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A Transforming Growth Factor β (TGF-β) Receptor from Human Placenta Exhibits a Greater Affinity for TGF-β2 than for TGF-β1[†,‡]

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ABSTRACT: Affinity-labeling techniques have been used to identify three types of high-affinity receptors for transforming growth factor β (TGF-β) on the surface of many cells in culture. Here we demonstrate that membrane preparations from tissue sources may also be used as an alternative system for studying the binding properties of TGF-β receptors. Using a chemical cross-linking technique with ^{125}I-TGF-β1 and ^{125}I-TGF-β2 and bis(sulfosuccinimidyl)suberate (BS^3), we have identified and characterized two high-affinity binding components in membrane preparations derived from human term placenta. The larger species, which migrates as a diffuse band of molecular mass 250-350 kDa on sodium dodecyl sulfate-polyacrylamide electrophoresis gels, is characteristic of the TGF-β receptor type III, a proteoglycan containing glycosaminoglycan (GAG) chains of chondroitin and heparan sulfate. The smaller species of molecular mass 140 kDa was identified as the core glycoprotein of this type III receptor by using the techniques of enzymatic deglycosylation and peptide mapping. Competition experiments, using ^{125}I-TGF-β1 or ^{125}I-TGF-β2 and varying amounts of competing unlabeled TGF-β1 or TGF-β2, revealed that both the placental type III proteoglycan and its core glycoprotein belong to a novel class of type III receptors that exhibit a greater affinity for TGF-β2 than for TGF-β1. This preferential binding of TGF-β2 to placental type III receptors suggests differential roles for TGF-β2 and TGF-β1 in placental function.

Transforming growth factor β (TGF-β)[1] represents a family of multifunctional regulatory proteins involved in cellular differentiation and proliferation. TGF-β1 and TGF-β2 have been isolated from tissue sources, whereas the amino acid sequences of TGF-β3, TGF-β4, and TGF-β5 have been deduced from cDNA clones. TGF-βs, as with other polypeptide hormones, appear to act by binding to specific cell surface

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[1] Abbreviations: TGF-β, transforming growth factor β; GAG, glycosaminoglycan; BS^3, bis(sulfosuccinimidyl) suberate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; EDTA, disodium ethylenediaminetetraacetate; STI, soybean trypsin inhibitor; TCA, trichloroacetic acid; PMSF, phenylmethanesulfonyl fluoride; hCG, human chorionic gonadotropin; hPL, human placental lactogen.

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receptors that transduce the regulatory signal [for reviews, see Roberts and Sporn (1989) and Massagué (1990)]. The nature of the TGF-β signaling pathway(s) remains uncertain; however, some mechanism(s) may be linked to the phosphorylation state of the retinoblastoma protein (Laiho et al. 1990a) or to a GTP-binding protein (Howe et al., 1990).

Although the signaling activity of TGF-β receptors remains obscure, it has been possible to identify putative TGF-β receptors by chemical cross-linking of ^{125}I-TGF-β to specific, high-affinity binding components on cell surfaces (Massagué, 1985; Massagué & Like, 1985; Fanger et al., 1985; Segarini, 1989). This affinity-labeling technique has revealed three types of high-affinity binding components, operationally termed TGF-β receptors, on the cell surface of most established cell lines examined (Massagué & Like, 1985; Cheifetz et al., 1986; Wakefield et al., 1987). Types I and II receptors are glycoproteins identified as affinity-labeled complexes having molecular masses of 65 kDa and 85–95 kDa, respectively, and both bind TGF-β1 with a higher affinity than TGF-β2 (Cheifetz et al., 1987, 1988a). The type III component is a membrane proteoglycan of approximately 250–350 kDa containing glycosaminoglycan (GAG) chains of chondroitin and heparan sulfate. Its core glycoprotein is identified as an affinity-labeled complex of molecular mass 100–140 kDa that contains the functional binding site for TGF-β (Segarini & Seyedin, 1988; Cheifetz et al., 1988b). In general, type III receptors bind TGF-β1 and TGF-β2 with similar affinities (Cheifetz et al., 1987, 1988a; Cheifetz & Massagué, 1989), although there is evidence for a type III subset, termed class B, found on NRK and Swiss 3T3 fibroblasts that preferentially binds TGF-β2 (Segarini et al., 1987).

Until recently, the only criteria that could be used to define TGF-β signaling receptors was the correlation between receptor affinities for TGF-β1 and TGF-β2 and the potencies of TGF-β1 and TGF-β2 in biological assays. On this basis, Massagué and co-workers had originally proposed that the type III (250–350 kDa) receptor mediates the effect of TGF-β on regulation of extracellular matrix production and inhibition of epithelial cell proliferation (Cheifetz et al., 1987, 1988a), whereas the type I (65-kDa) receptor mediates the effect of TGF-β on the growth of mouse hematopoietic progenitor cells (Ohta et al., 1987). Recently, however, mutants of epithelial cells have been isolated that selectively lose either the type I or both the types I and II receptors as they become resistant to growth inhibition by TGF-β (Boyd & Massagué, 1989; Laiho et al., 1990b). In addition, certain cells that do not display detectable type III receptors are still able to respond to TGF-β (Segarini et al., 1989). Therefore, it has now been proposed that the type III receptor may not be a true, signaling receptor for many TGF-β activities including regulation of DNA synthesis and extracellular matrix production (Segarini et al., 1989). The recent discovery of both membrane-anchored and soluble forms of the type III proteoglycan supports the notion of novel nonsignaling roles, such as high-affinity storage sites for TGF-βs, activators of latent TGF-β, or clearance receptors, for these molecules (Andres et al., 1989).

