Aaronson, S. A. (1990) *Science* **248**, 1541–1544.
17. Jhappan, C., Stahle, C., Harkins, R. N., Fausto, N., Smith, G. H. & Merlino, G. T. (1990) *Cell* **61**, 1137–1146.
18. Kondaiah, P., Van Obberghen-Schilling, E., Ludwig, R. L., Dhar, R., Sporn, M. B. & Roberts, A. B. (1988) *J. Biol. Chem.* **263**, 18313–18317.
19. Hanks, S. K., Armour, R., Baldwin, J. H., Maldonado, F., Spiess, J. & Holley, R. W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 79–82.
20. Brunner, A. M., Marquard, H., Malacko, A. R., Lioubin, M. N. & Purchio, A. F. (1989) *J. Biol. Chem.* **264**, 13660–13664.
21. Flanders, K. C., Roberts, A. B., Ling, N., Fleurdelys, B. E. & Sporn, M. B. (1988) *Biochemistry* **27**, 739–746.
22. Roberts, A. B., Rosa, F., Roche, N. S., Coligan, J. E., Garfield, M., Rebbert, M. L., Kondaiah, P., Danielpour, D., Kehrl, J. H., Wahl, S. M., Dawid, I. G. & Sporn, M. B. (1989) *Growth Factors* **2**, 135–147.
23. Cheifetz, S., Bassols, A., Stanley, K., Ohta, M., Greenberger, J. & Massagué, J. (1988) *J. Biol. Chem.* **263**, 10783–10789.
24. Cheifetz, S., Weatherbee, J. A., Tsang, M. L.-S., Anderson, J. K., Mole, J. E., Lucas, R. & Massagué, J. (1987) *Cell* **48**, 409–415.
25. Segarini, P. R., Roberts, A. B., Rosen, D. M. & Seyedin, S. M. (1987) *J. Biol. Chem.* **262**, 14655–14662.
26. Mitchell, E. J., Fitz-Gibbon, L. & O'Connor-McCourt, M. D. (1992) *J. Cell. Physiol.* **150**, 334–343.
27. Mitchell, E. J. & O'Connor-McCourt, M. D. (1991) *Biochemistry* **30**, 4350–4356.
28. Cheifetz, S. & Massagué, J. (1991) *J. Biol. Chem.* **266**, 20767–20772.
29. Danielpour, D. & Sporn, M. B. (1990) *J. Biol. Chem.* **265**, 6973–6977.
30. O'Connor-McCourt, M. D. & Wakefield, L. M. (1987) *J. Biol. Chem.* **262**, 14090–14099.
31. Segarini, P. R., Rosen, D. M. & Seyedin, S. M. (1989) *Mol. Endocrinol.* **3**, 261–272.
32. Ostman, A., Andersson, M., Hellman, U. & Heldin, C.-H. (1991) *J. Biol. Chem.* **266**, 10073–10077.
33. Sha, X., Yang, L. & Gentry, L. E. (1991) *J. Cell Biol.* **114**, 827–839.
34. Sporn, M. B. & Roberts, A. B. (1989) *J. Am. Med. Assoc.* **262**, 938–941.
35. Geiser, A. G., Burmester, J. K., Webbink, R., Roberts, A. B. & Sporn, M. B. (1992) *J. Biol. Chem.* **267**, 2588–2593.