Journal of Cellular Biochemistry 61:230–237 (1996)

TGF-β Receptors Are Diminished After Retinoid Exposure in Rat Liver Epithelial Cells

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Abstract When rat liver epithelial cells were exposed to retinoic acid or retinol for 24 hr, the levels of transforming growth factor-β (TGF-β) receptors were reduced in a dose-dependent way. The decrease appeared after 12 hr of incubation with the retinoids and binding levels remained low until 24 hr after the removal of the molecules. Retinoid treatment induced a fourfold enhancement of transglutaminase (TGase) activity in the cell membranes, and cystamine, an inhibitor of TGase, prevented the decrease of the receptors. Neutralization of TGF-β by a monoclonal antibody did not suppress the decrease of the binding levels, indicating that decreased TGF-β binding capacity was not due merely to the internalization of ligand-bound receptors promoted by a stimulation of TGF-β synthesis. Thus, retinoid treatment resulted in an intense disappearance of the functional receptors from the membranes that seemed to be mediated by increased TGase activity. This phenomenon can represent a strong signal attenuation for TGF-β following retinoid exposure.

Key words: retinoic acid, retinol, binding, transglutaminase, hepatic

Retinoids play an essential role in controlling cell growth and differentiation by acting on gene expression and protein synthesis [Blomhoff et al., 1990; Wolf, 1990]. Retinoids are required in normal growth of bone, reproduction, or embryonic development. They can also block the growth of malignant cells, and the chemopreventive action of retinoids in carcinogenesis is now well recognized and has proven efficient in the treatment of cancers [Verma, 1991; Corbeil et al., 1994].

Transforming growth factor-βs (TGF-βs) are also multifunctional molecules that have been involved in cell growth and differentiation. There are three distinct but highly related mammalian isoforms of TGF-βs, named -β1, -β2, and -β3. The mature active forms are homodimers of two 12–15 kDa subunits, but in most cells TGF-βs are released as a biologically inactive precursor (latent TGF-β) consisting of the dimeric mature protein associated with a dimer of the N-terminal proregion. The three isoforms of TGF-β play key roles in embryogenesis and carcinogenesis [Attisano et al., 1994].

Received July 25, 1995; accepted October 16, 1995.

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Defining the relationships between the retinoids and TGF-βs has been a topic of tremendous interest in the past years. Several studies have shown that TGF-β synthesis can indeed be modulated by all-trans retinoic acid (RA) treatment in different models: In vivo, treatment with RA induces TGF-βs synthesis in epithelia of the rat [Glick et al., 1991]. In vitro, increased synthesis of TGF-β1 or TGF-β2 has been described in HL-60 cells [Falk et al., 1991] or chicken chondrocytes [Jakowlew et al., 1992], but RA has also been shown to reduce TGF-β production in Ito cells [Davis et al., 1990]. Moreover, retinoids cannot only increase TGF-β production in bovine endothelial cells, but also mediate its activation by the induction of transglutaminase (TGase) [Kojima et al., 1993; Kojima and Rifkin, 1993]. Thereby, in many instances, TGF-β can mediate the effects of RA in epithelial cell growth and differentiation.

The active form of TGF-β binds to three major cell surface proteins in most cells, designated as type I, II, and III receptors [Attisano et al., 1994]. Type I and II receptors are glycoproteins of approximately 55 and 75 kDa, and have both been identified as serine/threonine kinase receptors. They seem to be involved in signal transduction and to interact with each other in heterodimeric complexes to mediate the signal [Attisano

et al., 1994]. Type III, named betaglycan, is an 853 amino acid proteoglycan, that does not seem to be involved in signal transduction, but plays a key role in TGF-β action mechanism by binding the ligand and presenting it to the signaling type II receptor [Attisano et al., 1994]. These proteins have been cloned, but there is only meager information on the regulation of their synthesis.

In this study, we have examined the effect of retinoid exposure on the levels of receptors for TGF-β in cultures of rat liver epithelial cells (named REL cells) and CCL 64 cells. We observed that in REL cells, retinoid treatment led to a sharp decrease of the three types of receptors, and that TGase seemed to be involved in this event.

MATERIALS AND METHODS

Materials

TGF-β1, cystamine, and retinoic acid (RA) were obtained from Sigma Chemical Co. (St. Louis, MO). ^{125}I-TGF-β1 (120-180 μCi/μg) was purchased from Dupont (Wilmington, DE). All culture reagents and retinol (RET) were obtained from ICN (Costa Mesa, CA). Neutralizing antibody against TGF-β1, 2, 3 was purchased from Genzyme (Cambridge, MA).

