Heterodimeric Transforming Growth Factor β

BIOLOGICAL PROPERTIES AND INTERACTION WITH THREE TYPES OF CELL SURFACE RECEPTORS*

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Type β transforming growth factors (TGF) are disulfide-linked homo- and heterodimers of two related polypeptide chains, β1 and β2. The homodimers TGF-β1 and TGF-β2 are widely distributed, but the heterodimer TGF-β1.2 has been found only in porcine platelets (Cheifetz, S., Weatherbee, J. A., Tsang, M. L.-S., Anderson, J. K., Mole, J. E., Lucas, R., and Massagué, J. (1987) Cell 48, 409–415). Here we characterize the receptor binding and biological properties of TGF-β1.2 and compare them with those of TGF-β1 and TGF-β2. Three types of cell surface receptors previously identified by affinity labeling with 125I-TGF-β1 are available for binding to TGF-β1.2. These three types of receptors are detected as 65-kDa (type I), 85–95-kDa (type II), and 250–350-kDa (type III) affinity-labeled receptor complexes on electrophoresis gels. They coexist in many cell types, have high affinity for TGF-β1, and varying degrees of affinity for TGF-β2. Of the 11 cell lines screened in the present study none showed evidence for additional receptor types that would bind TGF-β2 but not TGF-β1. In receptor competition studies, TGF-β1, TGF-β1.2, and TGF-β2 competed for binding to type I and type II receptors with a relative order of potencies of 16:5:1 and 12:3:1, respectively, whereas TGF-β1 and TGF-β1.2 were of equal potency for the type III receptors. The three forms of TGF-β were equally potent at stimulating the biosynthesis of extracellular sulfated proteoglycan in BRL-3A rat liver epithelial cells, a response that presumably involves the type III receptor present in these cells. In contrast, the ability of the three ligands to inhibit the growth of B6SUt-A multipotential hematopoietic progenitor cells which display only type I receptors decreased in the order TGF-β1, TGF-β1.2, and TGF-β2 with a relative potency of 100:30:1. The results indicate that the presence of one β1 chain in TGF-β1.2 increases (with respect to TGF-β2) the biological potency and binding affinity toward receptor types I and II, but the presence of a second β1 chain in the dimer is required for full potency.

The TGF-β family of polypeptides that regulate cell growth and phenotype includes numerous factors found in insects and vertebrates (reviewed in Ref. 1). Within this family, the TGFs-β are a group of disulfide-linked dimers of 25 kDa distinguished by their ability to control proliferation and differentiation of cells derived from a variety of origins such as connective, epithelial, endothelial, chondrogenic, myogenic, adipogenic, hematopoietic, and neural tissues (reviewed in Refs. 1–3). Two homodimeric forms of TGF-β, TGF-β1 and TGF-β2, exist whose chains show about 70% amino acid sequence similarity; TGF-β1 and TGF-β2 are found in numerous mammalian tissues (4–8). In addition, co-expression of the TGF-β1 and TGF-β2 chains in the same cell can give rise to the heterodimer, TGF-β1.2. Thus far, TGF-β1.2 has been found only in porcine platelets stored together with TGF-β1 and TGF-β2 (5). We have speculated that the expression of multiple forms of TGF-β that have varying degrees of affinity for different types of cell surface receptors may provide a means to finely tune the response of multicellular systems to these polypeptides (5, 9). Cell types that respond differently to TGF-β1 and TGF-β2 have indeed been found including various lines of multipotential hematopoietic progenitor cells (9). The existence of the heterodimer TGF-β1.2 increases the regulatory potential of the TGF-β system, but the limited availability of this product had not allowed the study of its properties until now.

TGF-β1 and TGF-β2 bind with high affinity to three distinct components on the surface of target cells. These three components display affinity constants for TGF-β1 in the picomolar range, are not recognized by unrelated polypeptide hormones, and have properties of integral membrane glycoproteins. Based on these characteristics, they have been operationally defined as TGF-β receptors and have been classified according to their structural and functional properties (reviewed in Ref. 10). Two of the three types of TGF-β receptors, types I and II, have higher affinity for TGF-β1 than TGF-β2 (5, 9). These two receptor types can be distinguished by the proteolytic peptide maps of their affinity-labeled domains (11). Type I TGF-β receptors are identified as affinity-labeled complexes of 65 kDa. Type II receptors yield affinity-labeled complexes of 85 kDa in rat, mouse, and mink cells, 95 kDa in human and monkey cells, and 110 kDa in chick fibroblasts. A third TGF-β receptor type, type III, is a 250–350-kDa glycoprotein with high affinity for both TGF-β1 and TGF-β2. In some cell lines type III receptors exist as part of a larger complex stabilized by disulfide bonds (12). With the exception of retinoblastoma and neuroblastoma tumors that lack TGF-β receptors (13), at least one and most frequently all three TGF-β receptor types coexist in nearly 100 cell lines.