With a few exceptions (Kyprianou & Isaacs, 1988; MacKay et al., 1989, 1990; Gruppuso et al., 1990), most research on the characterization of TGF-β receptors has been done with established or primary cells in culture. We were particularly interested in determining whether TGF-β-binding components in membrane preparations from tissue sources could be characterized. Human term placental membrane preparations were chosen because of their ready availability and their broad usage for receptor assays and receptor purification [e.g., the epidermal growth factor receptor (Downward et al., 1984)]. Moreover, since the factors involved in placental growth, development, and immunosuppression are poorly understood, it is important to investigate the role(s) of TGF-β during pregnancy. In the present study, we have identified and characterized novel type III TGF-β receptors in human placental membrane preparations that exhibit a greater affinity for TGF-β2 than TGF-β1.

Experimental Procedures

Placental Membranes. Human placentas obtained at term after Caesarean section from nonlabored mothers were held on ice for approximately 30 min during transport to the laboratory. Membranes were prepared as described (Valentine et al., 1987). Briefly, fresh spongy placental tissue was dissected away from the cord and large vessels and thoroughly washed with buffer 1 (100 mM NaCl, 5 mM EGTA, 5 mM EDTA, 25 mM Tris-HCl, pH 7.4) containing the following protease inhibitors: 2 μg/mL aprotinin, 2 μg/mL STI, 2 μg/mL leupeptin, 25 mM benzamidine, 1 mM PMSF. Throughout the procedure, the tissue was maintained at 0–4 °C. The tissue was first homogenized in a Waring blender for 90 s with buffer 2 (250 mM sucrose, 5 mM EGTA, 5 mM EDTA, 25 mM Tris-HCl, pH 7.4) containing the above protease inhibitors in a final volume of 1 L. An additional 1-L volume of buffer 2 was then added, followed by further homogenization with a Brinkman Polytron with use of a single burst for 90 s at setting 7. The homogenate was centrifuged at 7500*g* for 30 min at 4 °C, and the supernatant was made to 100 mM NaCl and 0.2 mM $MgSO_4$ by addition of salts. This material was centrifuged at 30000*g* for 60 min at 4 °C, and the creamy white membrane pellets were resuspended in a total volume of 500 mL of buffer 3 (5 mM EGTA, 5 mM EDTA, 50 mM Tris-HCl, pH 7.4, containing the above protease inhibitors) with use of a glass/Teflon Dounce homogenizer. The membranes were washed two times, and the final pellet was resuspended in approximately 50 mL of buffer 3 and frozen in aliquots at −80 °C. Protein content was estimated by the method of Lowry et al. (1951).

Iodination of TGF-β. TGF-β1 and TGF-β2 (both purified from porcine platelets) were purchased from R & D Systems (Minneapolis, MN). ^{125}I-Labeled TGF-β1 and ^{125}I-labeled TGF-β2 were prepared by the chloramine T method as described (Ruff & Rizzino, 1986) with use of 1 μg of carrier-free TGF-β (redissolved into 15 μL of 30% acetonitrile/0.1% trifluoroacetic acid) and 1 mCi of Na[^{125}I] (13 mCi of ^{125}I/μg of iodine; Amersham International). The TCA precipitability of ^{125}I-TGF-β was typically 85% with specific activity in the range of 100–200 μCi/μg. Stock solutions of unlabeled 400 nM TGF-β (in the presence of BSA carrier as supplied by R & D Systems) were prepared by addition of 4 mM HCl/1 mg/mL BSA to the 1-μg aliquots of lyophilized TGF-β supplied.

Ligand Binding and Affinity-Labeling of Placental Membranes. Aliquots of frozen placental membranes were thawed and centrifuged at 100000*g* at 4 °C for 10 min with a Beckman TL 100 ultracentrifuge and a TL 100.2 fixed angle rotor. Membrane pellets were resuspended to 3.0 mg/mL with Dulbecco's PBS, pH 7.4, by use of a glass/Teflon Dounce homogenizer. Aliquots of 50 μL or 100 μL of membranes were placed into siliconized 1.5-mL polypropylene test tubes on ice and incubated with ^{125}I-TGF-β1 (or ^{125}I-TGF-β2) alone or with varying concentrations of unlabeled TGF-β1 or TGF-β2 as indicated. Following a 3-h incubation on ice, membranes were cross-linked by a 10-min (or as otherwise noted) exposure to 1 mM bis(sulfosuccinimidyl) suberate (BS^3) (Pierce,