Cell Culture

The isolation of the clone of REL cells was described previously [Lagarrigue et al., 1992]. CCL 64 cells were purchased from the ECACC (Salisbury, UK). REL cells and CCL 64 cells were grown in Dulbecco's medium containing 10% fetal calf serum, 2 mM L-glutamine, 50 UI/ml penicillin, 50 μg/ml streptomycin, and 10 μg/ml gentamycin, at 37°C in a humidified atmosphere. Incubations were performed in this medium and products were diluted in dimethylsulfoxide or ethanol. The final solvent concentration was not higher than 0.5% (v/v) and did not affect cell viability.

^{125}I-TGF-β1 Crosslinking

Cells were seeded at 4×10^5 cells per dish, allowed to spread for 24 hr, and incubated for 24 hr without or with the molecules. At the end of the incubations, cultures were carefully washed twice in phosphate buffer saline (PBS). Cells from companion wells incubated without or with the molecules were trypsinized and counted on a Coulter-Counter Channelyzer. For affinity crosslinking assay, affinity labeling was performed as described by Cheifetz et al. [1986]. The cells were incubated in binding buffer (40 mM Hepes, 128 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 5 mM $MgSO_4$, 5 mg/ml BSA, pH 7.4) containing 1×10^6 cpm of ^{125}I-TGF-β1 for 3.5 hr at 4°C. Cells were then washed three times with binding buffer and treated with 700 μl of 0.15 mM disuccinimidyl suberate (Pierce, Rockford, IL) for 15 min on ice. Following centrifugation (15,000 × *g* for 10 min at 4°C), cellular material was solubilized for 40 min at 4°C in 10 mM Tris buffer, pH 7, 1 mM EDTA, 1% Triton, 1 mM phenylmethylsufonyl fluoride (PMSF), and 50 μg/ml leupeptin. SDS-PAGE of the Triton-solubilized receptors reduced with 1% β-mercaptoethanol was performed according to the procedure of Laemmli [1974], using 7.5% polyacrylamide gel. After electrophoresis, the gels were stained with Coomassie blue, dried, and exposed to Kodak XAR film with an enhancing screen.

The autoradiographies were scanned and the intensity of the bands quantified by a computer image processing system (Biocom 500, France).

Preparation of Cytosol, Membrane, and Total Protein Fractions for Type II Receptor Immunoblotting

Confluent cell monolayers were washed twice in PBS, scraped off, and ultrasonicated on ice in the presence of 10 mM Tris buffer, pH 7, containing 0.25 M sucrose, 1 mM EDTA, 1 mM PMSF, and 10 μg/ml leupeptin. The samples were centrifuged at 15,000 × *g* for 15 min at 4°C and the supernatants were collected (cytosol fraction). The pellets were washed with the same buffer and solubilized for 40 min at 4°C in 10 mM Tris buffer, pH 7.0, containing 1 mM EDTA, 1% Triton, 1 mM PMSF, and 50 μg/ml leupeptin. After centrifugation at 15,000 × *g* for 15 min at 4°C, the supernatants were collected (membrane fraction).

Total cellular proteins were extracted by lysis buffer (10 mM Tris buffer, pH 7.5, 1% SDS, 1 mM EDTA, 1 mM PMSF, 10 μg/ml leupeptin). Each sample corresponding to 100×10^3, 150×10^3, $1{,}000 \times 10^3$ cells/lane for total, cytosol, and membrane fractions, respectively, was adjusted to 2% SDS, 10% glycerol, and 50 mM β-mercaptoethanol before 7.5% SDS polyacrylamide gel electrophoresis analysis.

The proteins were transferred to a nitrocellulose membrane (Schleicher and Schuell) at 100 mA for 16 hr in 25 mM Tris, pH 8.0, 192 mM

glycine, and 20% methanol. Immunodetection of TGF-β type II was carried out according to the manufacturer's recommendations with the anti TGF-β type II receptor antibody, TGF-β (R-1) (C-16) (Santa Cruz Biotechnology, Santa Cruz, CA).

