Cellular Receptors for Type β Transforming Growth Factor

LIGAND BINDING AND AFFINITY LABELING IN HUMAN AND RODENT CELL LINES*

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Type β transforming growth factor (βTGF) purified from human platelets to homogeneity as judged by NH2-terminal amino acid sequence analysis has been labeled with 125I to characterize its interaction with cellular receptors. Binding of 125I-βTGF to target cells is temperature- and time-dependent, specific, saturable, and reversible. About 1.6–1.9 x 10^5 binding sites/cell with high affinity for βTGF (Kd = 5.6–7.8 x 10^-11 M and 9.1–14 x 10^-11 M, respectively) are found in NRK-49F and BALB/c 3T3 cells. βTGF receptors do not appear to undergo acute down-regulation by the ligand. Specific binding of 125I-βTGF has been observed in several human, rat, and mouse fibroblast lines and in some, but not all, tumor-derived cell lines examined. 125I-βTGF has been cross-linked to intact cells and isolated membrane preparations using disuccinimidyl suberate. Cells and isolated membranes from human, rat, and mouse origin affinity labeled with 125I-βTGF exhibit a major labeled species of approximately 280 kilodaltons that has the properties of high affinity and specificity expected from a physiologically relevant βTGF receptor. Minor labeled species of 70–90 kilodaltons are also labeled by 125I-βTGF, but they correspond to molecular species with low apparent affinity (Kd ~ 10^-9 M) for 125I-βTGF.

The signals elicited by receptors for polypeptide growth factors are capable of inducing cellular transformation (1). The oncogenic potential of genes encoding products related to platelet-derived growth factor (PDGF) and the receptor TGFs and type 01 TGF receptor. Minor labeled species of 70–90 kilodaltons are also labeled by 125I-βTGF, but they correspond to molecular species with low apparent affinity (Kd ~ 10^-9 M) for 125I-βTGF.

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βTGF is a polypeptide found in transformed cells that also produce αTGF (5, 6) and in normal tissues, including blood platelets (13–16). It consists of two identically sized 11–12 kDa chains linked by disulfide bonds (13, 14, 16, 17). By analogy to other polypeptide hormone systems, the cellular actions of βTGF are presumably exerted through its interaction with specific cell-surface receptors. This report shows that in various cell types, 125I-βTGF binds to a single class of saturable, high-affinity receptors specific for this ligand. Like the receptors for other polypeptide growth factors, receptors for βTGF are found in relatively low numbers in target cells. To understand the mode of action of βTGF, it is important to gain information on the structural and functional properties of these receptors. However, the low abundance of this receptor type is a limiting factor in efforts to characterize its properties. To obviate this limitation, we have used receptor affinity labeling methodology consisting of cross-linking βTGF receptors with receptor-bound 125I-βTGF using a bifunctional reagent. This methodology has been useful in characterizing receptors for insulin (15, 19), insulin-like growth factors I and II (20–22), αTGFs (10, 23), platelet-derived growth factor (24), and other polypeptide hormones (25–27). This report describes the identification and properties of affinity labeled cellular components which have the characteristics of a physiologically relevant receptor for βTGF.

EXPERIMENTAL PROCEDURES* RESULTS

Structural and Biological Integrity of Radiiodinated βTGF—The preparations of platelet βTGF used in these studies contain one major 23-kDa polypeptide consisting of two identically sized chains of 11–12 kDa linked by disulfide bonds, as judged by dodecyl sulfate-polyacrylamide gel electrophoresis with and without reductant (dithiothreitol) and silver staining of the gels (Fig. 1A). Two separate microsequencing analyses of reduced and S-carboxymethylated βTGF yielded one single N-terminal amino acid sequence, NH2-Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-Ser-Thr-Glu-Lys-Asn-Cys-Cys-Val-X-X-Leu-Tyr-Ile-Asp, under conditions in which about 30–35% of the protein loaded onto the

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1. The "Experimental Procedures" are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-2464, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Identification of βTGF Receptors

**FIG. 1. Structural and biological properties of 125I-βTGF.**

* A, dodecyl sulfate-polyacrylamide gel electrophoresis of purified βTGF. Samples containing 0.2 μg (lanes a and b) or 0.3 μg (lanes d and c) of protein corresponding, respectively, to two separate preparations of βTGF purified from human platelets were electrophoresed in the absence of reductant (lanes a and e) or in the presence of 50 mM dithiothreitol (lanes b and d). After electrophoresis, the gel was fixed and silver-stained. Molecular size standards (80 ng each) (lane c) included bovine serum albumin (68 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14 kDa). Staining material at the top of lanes b, c, and d is an artifact of the dithiothreitol-containing buffer. kDa, kilodalton.