Rockford, IL) on ice. The reaction was stopped by the addition of $^1/_5$ vol of 500 mM glycine and further incubation on ice for 5 min. Affinity-labeled membranes were either (i) directly solubilized for SDS-PAGE by adding $^1/_5$ volume of 5× electrophoresis sample buffer [0.25M Tris-HCl, pH 6.8, 5% (w/v) SDS, 5% (v/v) β-mercaptoethanol, 50% (v/v) glycerol, 0.0004% (w/v) bromophenol blue] and heating at 100 °C for 5 min or (ii) treated with Triton X-100 [to 1% (v/v) final concentration] and incubated at 4 °C for 30 min. Triton X-100 soluble components remained in the supernatant after centrifugation at 12000*g* for 10 min. Supernatants were treated with sample buffer for SDS-PAGE as described above.

Enzymatic Deglycosylation of Affinity-Labeled Placental Membranes. The enzymatic removal of the GAG chains was performed according to Cheifetz et al. (1988b) with as a starting material the Triton-soluble extracts of placental membrane preparations affinity labeled with either ^{125}I-TGF-β1 or ^{125}I-TGF-β2. Essentially, 100-μL (0.3-mg) aliquots of placental membranes were affinity-labeled with radiolabeled TGF-β and then extracted with 1% (v/v) Triton X-100. The detergent-soluble components were diluted 1:1 with acetate buffer to give final concentrations of 10 mM Tris-HCl, pH 7.5, 50 mM sodium acetate, pH 7.5, 10 mM $CaCl_2$, 0.2 mM EDTA, 0.2% (v/v) Triton X-100, 1 mg/mL BSA containing protease inhibitors, 5 μg/mL leupeptin, 50 μg/mL benzamidine, 50 μg/mL STI, 10 μg/mL apoprotinin, and 1 mM PMSF. Samples were incubated for 24 h at 37 °C with a combination of heparitinase at 2 units/mL and chondroitinase ABC at 0.5 unit/mL. Samples were then treated with sample buffer as described above or further digested with endoglycosidase H or endoglycosidase F exactly as described in Cheifetz et al. (1988b) except for the differences in the concentrations of the protease inhibitors as noted above. Chondroitinase ABC (EC 4.2.2.4) was purchased from Seikagaku Kogyo Co. (Tokyo), and heparitinase (EC 4.2.2.8) was from ICN Biomedicals (Cleveland, OH). Endoglycosidase F (EC 3.2.1.96) and endoglycosidase H (EC 3.2.1.96) were purchased from Boehringer Mannheim (Canada).

SDS-PAGE and Autoradiography. SDS-PAGE was performed by the method of Laemmli (Laemmli & Favre, 1973) with 3–10% (v/v) polyacrylamide-gradient gels unless otherwise indicated. Following electrophoresis, gels were stained with Coomassie Brilliant Blue, destained, dried, and exposed to Kodak Omat AR film at −80 °C with use of DuPont Cronex Lightning Plus intensifying screens for between 2 and 10 days. Quantitative densitometry of autoradiograms (exposed within the linear range of the film response) was carried out with use of an LKB Ultroscan XL laser densitometer. Relative peak heights (at maximum) were used in the calculations of relative binding of ^{125}I-TGF-β to the 250–350-kDa and 140-kDa receptor bands. ^{14}C-Labeled molecular mass standards were from Bethesda Research Laboratories (Gaithersburg, MD) and include lysozyme, 14 kDa; β-lactoglobulin, 18 kDa; carbonic anhydrase, 29 kDa; ovalbumin, 43 kDa; bovine serum albumin, 68 kDa; phosphorylase B, 97 kDa; and myosin heavy chain, 200 kDa.

Peptide Mapping. Peptide mapping of affinity-labeled receptor bands was carried out by the method of Cleveland et al. (1977) as modified by Cheifetz et al. (1986). Endoproteinase Glu-C (EC 3.4.21.19) (*Staphylococcus aureus* V-8 protease) was purchased from Boehringer Mannheim (Canada).

Results

Affinity Labeling of TGF-β-Binding Components on Human Placental Membrane Preparations. Affinity labeling of human (term) placental membrane preparations reveals two high-affinity binding components whose binding to either ^{125}I-TGF-β1 or ^{125}I-TGF-β2 is specifically blocked by excess unlabeled TGF-β1 or TGF-β2 (Figure 1). The high molecular mass band extending between approximately 250 and 350 kDa is characteristic of the proteoglycan form of the type III receptor for TGF-β, and the second band of molecular mass approximately 140 kDa is characteristic of the core glycoprotein of this type III receptor. Supporting evidence that the molecular mass 140-kDa band is indeed the core glycoprotein of the 250–350 kDa type III receptor is presented in Figures 3 and 4. Accordingly, we will refer to the 140-kDa species as the core glycoprotein of the type III proteoglycan throughout this report. Affinity labeling of the TGF-β receptors on placental membranes was optimized by evaluating the effect of various times of incubation (between 0.5 and 60 min) in the presence of 1 mM BS^3. Cross-linking for extended times (longer than 10 min) had no obvious effect on the 250–350-kDa type III band but resulted in broadening of the 140-kDa core receptor band (data not shown). Thus, for consistency, a 10-min incubation with the cross-linking agent BS^3 was adopted for these studies.