TGase Assay

At the end of the different incubations, cells were washed three times and scraped from the Petri dishes in cold Ca^{2+} and Mg^{2+}-free Hepes buffer (129 mM NaCl, 5 mM KCl, 0.3 mM Na_2HPO_4, 1 mM $NaHCO_3$, 5 mM glucose, and 25 mM Hepes, pH 7.4, 1 mM PMSF, 10 μg/ml leupeptin). The supernatant obtained after ultrasonication on ice and centrifugation (15,000 × *g*, 15 min, 4°C) represents the cytosolic fraction. The pellet was washed and solubilized in Hepes buffer containing 10 mM CHAPS (3-[3-cholamidopropyl) dimethylammonio]-1-propane sulfonate), centrifuged at 15,000 × *g* for 15 min at 4°C, and the supernatant collected as the soluble membrane extract.

TGase activity was measured as the Ca^{2+}-dependent incorporation of ^{14}C-putrescine (Amersham, Corp., Arlington Heights, IL) into N,N-dimethylcasein, according to the protocol described by Kojima et al. [1993], except that Whatman GF/C glass filters were washed four times with 10 ml of 5% TCA. Protein concentration was measured by the method of Lowry et al. [1951].

RESULTS

To examine the effects of retinoid exposure on the expression of TGF-β receptors, we used the method of ^{125}I-TGF-β crosslinking. The three types of receptors commonly found in most cells can be detected in REL cells [Mercier et al., 1995]. Figure 1A and B shows that a treatment of 24 hr with increasing doses of RA or RET resulted in a progressive reduction of the ^{125}I-TGF-β binding to the receptors. The effects of the molecules were very similar. We assessed by densitometric image analysis that, when REL cells were incubated with 1 μM RA, the percentage of type I receptor remaining was 54 ± 14, 37 ± 3 for type II and 42 ± 10 for type III as compared to controls (mean of four experiments). For 1 μM RET, the values were very close: 55 ± 9 for type I, 42 ± 7 for type II, and 49 ± 10 for type III. In contrast, when CCL 64 cells were incubated in the same conditions, the binding levels were not reduced at any dose as compared to controls (Fig. 1C). Time course experiments on REL cells showed that an interval of 12 hr was necessary to observe a decrease (Fig. 2). When RA was removed after a 24 hr exposure and binding was checked after varying time intervals, we observed that each type of receptor increased progressively with time but remained lower than control values up until 24 hr (Fig. 3).

The abundance of the type II receptor at the cell membrane was directly assessed by Western blot analysis. Figure 4 shows a typical autoradiography. Type II was mainly found in the cytosolic fraction, which remained unchanged when RA or RET was added. Yet RA and RET treatment reduced the amount of protein at the cell surface, implying that the decrease observed

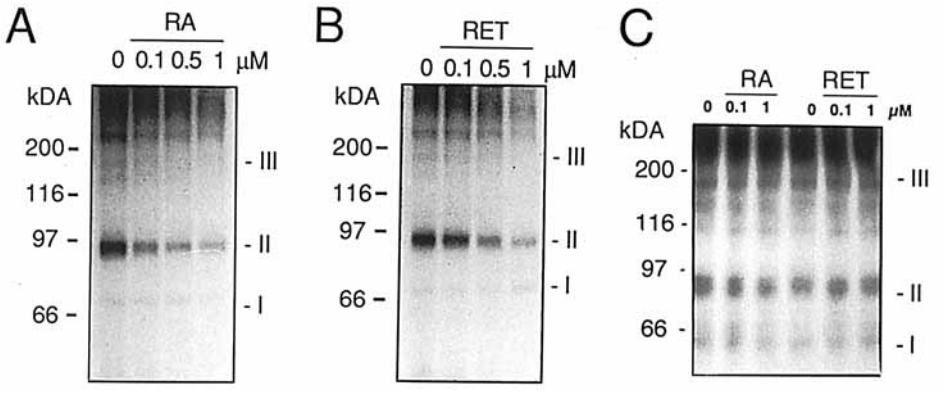

Fig. 1. Effect of retinoid exposure on TGF-β receptor levels in REL cells and CCL 64 cells. Confluent REL cells (**A** and **B**) or CCL 64 cells (**C**) were incubated for 24 hr with the retinoids at the indicated concentrations. They were then incubated for 3.5 hr at 4°C with 50 pM ^{125}I-TGF-β1. Cells were treated with 0.15 mM disuccinimidyl suberate, extracted with 1% Triton X-100, in the presence of protease inhibitors, and subjected to electrophoresis on 7.5% SDS-PAGE. The autoradiogram from a fixed, dried gel is shown. The position and molecular mass of protein standards electrophoresed on parallel lanes and of the three types of receptors are indicated.

with the crosslinking method was not due to a reduction of the affinity for the ligand, but to a reduction of the receptors detectable. The fact that no change was observed in the total extracts under retinoid exposure suggested that the compounds did not reduce the receptor synthesis.