* B, dodecyl sulfate-polyacrylamide gel electrophoresis of '*'I-βTGF. Aliquots (80,000 cpm) were electrophoresed in the presence of dithiothreitol (lane a) or without reductant (lane b). An autoradiogram (4 h) from the fixed, dried gel is shown.

* C, induction of [3H]thymidine incorporation into DNA by native (○) and '*'I-labeled (●) βTGF. The indicated concentrations of growth factor were added together with (methyl-3H)thymidine (0.5 μCi/well) to sparse monolayers of NRK-49F cells in 16-mm wells that had been growth-arrested by incubation with medium containing 0.1% calf serum. [3H]Thymidine incorporated into trichloroacetic acid-insoluble material was determined 24 h later. [3H]Thymidine incorporated into DNA in control cells treated with 5% calf serum was 218 pmol/10^6 cells. Data are the average of duplicate determinations.

* D, induction of anchorage-independent proliferation by native βTGF (○) and '*'I-βTGF (●). NRK-49F cells were plated in medium containing 0.35% agar, 0.3 nM EGF, and the indicated concentrations of βTGF or '*'I-βTGF. Assays were read 8 days later. The percent of cells growing into large colonies (>100 cells/colony) in each condition is plotted. Data are the average of duplicate plates.
Identification of βTGF Receptors

The presence of a confluent cell monolayer in the dishes markedly polypeptide growth factors. and in BALB/c 3T3 cells (not illustrated). These results concentration-dependent manner in NRK-49F cells (Fig. 2) cells. Only flGF inhibited the binding of 125I-flGF in a concentration-dependent manner in NRK-49F cells (Fig. 2) and in BALB/c 3T3 cells (not illustrated). These results indicate that 125I-βTGF binds specifically to target cells and that binding occurs at receptors that do not recognize other polypeptide growth factors.

βTGF and 125I-βTGF bind to nonsiliconized glass and various types of plastic surfaces. Significant adsorption of 125I-βTGF to plastic culture dishes was observed in the absence of cells. However, binding of 125I-βTGF to plastic dishes was not decreased by a 100-fold molar excess of βTGF. The presence of a confluent cell monolayer in the dishes markedly prevented nonspecific adsorption of 125I-βTGF. Thus, the amount of radioactivity nonspecifically bound to culture dishes incubated with 170 pm 125I-βTGF was decreased from about 4% of the input radioactivity in dishes without cells to 0.5% of the input radioactivity in dishes containing a confluent monolayer of cells (not illustrated).

Time-course and Reversibility of 125I-βTGF Binding—Fig. 3 illustrates the kinetics of 125I-βTGF association to cellular receptors at 4 and at 37 °C. At 4 °C 125I-βTGF binding was half-maximal by 30–60 min and reached saturation 4 h after radioligand addition. The binding kinetics at 37 °C were considerably faster, maximal binding being obtained about 25 min of incubation and remaining constant thereafter for at least 4 h (Fig. 3).

Progressive dissociation of cell-bound 125I-βTGF was obtained when cells preincubated with radioligand were washed free of unbound hormone and incubated at 37 °C in the presence of binding buffer (Table I). The relative dissociation was approximately the same regardless of whether cells had been preincubated with 125I-βTGF for 0.5 or 3.5 h at 37 °C, or for 3.5 h at 4 °C. Very little (12%) dissociation of prebound 125I-βTGF was obtained in cells incubated for 3.5 h at 4 °C (Table I).

Affinity and Number of βTGF Receptors—Binding isotherms obtained with various concentrations of 125I-βTGF and native βTGF indicated that saturation of βTGF receptors at 4 °C is achieved at 0.3–0.6 nM βTGF in both NRK-49F cells (Fig. 4A) and BALB/c 3T3 cells (Fig. 5A). Scatchard analysis of 125I-βTGF binding to NRK-49F cells and BALB/

**TABLE I**

| 125I-βTGF binding conditions | Dissociation conditions | Amount released |
|------------------------------|------------------------|-----------------|
| Temperature                  | Time                   | % prebound cpm  |
| 0.5 h at 37 °C               | 37                     | 10              | 22              |
| 3.5 h at 37 °C               | 37                     | 10              | 76              |
| 3.5 h at 4 °C                | 37                     | 10              | 19              |
| 0.5 h at 37 °C               | 4                      | 10              | 71              |
| 0.5 h at 37 °C               | 4                      | 10              | 14              |