* The abbreviation used is: TGF, transforming growth factor.

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¶ The molecular weight values given for the affinity-labeled receptor complexes are estimates based on the migration of these complexes on electrophoresis gels run against standards. The receptor molecular weight values include 12 kDa that correspond to one [125I]-TGF-β1 monomer chain cross-linked to the receptor protein in dithiothreitol-reduced samples.
and tissues that we have screened (11-15).

In the present studies we have characterized the biological activity of TGF-β1.2 and its interaction with the three receptor types. The results provide new information on the contribution of individual TGF-β chains to the receptor binding and biological properties of the TGFs-β.

EXPERIMENTAL PROCEDURES AND RESULTS

Receptor Binding and Biological Properties of TGFβ-1.2—The ability of TGF-β1.2 to compete with ¹²⁵I-TGF-β1 for binding to and affinity labeling of cell surface receptors was compared with that of human platelet TGF-β1 (which has the same amino acid sequence as porcine TGF-β1 (21)) and that of porcine platelet TGF-β2 in affinity-labeling competition experiments (Fig. 5). BRL-3A rat liver epithelial cells were selected for these experiments because they exhibit relative levels of affinity-labeled receptor types I, II, and III that facilitate comparison between labeled species in the same autoradiogram.

The potency of TGF-β1.2 in inhibiting the labeling of receptor types I and II was intermediate between those of TGF-β1 and TGF-β2 (Fig. 5). Densitometric scans of the autoradiograms revealed reproducible differences between the binding to these two receptor types. Thus, the approximate order of potencies of TGF-β1, TGF-β1.2, and TGF-β2 to compete for type I receptors was 16:5:1. At low concentrations

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**Fig. 5. Receptor competition of TGF-β1, TGF-β1.2, and TGF-β2 in BRL-3A cells.** Confluent monolayers of BRL-3A cells were incubated for 4 h at 4 °C with 25 pM ¹²⁵I-TGF-β1 alone or in the presence of indicated concentrations of TGF-β1, TGF-β1.2, or TGF-β2. Affinity labeling was done as described in Fig. 2. One representative experiment of five is shown. A, autoradiograms from electrophoresis gels containing the affinity-labeled samples. B, densitometric analysis of the bands corresponding to the type I (65 kDa), type II (85 kDa), and type III (280 kDa) TGF-β receptors. Symbols are: ○, competing TGF-β1; ●, TGF-β1.2; and △, TGF-β2. Data are expressed as percent inhibition of the labeling of each band relative to the same band in samples affinity-labeled with ¹²⁵I-TGF-β1 alone.
Heterodimeric TGF-β

TABLE I

| Cell line          | Function                        | Parameter measured | Effect of TGF-β | ED_{50} TGF-β1 | TGF-β2 |
|--------------------|---------------------------------|--------------------|-----------------|----------------|--------|
| Mv1Lu mink lung epithelial | Extracellular matrix production | Fibronectin synthesis | Stimulation     | 30             | 20     |
| 3T3-L1 mouse preadipocyte | Extracellular matrix production | Type I collagen synthesis | Stimulation     | 6              | 4      |
| NRK-49F rat kidney fibroblasts | Extracellular matrix production | Proteoglycan synthesis | Stimulation     | 5              | 5      |
| BRL-3A rat liver epithelial | Extracellular matrix production | Proteoglycan synthesis | Stimulation     | 25             | 25     |
| 3T3-L1             | Cell adhesion                    | Fibronectin receptor synthesis | Stimulation | 30             | 5      |
| Mv1Lu              | Proliferation                    | ![Image](image_url) |                | 10             | 1000   |
| B6SUt-A and 32 DCL3 mouse hematopoietic progenitors | CSF-induced proliferation/differentiation | [H]Thymidine incorporation | Stimulation | 5              | 5      |
| NRK-49F            | Growth in soft agar              | Colony formation    | Stimulation     | 35             | 20     |
| 3T3-L1             | Adipogenic differentiation        | Glyceraldehyde dehydrogenase activity | Inhibition     | 7              | 5      |
| L6E9 rat skeletal myoblast | Myogenic differentiation         | Creatine phosphokinase activity | Inhibition     | 7              | 5      |

*References are to previously published work describing the relative potency of TGF-β1 and TGF-β2.