To investigate the possibility that the decrease was a consequence of an internalization of the receptors due to an interaction with the ligand, we designed several experiments using a TGF-β neutralizing antibody. Cells were coincubated at 37°C for 24 hr with increasing concentrations of TGF-β1 corresponding to 2–10-fold the total production of TGF-β in these cells [Mercier et al., 1995] and 50 μg/ml neutralizing antibody, and the level of binding was examined by crosslinking. Figure 5A shows that the antibody is indeed able to counteract the binding of the ligand and the internalization of the complexes, since the level of receptors remains unchanged in all the samples containing the antibody.

In a further step, cells were coincubated with the retinoids and the antibody for 24 hr, and the receptors checked by crosslinking. Figure 5B shows that, in this case, the antibody cannot counteract the decrease of the receptors. Therefore, we excluded the possibility that the decrease observed was a mere consequence of the internalization of ligand–receptor complexes.

As TGase (E.C.2.3.2.13) is an enzyme that is known to be induced by retinoid exposure [Cai et al., 1991; Nara et al., 1989] and is expressed in hepatic cells [Ikura et al., 1988; Piacentini et al., 1992], we checked whether TGase could be involved in this phenomenon. TGase is inhibited by primary amines, and particularly cystamine [Folk, 1980]. We therefore coincubated REL cells with retinoids and cystamine. Cystamine 100 μM was the highest dose used, as higher concentrations appeared toxic. As shown in Figure 6A, while cystamine alone did not modify the level of any type of receptors in REL cells, addition of cystamine prevented the decrease of the receptor expression in cells treated with the retinoids. Densitometry showed that, when cells were coincubated with retinoids and 100 μM

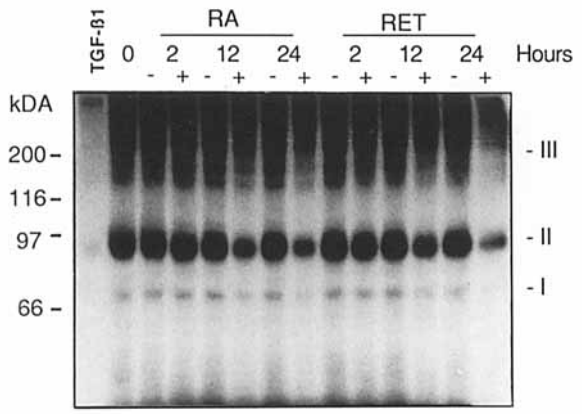

Fig. 2. Time course of the effect of retinoids on receptor levels in REL cells. Confluent cells were incubated for the specified times in the presence of 1 μM RA or 1 μM RET (+), or in the absence of retinoids (−). The first two lanes show the expression of the receptors at time 0; cells were labeled with 50 pM ^{125}I-TGF-β1 with (*lane 1*) or without (*lane 2*) 3,000 pM unlabeled TGF-β1. Crosslinking was performed as described in Figure 1.

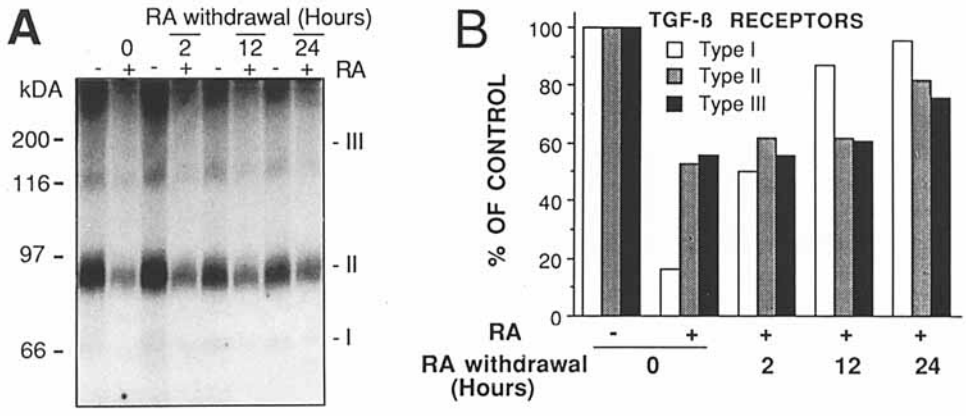

Fig. 3. Reversibility of the effects of retinoids on TGF-β receptor levels. Confluent cells were incubated for 24 hr in the presence (+) or absence (−) of 1 μM RA. At the end of the incubation, RA-containing (+) or not containing (−) media were removed. Cells were reincubated for the indicated times in the usual medium and submitted to ^{125}I-TGF-β1 crosslinking (**A**). Densitometric image analysis of the bands was performed and is shown in **B**. Values are expressed as percent of controls which corresponded to cells incubated for the first 24 hr without RA, and reincubated for the indicated times in the usual medium.