*Confluent (2.3 x 10⁶ cells/well) monolayers of BALB/c 3T3 cells in 35-mm wells were incubated in the presence of 170 pm 125I-βTGF under the indicated conditions of time and temperature. Cell monolayers were then washed three times with ice-cold binding buffer, and cell-bound 125I-βTGF was allowed to dissociate by incubation in 1.5 ml of binding buffer under the indicated dissociation conditions. After dissociation, the radioactivity that remained specifically bound to the monolayers was determined as described under "Experimental Procedures."
Identification of βTGF Receptors

Affinity Labeling of NRK-49F Cells and Membranes with ^125^I-βTGF—We attempted to affinity label cellular receptors for βTGF by cross-linking intact cells and isolated membrane preparations with cell- or membrane-bound ^125^I-βTGF using the homobifunctional agent, disuccinimidyl suberate (DSS) (18). Fig. 6 shows an autoradiogram from a dodecyl sulfate-polyacrylamide electrophoresis gel of NRK-49F cells and isolated NRK-49F membranes cross-linked with ^125^I-βTGF. Cells and membranes were sequentially incubated in the presence of ^125^I-βTGF and DSS. The cross-linked samples were extracted with the nonionic detergent Triton X-100, and the detergent-solubilized material was subjected to electrophoresis and autoradiography. One major labeled species that migrated slowly (M_r = 250–300 x 10^6) on 7% polyacrylamide gels was observed in samples from affinity labeled intact NRK-49F cells (Fig. 6a). A relatively low (1.5 nM) concentration of βTGF (Fig. 6b) inhibited the labeling of this species by about 70% as determined by excising the corresponding gel segments and counting their radioactivity in a γ counter. This labeled species was not observed in cells that were incubated with ^125^I-βTGF but not exposed to DSS (Fig. 6a) or in control experiments in which incubations with ^125^I-βTGF and DSS were performed in the absence of cells (not illustrated). Affinity labeled NRK-49F membranes (Fig. 6, d–f) exhibited a major labeled species with molecular size and labeling properties similar to those of the major species corresponding to a membrane-associated cellular receptor with high affinity for βTGF.

Minor labeled bands were observed in the 70–90-kDa region of the gels (Fig. 6 and following ones). Their labeling could not be inhibited by 1.5 nM βTGF present during incubation of cells or membranes with ^125^I-βTGF. However, the labeling of some of these bands was effectively inhibited by higher concentrations of βTGF (see below). Thus, these labeled species seem to correspond to cellular components with lower apparent affinity for ^125^I-βTGF. Some of these labeled bands are likely nonspecific since they appeared even in cells and membrane samples that had not been exposed to DSS following incubation with ^125^I-βTGF (Fig. 6, a and d).


d = ^125^I-βTGF binding to BALB/c 3T3. A, confluent (2.3 x 10^5 cells/well) monolayers of BALB/c 3T3 cells were incubated for 4 h at 4 °C in the presence of ^125^I-βTGF plus various concentrations of native βTGF as in Fig. 4. Specifically bound βTGF was then determined. B, Scatchard plot corresponding to the data in panel A.

FIG. 5. Saturation curves of ^125^I-βTGF binding to BALB/c 3T3. A, confluent (2.3 x 10^5 cells/well) monolayers of BALB/c 3T3 cells were incubated for 4 h at 4 °C in the presence of ^125^I-βTGF plus various concentrations of native βTGF as in Fig. 4. Specifically bound βTGF was then determined. B, Scatchard plot corresponding to the data in panel A.
were extracted with a solution containing 1% Triton X-100 and without the cross-linking agent, DSS. The affinity labeled samples crude membrane fraction. Suspensions of whole cells (2 × 10^5) was added to some samples (c and f) during incubation with 0.25 mM DSS as described under "Experimental Procedures." Native βTGF (1.5 nm) was added to some samples (c and f) during incubation with 125I-βTGF. Samples a and d were treated with dimethyl sulfoxide without the cross-linking agent, DSS. The affinity labeled samples were extracted with a solution containing 1% Triton X-100 and subjected to electrophoresis on 7% (lanes a–c) or 6.5% (lanes d–f) polyacrylamide gels. Autoradiograms (3 days) from the resulting fixed, dried gels are shown. The positions of molecular size markers are indicated. Arrows point at the 280-kDa-labeled species. kDa, kilodalton.

considered at present an approximate value.