DISCUSSION

The results of the present study provide information on three aspects of the interaction of TGF-βs with receptors in
target cells. First, the results show that in a sample of 11 cell lines the only receptors with which TGF-β2 can detectably interact are the three types of cell surface components previously identified as receptors for TGF-β1. Second, the results define biological and receptor binding properties of the heterodimer, TGF-β1.2. Third, the results with TGF-β1.2 extend preliminary evidence correlating induction of particular cellular responses with occupancy of different types of TGF-β receptors.

The results of binding experiments show that all the binding of 125I-TGF-β2 to cells can be competed as effectively by TGF-β1 as by TGF-β2. In reverse experiments, TGF-β2 inhibits strongly the labeling of type III receptors by 125I-TGF-β1 but inhibits more poorly the labeling of receptor types I and II. This is consistent with the observation that TGF-β2 is less potent than TGF-β1 in competing with 125I-TGF-β1 for binding to intact cells and that 125I-TGF-β2 affinity labels well the type III receptor but much less effectively the receptor types I and II. Precipitation of cells with TGF-β1 to saturate binding sites for this ligand failed to uncover residual sites that would preferentially bind TGF-β2. In contrast to the ubiquitous and easily detectable occurrence of TGF-β receptor types I, II, and III, the results indicate that if additional types of receptors exist that bind preferentially TGF-β2 over TGF-β1 they would have to be present at levels below the detectability limit of our assays (<300 copies/cell, Ref. 9) or be restricted to cell types not screened in this study.

The approach used here and in our previous work to identify TGF-β binding proteins has been used recently by Segarini et al. (26). Their results confirm ours in identifying three affinity-labeled receptor types of 65, 85, and 250 kDa, but with one significant difference. This difference lies in the inability of TGF-β1 to compete in their studies with 125I-TGF-β2 for binding to and labeling of all three receptor components in NRK-49F and Swiss 3T3 cells. Based on this result, Segarini et al. (26) have proposed that each one of the three receptor types is in turn a mixture of various classes of binding sites and TGF-β2, and the source and passage number of the NRK-49F cells used in both studies were the same. However, differences in the results might arise from differences in 125I-labeled ligand preparations. We use milder oxidizing conditions than those previously described for iodination of TGF-β (18).

The availability of the heterodimer TGF-β1.2 has allowed a better characterization of the interaction of the TGF-βs with their cell surface receptors. The results show that the presence of one β1 chain in TGF-β1.2 makes it a better ligand for receptor types I and II and a more potent agonist than TGF-β2. However, only the TGF-β1 homodimer displays full potency. It is of interest that the relative potencies of TGF-β1, TGF-β1.2, and TGF-β2 for binding to type I receptors are 16:5:1, but the differences between these three ligands are amplified at the level of the biological response in B6SuT-A hematopoietic progenitor cells reaching an order of potencies of 100:30:1 (see Fig. 7). This observation is reproducible and is not due to a faster rate of degradation of TGF-β2 by B6SuT-A cells (9) or a wider divergence in the receptor affinities of TGF-β1 and TGF-β2 at 37 °C than at 4 °C (data not shown). The basis for this phenomenon is not known. It is possible that each of the two chains in a TGF-β molecule binds one receptor molecule, ligand-induced receptor dimerization being necessary for receptor activation and cell stimulation. In this case, the relative potency for receptor activation by a given form of TGF-β would be closer to the product of the relative binding potencies of the individual TGF-β chains.

Comparisons between the biological potency and the receptor binding affinity represent a first approximation to the question of which receptors may be involved in mediating individual actions of the TGFs-β. The simplest interpretation of the available data suggests that occupancy of type I receptors may be involved in inhibition of proliferation in hematopoietic progenitor cells, while all the other responses measured including regulation of cell adhesion proteins and their receptors, and inhibition of adipogenic differentiation, myogenic differentiation, and epithelial cell proliferation may be mediated by type III receptors. Alternative approaches to probe more directly the biological properties of the various TGF-β receptors may uncover a scheme more complex than the one outlined here. In this context, it is intriguing that growth inhibition by TGFs-β correlates with interaction of these polypeptides with type I receptors in B6SuT-A cells, but with type III receptors in Mv1Lu epithelial cells even though this cell line also has type I receptors. It is possible that growth inhibition is the end point of pathways that are different in these two cell types. However, cooperation between the signals of the two receptors in epithelial cells or sharing of postreceptor mechanisms by different receptors are open possibilities as well.