In the experiments shown in panels A and B of Figure 1, the two high-affinity receptor components for TGF-β are clearly seen when the membrane preparations were extracted with 1% (v/v) Triton X-100 following affinity labeling. In contrast, in experiments where the affinity-labeled membrane preparations were directly solubilized into electrophoresis sample buffer, several bands are seen, although only the 250–350- and 140-kDa bands are specifically blocked by excess unlabeled TGF-β (Figure 1C). These other bands are most likely due to nonspecific binding of TGF-β to Triton-insoluble proteins. We did not detect any type I receptor, although in some experiments a weak band at about 85 kDa (as in Figure 1A) was seen that may be the type II receptor. Recently, we have detected types I and II receptors by affinity labeling of primary human placental cytotrophoblasts in monolayer culture; however, the signals are very weak relative to that of the type III receptor (Mitchell, Fitz-Gibbon, and O'Connor-McCourt, manuscript in preparation). Similarly, the type III receptor was the predominant band detected by affinity labeling of prostate membrane preparations (Kyprianou & Isaacs, 1988). In contrast, only types I and II and not type III were detected in liver membranes (Gruppuso et al., 1990).

Affinity-Labeled Human Placental Membrane Type III TGF-β Receptor and Its Core Glycoprotein Have a Higher Affinity for TGF-β2 than for TGF-β1. Competition experiments, with either ^{125}I-TGF-β1 or ^{125}I-TGF-β2 and varying concentrations of unlabeled TGF-β1 or TGF-β2 indicate that both forms of the placental membrane TGF-β receptor, i.e., the type III proteoglycan and its core glycoprotein, have a greater affinity for TGF-β2 than for TGF-β1. In the typical results shown in Figure 1A, B, it can be seen that 10 nM TGF-β1 blocks binding of 150 pM ^{125}I-TGF-β1 to the type III proteoglycan and its core by approximately the same extent as 1 nM TGF-β2. Similarly, 10 nM TGF-β1 blocks binding of 150 pM ^{125}I-TGF-β2 to the type III proteoglycan and core receptor species by approximately the same extent as 2 nM TGF-β2. We have used the Swiss 3T3 and BRL-3A cell lines as our standards to ensure that the TGF-β1 and TGF-β2, used in our placental membrane studies, compete equally against the type III receptors present on these cells (not shown).

In order to obtain a better estimate of the relative affinities of these two receptors for TGF-β1 and TGF-β2, autoradiograms from competition experiments were subjected to