Most of the intact cell experiments in these affinity labeling studies were performed with cell suspensions rather than intact cell monolayers to reduce the incubation volume and, consequently, the amounts of homogeneous βTGF and 125I-βTGF to be used. However, some affinity labeling experiments were done with intact cell monolayers to test whether cell detachment with EDTA-containing buffer might have altered the properties of cellular components labeled with 125I-βTGF. The results shown in Fig. 7 correspond to one such experiment. Comparison between the results obtained with BALB/c 3T3 and NRK-49F cells in this experiment (Fig. 7) and in

### Table II

| Cell line       | Cell type                        | Specific binding of 150 pm 125I-βTGF* |
|-----------------|----------------------------------|--------------------------------------|
|                  |                                  | fmol/10^6 cells | % total binding |
| WI-38           | Human lung fibroblast             | 6 ± 1 (n = 3) | 65          |
| GM-316          | Human skin fibroblast             | 24 ± 2 (n = 3) | 79          |
| HOS             | Human osteosarcoma                | 9 ± 2 (n = 2) | 60          |
| A431            | Human epidermoid carcinoma        | 9 ± 1 (n = 3) | 54          |
| A675            | Human melanoma                    | <2 (n = 2) | <10         |
| NRK-49F         | Rat kidney fibroblast             | 20 ± 1 (n = 5) | 81          |
| H35             | Rat hepatoma                      | <2 (n = 2) | <10         |
| FeSV-Fre        | Rat embryo fibroblast, feline sarcoma virus-transformed | 4 ± 1 (n = 2) | 43          |
| BALB/c 3T3      | Mouse embryo fibroblast           | 16 ± 1 (n = 5) | 79          |
| Swiss 3T3       | Mouse embryo fibroblast           | 12 ± 1 (n = 3) | 65          |
| MuSV-3T3        | Mouse embryo fibroblast, murine sarcoma virus-transformed | 6 ± 1 (n = 2) | 52          |

*Cell monolayers (1.7–2.6 × 10^4 cells/well) in 35-mm wells were incubated for 2 h at 10 °C in the presence of 150 pm 125I-βTGF. Cell-associated radioactivity was then determined. Specific binding is the difference between total binding in the absence of excess βTGF and binding in the presence of 40 nM βTGF. Data are the average ± S.D. of results obtained in the indicated number of experiments.

**Fig. 6.** Dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography of NRK-49F cells and membranes affinity labeled with 125I-βTGF. Near-confluent monolayers of NRK-49F cells were detached from the culture vessels and either subjected directly to the affinity labeling protocol or homogenized to obtain a crude membrane fraction. Suspensions of whole cells (2 × 10^5) cells in 0.5 ml of buffer (lanes a–c) or isolated membranes (150 µg of membrane protein in 0.3 ml of buffer) (lanes d–f) were affinity labeled by sequential incubations in the presence of 150 pM 125I-flGF and 0.25 mM DSS as described under "Experimental Procedures." Native βTGF (1.5 nm) was added to some samples (c and f) during incubation with 125I-βTGF. Samples a and d were treated with dimethyl sulfoxide without the cross-linking agent, DSS. The affinity labeled samples were extracted with a solution containing 1% Triton X-100 and subjected to electrophoresis on 7% (lanes a–c) or 6.5% (lanes d–f) polyacrylamide gels. Autoradiograms (3 days) from the resulting fixed, dried gels are shown. The positions of molecular size markers are indicated. Arrows point at the 280-kDa-labeled species. kDa, kilodalton.

**Fig. 7.** Affinity labeling of intact monolayers of human, rat, and mouse fibroblasts with 125I-βTGF. Human WI-38 cells (1.9 × 10^5 cells/well), rat NRK-49F cells (1.7 × 10^5 cells/well), and mouse BALB/c 3T3 cells (2.2 × 10^5 cells/well) were incubated in the presence of 150 pm 125I-βTGF alone (–) or with 40 nM native βTGF (+). Monolayers were then washed and cross-linked to bound 125I-βTGF with 0.25 mM DSS. After cross-linking, cells were scraped off the dishes, sedimented, and extracted with 1% Triton X-100 before they were subjected to electrophoresis on 5–7% polyacrylamide gels and autoradiography. A resulting autoradiogram (4 days) is shown. The positions corresponding to molecular size markers are indicated. Arrow points at the 280-kDa-labeled species. kDa, kilodalton.
experiments using cell suspensions (Fig. 8A) shows that apparently the same cellular components were affinity labeled with \(^{125}\text{I}\)-PTGF, regardless of whether intact cell monolayers or cell suspensions were used.