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Supplement to Heterodimeric Transforming Growth Factors: Biological Properties and Interaction with Three Types of Cell Surface Receptors

by

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FINANCIAL PROVISION

Cell culture

Methods were similar to all cell lines were obtained from American Type Culture Collection and were grown according to the supplier's specifications. Antibiotics were used in the presence of 10% as previously described (18). Cells were cultured in 

Source of TGF-β

Heterodimeric TGF-β was purified from activated platelets as previously described (20). Recombinant TGF-β1 and TGF-β2 were purified as described by Segarini et al. (17) and purified platelet extracts were used as a source of TGF-β. The preparations of TGF-β used for homogenous were obtained by gel electrophoresis and silver staining, and were quantitated by densitometric analysis.

Isolation of TGF-β

The conditions for preparing isolated TGF-β were essentially as described by Frailis et al. (18) with the modification that the concentration of 125I-TGF-β was determined by a final volume of 1.0 ml of 2% bovine serum albumin in PBS at 4°C. The mixture was incubated for 2 h at 4°C in the absence of proteinase K. The TGF-β in 1.0 ml of either 0.1% bovine serum albumin or 0.1% acetic acid (TGF-β1) or 0.1% acetic acid (TGF-β2) was mixed with an equal volume of a 1.0 ml solution of 2.5% (by vol) 0.4 M KCl/50 mM Tris.HCl buffer (pH 7.4). The mixture was incubated for 2 h at 4°C. In both procedures, the ability of non-specific binding to be competed was assessed in the presence of 100 μl of standard cell culture medium. The specific activity of the preparations of TGF-β was determined by (125I-TGF-β and 125I-TGF-β2) and 125I-TGF-β3, respectively.

Receptor Binding and Drug Kinetics

The protocols followed for measuring receptor binding at 4°C and preparing affinity labeled receptors have been described (21). The concentration of TGF-β used in each experiment is indicated in the legend.

Other Aspects

Conditions followed for measuring the TGF-β-induced expression of extracellular matrix proteins, including proteinsases (19), fibronectin, and type I collagen (20) and the activation of MAP kinase (21) and other kinases (22) that have been described in the legend. Inhibitors of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) were added to the culture media at the concentrations specified. The dependence of the activity of TGF-β on the presence of these inhibitors was assessed by measuring the levels of matrix metalloproteinase activity using a commercially available kit (23).

RESULTS

Receptors Available for TGF-β Binding

Previous work (23) has shown that TGF-β binds to cell surface receptors that have high affinity for TGF-β. Moreover, some of the biological effects of TGF-β1 and TGF-β2 are mediated by the ability of TGF-β1 and TGF-β2 to compete with 125I-TGF-β in binding to intact cells. However, the ability to inhibit TGF-β binding to TGF-β1 and TGF-β2 of intact cells have been examined by using a commercial kit (24). Similar results were obtained with intact cells (25) (Fig. 1). TGF-β1 and TGF-β2 competed effectively with 125I-TGF-β1 and the ability of TGF-β1 and TGF-β2 to compete with 125I-TGF-β1 for binding to intact cells.

These results indicate that most if not all of the TGF-β1 or TGF-β2 binding in NIH-3T3 cells or BM-13 cells occurs on receptors that are also recognized by TGF-β3. However, few or no receptors with high affinity for TGF-β1 and TGF-β2 were detected in NIH-3T3 cells or BM-13 cells that had been preincubated with 125I-TGF-β1 or 125I-TGF-β2. To investigate whether such receptors exist, we performed competition experiments of intact cells that were preincubated with 125I-TGF-β1 or 125I-TGF-β2. The ability of TGF-β1 and TGF-β2 to compete with 125I-TGF-β1 for binding to intact cells.