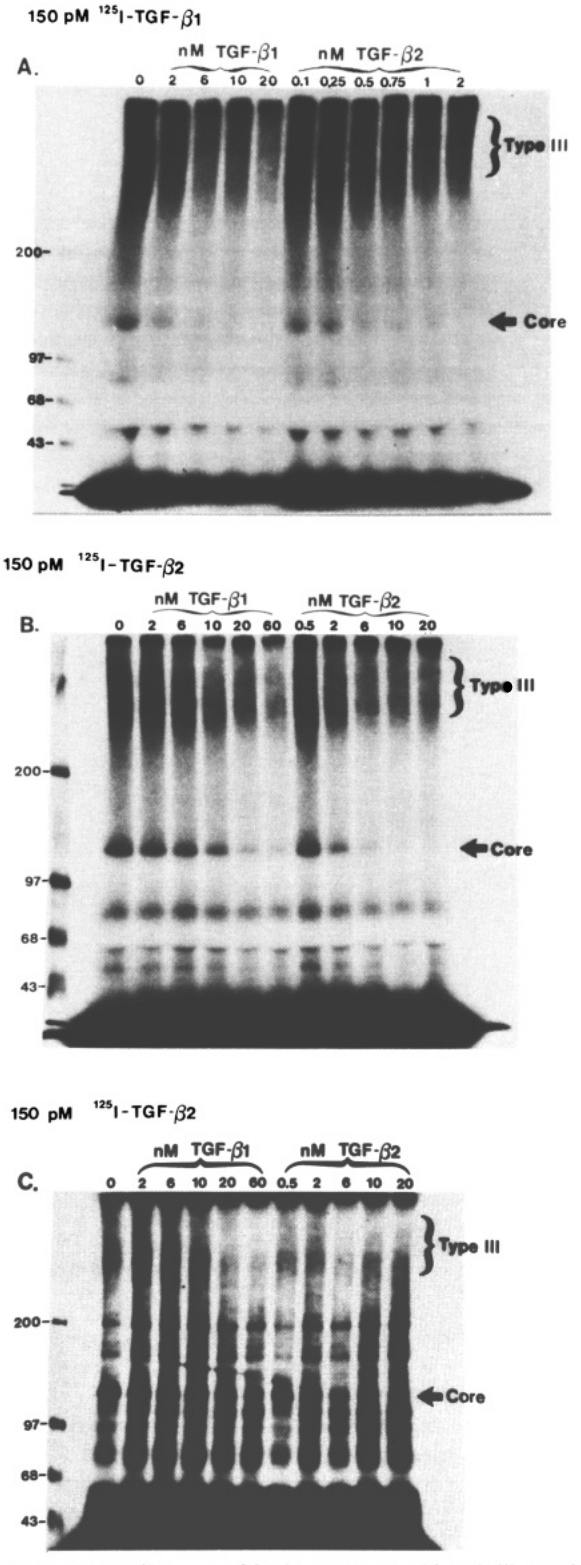

FIGURE 1: Autoradiograms of SDS–polyacrylamide gels illustrating the differential competition by TGF-β1 or TGF-β2 for TGF-β receptors affinity labeled with ^{125}I-TGF-β1 or ^{125}I-TGF-β2. Human placental membrane preparations were incubated with 150 pM radiolabeled TGF-β1 (A) or TGF-β2 (B and C) for 3 h at 4 °C in the absence (0) or presence of the indicated concentrations (in nanomolar) of unlabeled TGF-β1 or TGF-β2. Bound material was cross-linked by a 10-min exposure to 1 mM BS^3, and the membranes were then either extracted with 1% (v/v) Triton X-100 followed by treatment of the Triton-soluble components with sample buffer for SDS–PAGE (A and B) or directly prepared for SDS–PAGE (C). In these experiments the entire sample was electrophoresed on 3–10% linear gradient polyacrylamide gels that were dried and exposed for autoradiography. Molecular mass markers are shown in kilodaltons. Similar results were obtained with placenta from three individuals. During the course of these experiments, ^{125}I-TGF-β1 and ^{125}I-TGF-β2 from four and two separate iodinations, respectively, were used.

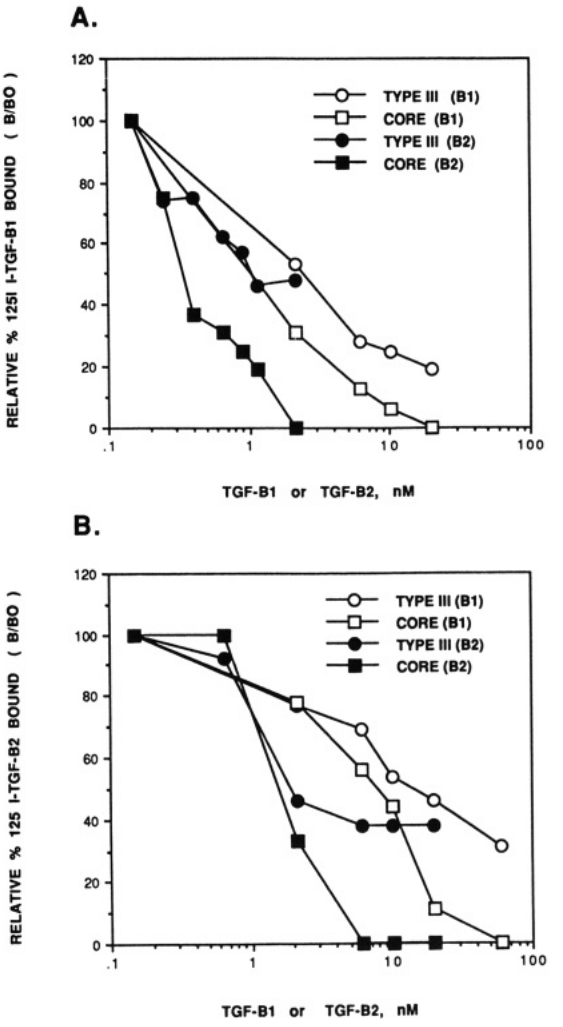

FIGURE 2: Competition curves of ^{125}I-TGF-β1 (A) or ^{125}TGF-β2 (B) by unlabeled TGF-β1 or TGF-β2 as derived from densitometric analysis. Each lane of the autoradiograms shown in panels A and B of Figure 1 was scanned, and the relative intensity of the ^{125}I-TGF-β labeling of the type III proteoglycan (250–350-kDa) and core (140-kDa) receptor bands (expressed as a percentage of ^{125}I-TGF-β bound to the same band in the absence of unlabeled ligand (% B/B_0)) was plotted against the concentration of competing unlabeled TGF-β1 or TGF-β2.

quantitative densitometry. Competition curves were derived thereby in which the binding in the presence of unlabeled ligand was expressed as a percentage of the ^{125}I ligand binding in the absence of competing ligand (% B/B_0). Panels A and B of Figure 2 illustrate the competition curves derived from the representative experiments in panels A and B of Figure 1. In the experiment with 150 pM ^{125}I-TGF-β1 (Figure 2A), it is seen that 50% of the ^{125}I-TGF-β1 bound is blocked by 2.5 nM unlabeled TGF-β1 for the type III proteoglycan or by 1.5 nM TGF-β1 for the core glycoprotein. In contrast, 50% of the ^{125}I-TGF-β1 bound is blocked by lower concentrations of TGF-β2, i.e., 1.0 nM unlabeled TGF-β2 and approximately 0.25 nM TGF-β2 for the type III proteoglycan and core glycoprotein, respectively. These results indicate that both of the receptor forms have a greater affinity for TGF-β2 than for TGF-β1.