As in other experiments in this study, the samples electrophoresed on the gel shown in Fig. 8A were extracts from the corresponding affinity labeled cells obtained with the nonionic detergent, Triton X-100. The purpose of this detergent extraction was to optimize electrophoretic resolution by reducing the amount of cellular protein loaded on the gels. Fig. 8B shows an autoradiogram from a gel containing the Triton-insoluble material corresponding to the samples run on the gel in Fig. 8A. A faint trace of nonspecifically labeled 70-kDa material was the only labeled species detected in these gels (Fig. 8B). In other control experiments (not shown), small samples of \(^{125}\text{I}\)-PTGF-labeled cells that had been directly solubilized with sodium dodecyl sulfate exhibited the same pattern of labeled species as the Triton extracts shown in Figs. 7 and 8. These observations indicate that Triton-insoluble cellular components with an average apparent molecular weight of 280 kDa were affinity labeled by increasing concentrations of \(^{125}\text{I}\)-PTGF in the presence of an excess of each of these growth factors (not illustrated).

Fig. 8. Detergent solubility of affinity labeled \(^{125}\text{I}\)-\(\alpha\)-TGF receptors. Suspensions (1.8-2.2 \times 10^5 cells in 0.5 ml of buffer) of mouse Swiss 3T3 and BALB/c 3T3 fibroblasts and rat NRK-49F fibroblasts were affinity labeled by incubation with 150 pm \(^{125}\text{I}\)-\(\alpha\)-TGF alone (\(-\)) or in the presence of 40 nM \(\beta\)-TGF (\(+\)), followed by treatment with 0.25 mM DSS. The affinity labeled cells were incubated in the presence of 1% Triton X-100 for 40 min at 4°C. The Triton-soluble extract was separated from insoluble material by centrifugation at 12,000 \times g for 15 min. After heating in the presence of 2% sodium dodecyl sulfate, the Triton-soluble (A) and Triton-insoluble (B) fractions were electrophoresed on 5-7% polyacrylamide gels in the presence of dodecyl sulfate. 4-day autoradiograms from the fixed, dried gels are shown. The positions corresponding to molecular size markers electrophoresed on parallel lanes are shown. The arrow points at the 280-kDa-labeled species.

Fig. 9. Specificity of labeling of cellular components by \(^{125}\text{I}\)-\(\alpha\)-TGF. Aliquots (0.5 ml) of a suspension (4.1 \times 10^5 cells/ml) of BALB/c 3T3 fibroblasts were incubated in the presence of increasing concentrations of \(^{125}\text{I}\)-\(\alpha\)-TGF alone as indicated (lanes a-d), in the presence of 150 pm \(^{125}\text{I}\)-\(\alpha\)-TGF plus various concentrations of native \(\alpha\)-TGF and other growth factors (NGF, IGF-I, EGF, insulin), or in the presence of 150 pm \(^{125}\text{I}\)-\(\alpha\)-TGF plus \(\beta\)-TGF, insulin, or nerve growth factor II (NGF-II), or nerve growth factor (NGF), all at 300 nM final concentration (lanes e-g). Cells were then treated with 0.25 mM DSS, extracted with 1% Triton X-100, and subjected to electrophoresis on 5-7% polyacrylamide gels in the presence of dodecyl sulfate. A 6-day autoradiogram from a fixed, dried gel is shown. Arrow points at the 280-kDa-labeled species.

Effect of Various Cross-linking Concentrations—Various concentrations of DSS over a 10-fold (50-500 \mu M) concentration range were assayed for their ability to cross-link \(^{125}\text{I}\)-\(\alpha\)-TGF to intact cells. The 280-kDa species was increasingly labeled by increasing concentrations of DSS, but even under the mildest cross-linking condition used (50 \mu M DSS), this labeled species exhibited a diffused migration on the electrophoresis gels (Fig. 10). In all the experiments described here, the diffused appearance of the 280-kDa band was in contrast to the sharpness of Coomassie Blue-stained protein bands in the high molecular weight region of the gels (not illustrated).