Similar results were obtained with intact cells expressing different types of TGF-β1 and TGF-β2 receptors. In agreement with previous work with other cell types, TGF-β1 and TGF-β2 were equally potent in inhibiting the binding of intact cells prelabeled with TGF-β1 or TGF-β2. However, TGF-β1 was about 10 times more potent than TGF-β2 as inhibitors of the labeling of TGF-β1 or TGF-β2 receptors, respectively.

In a comparison of these results, the TGF-β1 receptor was effectively labeled by TGF-β3, TGF-β3 and TGF-β3 were equally potent at inhibiting the labeling of intact cells prelabeled with TGF-β1 or TGF-β2, respectively. TGF-β1 was about 10 times more potent than TGF-β2 as inhibitors of the labeling of TGF-β1 or TGF-β2 receptors, respectively.

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Heterodimeric TGF-β

A. NRK-49F Cells

![Graph showing binding of TGF-β to NRK-49F cells.]

B. BRL-3A Cells

![Graph showing binding of TGF-β to BRL-3A cells.]

**Figure 1.** Competition Profiles of TGF-α1 and TGF-α2 Versus Inactivated TGF-α2 in NRK-49F and BRL-3A Cells

Confluent monolayers of NRK-49F cells (A) and BRL-3A cells (B) seeded in 12-well multilastor dishes were incubated for 4 h at 4°C with 32P-labeled native TGF-α2 or TGF-α2 in the presence of increasing concentrations of unlabeled bovine TGF-α1 (x) or TGF-α2 (y). Tritiated acid-soluble counts were determined (z) and are expressed as a percent of the counts bound in control wells receiving only the indicated ligand. Values are the average of four (NRK-49F) or two (BRL-3A) determinations. Divergence between individual values in each data point was less than 18%.

**Figure 2.** Affinity labeling of NRK-49F cells with 32P-TGF-α1 and 32P-TGF-α2.

Confluent cell monolayers were incubated for 4 or 12 h at 4°C with 32P-TGF-αs alone (25 μM) or in the presence of the indicated concentrations of unlabeled ligands. Reaction mixtures were separated by using SDS-polyacrylamide gels and visualized by autoradiography. The position of molecular weight markers run in parallel with the three receptors is indicated with values in kilodaltons. The experiment was repeated twice with the same results.

**Figure 3.** Affinity labeling of various cell lines with 32P-TGF-α.

Confluent monolayers of cells from human Vero-C, AKII, rat liver, BRL-3A, mouse Mailot 321, NIH Swiss 3T3, D10-11, or 151 (Male) origin were incubated for 4 h at 4°C with 25 μM [32P]-TGF-α2 alone (x) or with unlabeled TGF-α1 (y) or TGF-α2 (z) at a concentration of 5 μM (a) for 3T3 and D10-11 cells. TGF-αs were from bovine bone. Affinity labeling and electrophoresis were performed as described in Figure 2. Autoradiograms of resulting gels are shown. The migration position of type I (a), type II (b), and type III (c) receptors is indicated on the left side of each panel.

**Figure 4.** Effect of Lipid:Phosphorus in the Affinity Labeled- Receptor Profile of NRK-49F Cells.

Confluent cell monolayers were incubated for 4 or 12 h at 4°C with the ligand indicated below. They were then rinsed five times with ice-cold binding buffer and incubated for another 4 h at 4°C with the indicated ligand. The ligands were (a) 3 μM TGF-α1, (b) 3 μM TGF-α2, (c) 1 μM TGF-α1, (d) 3 μM TGF-α2, (e) 0.2 μM TGF-α1-TGF-α2, (f) 3 μM TGF-α1, (g) 0.2 μM TGF-α1-TGF-α2, (h) buffer alone, and (i) 0.2 μM TGF-α1-TGF-α2, 3 μM TGF-α1, and (j) buffer alone. After washing and incubation, liganded receptors were identified by autoradiography. Autoradiograms of resulting gels are shown. The migration position of type I, type II and type III receptors is indicated.
Figure 6. Effect of three forms of TGF-β on the biosynthesis of secretory sulfated proteoglycan in BRL-34 cells.

Subconfluent monolayers of BRL-34 were placed in serum-free culture medium for 8 h prior to treatment with the indicated concentrations of TGF-β, TGF-β, or TGF-β for 20 h. Cultures were switched to serum-free culture medium containing 0.5 mM sulfate, 100 μM 35S-sulfate, and the corrected by centrifugation at 10,000 g. After 6 h, incorporation of radioactivity was measured by scintillation counting.