The preferential affinity of these type III receptors for TGF-β2 is more clearly seen in competition experiments with ^{125}I-TGF-β2 as the radiolabeled ligand. In Figure 2B, it is seen that 50% of ^{125}I-TGF-β2 binding is blocked by 16 nM unlabeled TGF-β1 compared with 2 nM unlabeled TGF-β2 for the type III proteoglycan or by 8 nM TGF-β1 compared

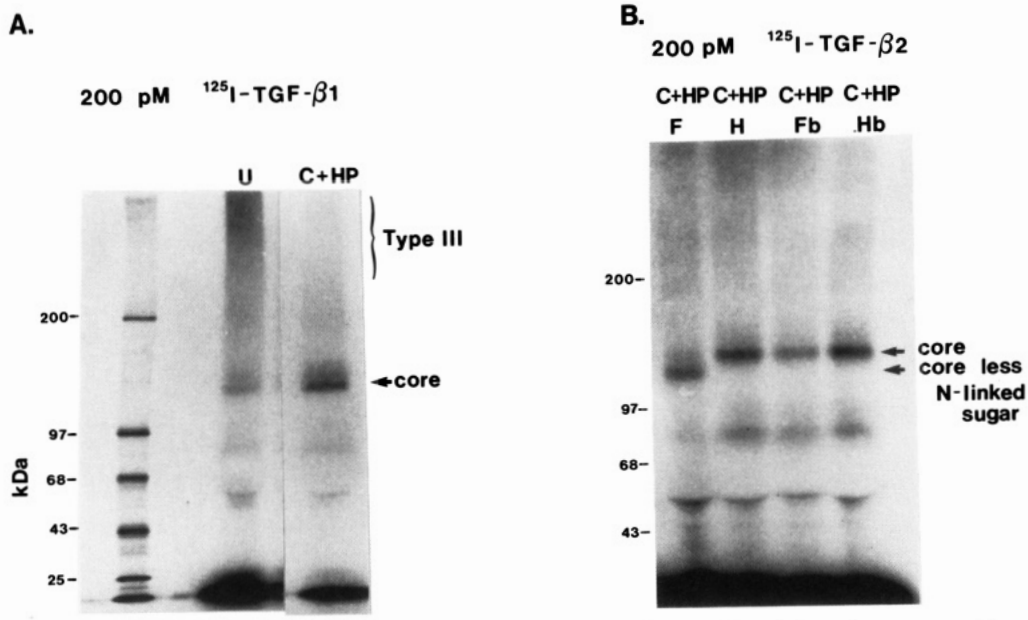

FIGURE 3: Enzymatic deglycosylation of Triton X-100 soluble extracts of affinity-labeled human placental membrane preparations. (A) Membranes were affinity-labeled with ^{125}I-TGF-β1 and incubated in the presence of chondroitinase ABC (C) and heparitinase (HP) for 24 h at 37 °C; lane U illustrates undigested affinity-labeled membrane extract. (B) Membranes were affinity-labeled with ^{125}I-TGF-β2 and incubated with chondroitinase ABC (C) and hepartinase (HP) as in panel A before further incubations with either endoglycosidase F (F) or endoglycosidase H (H) or their corresponding buffers alone, (Fb) and (Hb), respectively.

with 2 nM TGF-β2 for the core glycoprotein. Thus, these experiments with ^{125}I-TGF-β2 confirm that both forms of the type III TGF-β receptors on placental membranes have a greater affinity for TGF-β2 as compared to that for TGF-β1.

It is apparent from Figure 2 and results from similar experiments that the inhibition of binding of ^{125}I-TGF-β to the type III proteoglycan but not to the core levels off at approximately 20–45% (B/B_0). This indicates that there is a component with low-affinity or high-capacity binding to ^{125}I-TGF-β in the 250–350-kDa region on SDS–PAGE. In contrast, there is no similar residual binding of ^{125}I-TGF-β to the 140-kDa core band. This observation suggests that quantitative analysis of the core band may provide more meaningful values for the relative affinities of human placental membrane type III receptors for TGF-βs.