Thus, the anomalous migration of the 280-kDa band on dodecyl sulfate-polyacrylamide gels is not likely due to random cross-linking of the receptor species with other cell and membrane components, as observed with high (>0.5 mM) concentrations of DSS in other receptor affinity labeling studies (36, 37). The inability of N-hydroxysuccinimidyld p-azidobenzoate to cause the labeling of the 280-kDa species with \(^{125}\text{I}\)-\(\alpha\)-TGF (Fig. 10f) argues that the labeling induced by
I. Cross-linking conditions

FIG. 10. Effect of various cross-linking conditions on the labeling of cellular components by 125I-BTGF. Aliquots (0.5 ml) of a suspension (4 x 10^5 cells/ml) of BALB/c 3T3 cells were incubated in the presence of 150 pM 125I-BTGF. After washing the unbound ligand, cells were incubated in the presence of the indicated concentrations of DSS (lanes a-e) or 100 µM N-hydroxysuccinimide p-azidobenzoate (HSAB) (lane f). Cross-linking reactions were arrested by washing the cells with a Tris/HCl buffer. Affinity labeled samples were then extracted with Triton X-100 and subjected to electrophoresis on 5-7% polyacrylamide gels in the presence of dodecyl sulfate. Shown is an autoradiogram (6 days) obtained from a resulting fixed, dried gel. The arrow points at the 280-kDa-labeled species.

DSS is not simply an artifact of derivatization of free amino groups by succinimidy derivatives.

Effect of Preincubation with βTGF on 125I-βTGF Binding and βTGF Receptor Affinity Labeling—An acute decrease, or "down-regulation," of the number of receptors for various growth factors occurs soon after exposure of cells to the respective ligands at 37 °C (38-40). The acute down-regulation of these receptors often results in biphasic kinetics at 37 °C with a peak of maximal ligand binding within the first hour of incubation followed by a marked decrease in binding over the next few hours (38-40). In contrast to this phenomenology, repeated experiments showed that 125I-βTGF binding at 37 °C does not significantly decrease after reaching apparent equilibrium (Fig. 3). These results suggested that acute down-regulation of βTGF receptors may not occur in the cell lines used in these studies. To further investigate this possibility, monolayers of BALB/c 3T3 cells or NRK-49F cells were incubated in the presence of saturating (0.5-2 nM) concentrations of βTGF for 2 or 6 h. Cells were then washed for 1.5 h at 37 °C, and their ability to specifically bind 125I-βTGF was measured at 4 °C. It was found that the concentrations of βTGF tested decreased the binding of 150 pM 125I-βTGF to 69-84% of the values obtained in untreated cells (not illustrated).

However, control experiments using 125I-βTGF instead of native βTGF in the first incubation indicated that a portion of the observed decrease in subsequent 125I-βTGF binding was due to residual occupancy of cellular receptors by βTGF from the first incubation. Thus, residual receptor occupancy accounted for about half of the measured decrease in 125I-βTGF binding to cells preincubated with 0.5 nM βTGF (data not shown). These results suggest that down-regulation of βTGF receptors in BALB/c 3T3 cells occurred to a very limited extent (<15% of binding to control cells) in these experiments, if at all. Control experiments to monitor degradation of βTGF or 125I-βTGF (by radioreceptor assay and trichloroacetic acid precipitability) during incubation in the presence of a cell monolayer at 37 °C indicated that the concentrations of ligand used remained saturating for βTGF receptors in these cells throughout the entire incubation period (not shown).

We examined directly the effect of preincubation of NRK-49F cells with βTGF and 125I-βTGF on the susceptibility of the cells to affinity labeling by 125I-βTGF. Monolayers of
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NRK-49F cells were incubated for 20 min or 2 h in the presence of 0.5 nM βTGF or 0.5 nM 125I-βTGF. They were then extensively washed at 37 °C and affinity labeled with a saturating (150 pM) concentration of 125I-βTGF in the cold. Autoradiography of the resulting cell extracts, a progressive decrease in the intensity of labeling of the βTGF receptor could be detected with time of exposure to βTGF (Fig. 11). However, the labeling of this species was increased in cells that had been preincubated with 125I-βTGF instead of βTGF (Fig. 11). Thus, the decrease in receptor labeling after exposure to βTGF was most likely due to residual occupancy of receptor sites by βTGF carried over in the dishes from the preincubation step even after extensive washing at 37 °C.

DISCUSSION

This report demonstrates the presence of high-affinity receptors for βTGF in various cultured cell lines. The binding of 125I-βTGF to these receptors is time- and temperature-dependent, saturable, reversible, and competed for by βTGF but not by five other polypeptide growth factors tested.

The known biological effects of βTGF are half-maximal at 1-10 pM concentration and maximal at about 40 pM concentration (Fig. 1 and see Refs. 13, 14, 16, 17). Predictably, 125I-βTGF interacts with binding sites that exhibit a K_d in the picomolar range. According to the K_d values obtained here, however, occupancy of only about 20% of the βTGF receptors (by about 40 pM βTGF) is required for maximal biological action. Furthermore, this level of receptor occupancy could be an overestimate, considering that a substantial fraction of the added βTGF is likely to be degraded during the course of biosassays that last for up to several days, as in the case of the soft agar colony formation assay. It is therefore possible that a "spare" number of receptors exists for βTGF, as it has been proposed for other polypeptide hormone receptor systems such as the insulin receptor (41, 42). However, this possibility is difficult to rigorously assess because of the limitations in accurately determining the affinity of βTGF receptors at the biosassay temperature of 37 °C, a temperature at which complex receptor dynamics and significant degradation of radioligand added at low concentrations could occur.

The bifunctional agent DSS can cause the cross-linking of receptor-bound 125I-βTGF to a 280-kDa cellular component that has many of the properties expected from physiologically relevant, membrane-associated βTGF receptors. First, this 280-kDa component exhibits an apparent affinity for βTGF high enough to mediate the effects of this growth factor at low pM concentrations. Second, this receptor species is specific for βTGF and does not cross-react with other growth factors tested. βTGF purified from feline sarcoma virus-transformed rat cells was also able to inhibit the labeling of the 280-kDa receptor by platelet-derived 125I-βTGF (not illustrated). Third, this 280-kDa component is labeled in intact cells as well as in isolated membrane preparations. Fourth, it is found in mouse, rat, and human cell types that bind 125I-βTGF specifically and respond to βTGF. We have also detected this receptor species in Snyder-Thielen feline sarcoma virus-transformed rat embryo fibroblasts and in Moloney murine sarcoma virus-transformed 3T3 mouse cells (not illustrated), two cell lines that produce βTGF along with αTGF, and which may be stimulated by these factors in an autocrine manner (1). However, we have been unable to detect the 280-kDa βTGF receptor species in A549 human lung carcinoma and H35 rat hepatoma (not illustrated), two cell lines which do not exhibit high-affinity binding sites for 125I-βTGF. Finally, the relatively high efficiency (about 13%) of DSS to cross-link 125I-βTGF with the 280-kDa cellular component is consistent with the hypothesis that this component is the major receptor type for βTGF in the cell lines studied here.

Results of experiments using various concentrations of cross-linking agents (Fig. 10) do not support the possibility that the diffused appearance of the 280-kDa-labeled component is due to random cross-linking with other membrane components. In other control experiments (not illustrated), high molecular size receptors for insulin-like growth factors I and II, insulin, EGF, and αTGF affinity labeled by cross-linking with the respective 125I-ligands in BALB/c 3T3 and NRK-49F cells as described before (18-23) migrated on the gels as well-defined labeled bands in parallel with the diffused 280-kDa species labeled by 125I-βTGF. This observation argues that the anomalous migration of the 280-kDa receptor species is not an intrinsic problem of the methodology used. Like many membrane-associated proteins, the 280-kDa βTGF receptor identified here may be glycoprotein. In support of this possibility, recent experiments show that βTGF receptors bind specifically to immobilized lectin columns. The migration of certain glycoproteins on dodecyl sulfate-polyacrylamide gels is anomalous due to the heterogeneity of the carbohydrate moiety and variable binding of detergent (43, 44). It will be of interest to determine whether the 280-kDa βTGF receptor species identified in the present studies is a heterogeneously glycosylated protein.

A class of 70-90-kDa cellular components with low apparent affinity (K_d ~ 10^-8 M) for 125I-βTGF has also been detected in the affinity labeling experiments reported here. This class of low affinity binding sites may escape detection in 125I-βTGF binding studies because its apparent K_d is 2 orders of magnitude higher than the K_d for high-affinity βTGF binding sites. The significance of this class of binding sites with low affinity for 125I-βTGF is unclear. The known cellular effects of βTGF are exerted at concentrations of this growth factor (1-40 pM) which would not effectively bind to this class of sites. It is possible that these lower affinity sites play a role in as yet unidentified cellular events involving βTGF. Alternatively, these 70-90-kDa-labeled species may correspond to degradation products of the major 280-kDa receptor species that would pre-exist in the intact cells or occur during incubation with 125I-βTGF. The observed heterogeneity and variable labeling of these species in the present studies supports this possibility.