Structural Relationship between the Affinity-Labeled Human Placental Membrane TGF-β Receptor Forms As Demonstrated by Deglycosylation. The structural relationship between the TGF-β type III receptor and its core glycoprotein, which are present on cultured cell lines, was recently characterized by chemical and enzymatic deglycosylation (Segarini & Seyedin, 1988; Cheifetz et al., 1988b). We have carried out similar enzymatic deglycosylation experiments to confirm the relationship between the type III proteoglycan and its 140-kDa putative core glycoprotein found on placental membrane preparations. The experiment in Figure 3A illustrates affinity-labeled TGF-β receptors in placental membrane preparations digested with a combination of chondroitinase ABC, which removes GAG chains containing chondroitin/dermatan sulfate, and heparitinase, which cleaves heparan sulfate chains. Both the type III proteoglycan and the core glycoprotein receptor bands are seen in the undigested control, whereas the combination of enzymes removes the chondroitin/dermatan sulfate and heparan sulfate chains from the 250–350-kDa type III receptor, leaving predominantly a single core receptor band of 140 kDa. The affinity-labeled core receptor (following digestion with chrondroitinase ABC and heparitinase) could then be further cleaved with endoglycosidase F but not with endoglycosidase H (Figure 3B),

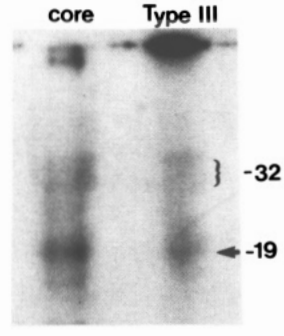

FIGURE 4: Comparative peptide maps of the placental type III proteoglycan and core TGF-β receptors. Gel slices containing affinity-labeled placental TGF-β type III proteoglycan (250–350-kDa) or core (140-kDa) receptors were subjected to *S. aureus* V-8 protease digestion as described under Experimental Procedures. Two major bands, 19 kDa and a doublet around 32 kDa, common to both receptor forms are seen.

indicating the presence of some N-linked carbohydrates of the complex type. We also observed that the Triton-solubilized core receptor bound to wheat germ agglutinin agarose beads (data not shown). These results show that the 140-kDa high-affinity binding protein for TGF-β, which is seen in placental membrane preparations, is indeed the core glycoprotein of the type III proteoglycan. It is not clear whether the placental type III core glycoprotein observed in the membrane preparations is truly a subpopulation of receptors that endogenously lack GAG chains in the intact placenta or whether a degree of deglycosylation has occurred during the placental membrane isolation procedure.

Structural Relationship between the Affinity-Labeled Human Placental Membrane TGF-β Receptor Forms As Demonstrated by Peptide Mapping. In order to compare the polypeptides of the type III proteoglycan and the core glycoprotein, we performed comparative peptide mapping by partial proteolysis of affinity-labeled type III proteoglycan and core glycoprotein receptor bands that had been sliced out of preparative gels. The same two bands, 19 kDa and a doublet around 32 kDa, are seen in an experiment where 5 μg of *S.*

aureus V-8 protease was used to digest the two receptor forms (Figure 4). These same two bands were seen in separate experiments using 1, 3, or 15 μg of *S. aureus* V-8 protease (not shown). These data indicate that the two proteins are most likely the same or at least have common TGF-β-binding domains. The higher M_r material near the top of the gel probably represents some undigested/partially digested material that does not migrate well into the high percentage (12–18%) acrylamide gel.

Discussion

Using an affinity-labeling technique with human placental membrane preparations, we have identified two high-affinity forms of the TGF-β type III receptor, the proteoglycan (250–350 kDa) and its core glycoprotein (140 kDa). Both of these forms of the placental TGF-β type III receptor belong to a novel class that exhibits a greater affinity for TGF-β2 than for TGF-β1. The affinity-labeling technique has been widely used with cells in culture to identify TGF-β receptor types; however, isolated kidney glomeruli (MacKay et al., 1990) and membrane preparations from prostate (Kyprianou & Isaacs, 1988), liver (Gruppuso et al., 1990), and, in this report, placenta are the only tissue sources for which TGF-β-binding components have been demonstrated. This is the first report for which affinity-labeled membrane preparations have been used in TGF-β competition studies with TGF-β1 and TGF-β2.

The importance of examining systems more closely related to the in vivo state is illustrated by the observation that the types of receptors for TGF-β expressed by established cell lines may differ significantly from those seen on primary cells of the same kind (Segarini et al., 1989). Also, MacKay et al. (1990) have examined isolated kidney glomeruli and characterized TGF-β receptors that are distinct in M_r and disulfide bridging when compared with the three major receptor types observed on established cell lines. In addition, whereas the type III component is prominent in placental (this report) and prostate (Kyprianou & Isaacs, 1988) membranes, types I and II predominant upon affinity labeling of liver membranes (Gruppuso et al., 1990). Consistent with this observation is our recent evidence with affinity-labeled human placental cytotrophoblasts that the signals for types I and II components are very weak relative to that for type III (Mitchell, Fitz-Gibbon, and O'Connor-McCourt, manuscript in preparation). Although, in general, the type III receptors on many kinds of established cell lines exhibit equal affinities for TGF-β1 and TGF-β2 (Cheifetz et al., 1988a, 1989), Segarini and co-workers first demonstrated a type III receptor having a greater affinity for TGF-β2 than TGF-β1 in two established cell lines (Segarini et al., 1987). Moreover, similar to our finding with term placental membranes, MacKay et al. (1990) observed a type III like component in glomeruli with a greater affinity for TGF-β2 than for TGF-β1. It may be the case that differential affinities of type III receptors for TGF-β1 and TGF-β2 that exist in the in vivo state are lost upon establishment of some, but not all, cell lines. It is noteworthy that α2-macroglobulin, the major serum-binding protein for TGF-β, also exhibits a greater affinity for TGF-β2 than for TGF-β1 (Danielpour & Sporn, 1990). These kinds of observations favor the examination of a variety of systems including tissue sources, such as placental membranes, for the characterization of TGF-β-binding components.

It is of interest that although there is a mixed population of cells represented in the placental membrane preparations, there is predominantly only a single M_r species of core receptor suggesting the presence of a single subclass of type III receptors. In contrast, Segarini and co-workers found three distinct core bands following enzymatic deglycosylation of ^{125}I-TGF-β1-labeled components on Swiss 3T3 fibroblasts and two core bands following deglycosylation of ^{125}I-TGF-β2-labeled components (Segarini, 1989). Three core proteins have also been observed in similar experiments with other cell lines and ^{125}I-TGF-β1 (Cheifetz et al., 1988b). These patterns of bands suggest that there may be three different kinds of core glycoproteins, two of which bind either TGF-β1 or TGF-β2 and a third that binds only TGF-β1. It is conceivable that, in certain cell types such as Swiss 3T3 fibroblasts, all three core proteins are expressed, whereas in some tissues containing various cell types, as seen here for term placenta, there is a preponderance of only one of these core glycoproteins. Eventually, it will be interesting to learn if indeed the three core glycoproteins are the products of three different genes and whether or not their existence is related to the three proposed subclasses of TGF-β type III receptors (Segarini et al., 1989). Recent studies (ten Dijke et al., 1990) suggest that the broad availability of recombinant TGF-β3 may reveal the existence of further subclasses of type III receptors, with differential selectivities for TGF-β1, TGF-β2, and TGF-β3.

Apart from its importance as a valuable tissue for receptor studies, placenta has attracted the interest of developmental biologists [as reviewed by Blay and Hollenberg (1989)]. Moreover, because of its "pseudomalignant" properties, it is also a model for carcinogenesis [as reviewed by Ohlsson (1989)]. Northern hybridization studies of TGF-β1, TGF-β2, and TGF-B3 during mouse development indicate that all three forms are expressed in placenta and that TGF-β2 is exceptionally abundant in placenta relative to other tissues examined (Miller et al., 1989a,b). In situ hybridization experiments show that the TGF-β2 is predominantly expressed in placental mesenchyme, probably blood vessels (Pelton et al., 1989). Both TGF-β1 (Lehnert & Akhurst, 1988) and TGF-β2 (Pelton et al., 1989) are postulated to play roles in developmental mesenchymal–epithelial tissue interactions via autocrine and/or paracrine mechanism(s). Immunocytochemical evidence suggests that TGF-β1 and TGF-β2 are present intracellularly within the murine trophoblast and extracellularly in the mesenchymal compartment (Thompson et al., 1989; Flanders et al., 1990). TGF-β1 has been recently shown to inhibit DNA synthesis in rat trophoblast cell lines (Hunt et al., 1989). The human term placenta is a complex organ that contains various cell types, the most abundant of which are fibroblastic and trophoblastic (Laga et al., 1973). The trophoblast, the villous epithelium present at the fetal–maternal interface, is responsible for the production of hCG and hPL and represents about 13% by weight of the term placenta (Laga et al., 1973). While we cannot prove that the type III class B receptors are contributed by the trophoblastic elements in our membrane preparations, we have found that human placental cytotrophoblasts in primary culture also display type III receptors having a greater affinity for TGF-β2 than for TGF-β1 (Mitchell, Fitz-Gibbon, and O'Connor-McCourt, manuscript in preparation). In addition to possible regulatory roles in trophoblastic growth and differentiation, a TGF-β2-like molecule appears to have a role as a potent immunosuppressive factor secreted by murine decidual cells (Clark et al., 1990), implying that TGF-β2 may be an essential factor in the maintenance of pregnancy. Our results, indicating a TGF-β type III receptor having a preferential affinity for TGF-β2, together with other recent reports (Miller et al., 1989a; Pelton et al., 1989; Clark et al., 1990), suggest that TGF-β2, in particular, is biologically significant in placenta. The availability of specific immunological and cDNA probes for the

various members of the TGF-β family and eventually their receptors will go a long way in aiding our understanding of the role of these growth factors in placenta. Such probes will enable the analyses of different placental tissue compartments and help to unravel the various functions of TGF-βs during pregnancy. Moreover, such studies will help to unravel the precise rose(s) for the type III proteoglycan molecule within the TGF-β receptor/signaling system(s).

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