The receptor for EGF and αTGF and the PDGF receptor undergo acute down-regulation when cells are exposed to the respective ligands at 37 °C. No evidence has been obtained in the present studies for biphasic binding kinetics of 125I-βTGF at 37 °C or for a markedly decreased βTGF receptor levels in BALB/c 3T3 or NRK-49F cells pretreated with various concentrations of 125I-βTGF. These results suggest that unlike the receptors for EGF/αTGF and PDGF, the receptors for βTGF may not undergo acute down-regulation.

Available information about the target cell selectivity of βTGF is limited because studies have focused on the ability of βTGF to induce anchorage-independent cell proliferation. Induction of anchorage-independent proliferation by βTGF is a complex cellular response with specific requirements including the competitive action other growth factors (5-8). These requirements may be different for each cell type, making difficult the identification of βTGF target cells. The identification of potential targets for βTGF can therefore be facilitated by structural identification and measurement of cellular receptors for this growth factor. For example, four fibroblast...
lines that respond differently to βTGF in soft agar assays, rat NRK-49F, human WI-38, mouse BALB/C 3T3, and Swiss 3T3, were all found to exhibit receptors for βTGF.

The studies reported here suggest that the physiologically relevant βTGF receptor consists of a 290-kDa-binding component. Obviously, other βTGF receptor subunits could exist that interact with this binding species and become dissociated upon solubilization and electrophoresis of affinity labeled cells and membranes. Further characterization and isolation of the βTGF receptor is needed to address this and other questions on the structure of βTGF receptors. The information derived from the present studies should help initiate efforts to quantitatively solubilize and isolate this receptor type.

After submission of this paper, Frolik et al. (45) and Tucke et al. (46) reported specific binding of 125I-βTGF to NRK-49F cells. These authors, however, report significant down-regulation of cells with unlabeled PTGF (45).

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were cell culture fluids of retrovirally transduced cells. The fluids were obtained by this method previously described to purify rat BTGF was the presence of 1 M 1-acetic acid extraction of washed platelets extracted from human platelets with acidic ethanol (15,16) and modified of 1 M method previously described to purify rat BTGF. The fractions containing cells (17). Briefly, the acid-ethanol extract was purified to remove the acid-ethanol extract and BTGF was purified from rat platelets. The fractions containing 125I-BTGF, native BTGF, or other hormones were assayed as indicated. Immunoprecipitation procedures for the first step consisted of the following: aliquots of Triton X-100 detergent-insoluble material containing radioactive hormone were added to a final concentration of 0.25 ml of dimethyl sulfoxide, 10 nM I-BTGF, and incubated at 4°C for 1 hr. Membrane protein affinity-labeling experiments. Membrane protein results were analyzed by autoradiography (36). The detergent-insoluble material containing radioactive hormone was added to a final concentration of 125I-BTGF binding to Triton X-100 detergent-insoluble material containing radioactive hormone was added to a final concentration of 105 cpm. Cells were washed and lysed by Triton X-100. Membrane protein affinity-labeling experiments. Membrane protein resulting in the detergent-insoluble material containing radioactive hormone was added to a final concentration of 125I-BTGF binding to Triton X-100 detergent-insoluble material containing radioactive hormone was added to a final concentration of 105 cpm. Cells were washed and lysed by Triton X-100. Membrane protein affinity-labeling experiments. Membrane protein resulting in the detergent-insoluble material containing radioactive hormone was added to a final concentration of 125I-BTGF binding to Triton X-100 detergent-insoluble material containing radioactive hormone was added to a final concentration of 105 cpm. Cells were washed and lysed by Triton X-100. Membrane protein affinity-labeling experiments. Membrane protein resulting in the detergent-insoluble material containing radioactive hormone was added to a final concentration of 125I-BTGF binding to Triton X-100 detergent-insoluble material containing radioactive hormone was added to a final concentration of 105 cpm. Cells were washed and lysed by Triton X-100. Membrane protein affinity-labeling experiments. Membrane protein resulting in the detergent-insoluble material containing radioactive hormone was added to a final concentration of 125I-BTGF binding to Triton X-100 detergent-insoluble material containing radioactive hormone was added to a final concentration of 105 cpm. Cells were washed and lysed by Triton X-100. Membrane protein affinity-labeling experiments. Membrane protein resulting in the detergent-insoluble material containing radioactive hormone was added to a final concentration of 125I-BTGF binding to Triton X-100 detergent-insoluble material containing radioactive hormone.