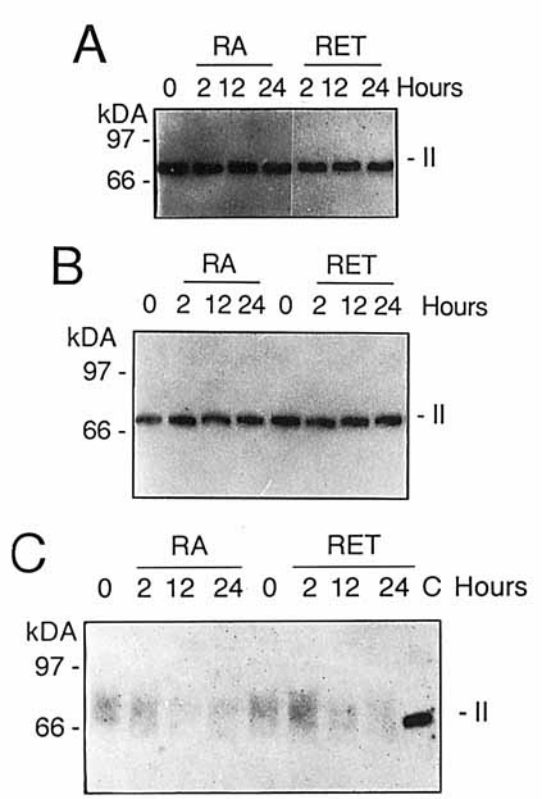

Fig. 4. Immunoblotting analysis of type II-TGF-β-receptor. Total (**A**), cytosolic (**B**), and membrane (**C**) protein extracts from cells incubated without or with 1 μM RA or 1 μM RET for the indicated times were analyzed with the type II-TGF-β-receptor antibody. In C, the last lane noted shows the receptor in a cytosolic extract. Samples loaded in A corresponded to 100 × 10^3 cells, in B to 150 × 10^3 cells, and in C to 1,000 × 10^3 cells.

cystamine, about 80% of the control binding level was recovered (Fig. 6B).

TGase activity was measured in REL and CCL 64 cells, and Table I summarizes the results obtained. In REL cells, TGase activity present in the membrane fraction was markedly enhanced by retinoid exposure (3.7-fold with RA; 4.8-fold for RET). In CCL 64 cells, although a TGase activity was detectable both in the membrane and cytosol fractions, retinoid treatment did not modify the activity as compared to controls. Thus, TGase activity was indeed higher in the membrane of REL cells after retinoid incubation.

DISCUSSION

The receptors for TGF-β have been the subject of intense interest, especially in the understanding of their diverse functions. Yet, information on their regulation remains fragmentary. If most studies have focused on the effect of RA on the synthesis of TGF-β or other growth factors [Dmitrovsky et al., 1990; Miller et al., 1990; Piazza and Ritter, 1993], only a few have described the effects of RA treatment on the expression of TGF-β receptors. In HL-60 cells exposure to RA increased the levels of TGF-β binding [Falk et al., 1991], and in the murine epidermal cell line JB6 addition of RA could prevent the decrease of receptors caused by TPA exposure [De Benedetti et al., 1991]. It has also been reported that glucocorticoids could increase the expression of the type III receptor in osteoblast-like cells, either post-transcriptionally [Centrella et al., 1991] or transcriptionally [Nakayama et al., 1994].

Our data showed that exposure to RA and RET led to a sharp decrease of the levels of the three types of receptors expressed in the REL cells. The results obtained with the cross-linking analyses can indicate either that the number of receptors was reduced, or the affinity for the ligand was decreased. Immunoblotting experiments for type II receptor showed that the quantity of detectable protein was indeed reduced. Surprisingly, we consistently detected a large amount of protein in the cytoplasmic fraction, a very small proportion being present at the cell membrane. The data obtained by this method showed a decrease of the protein after exposure to retinoids. The fact that the total extracts were not modified upon retinoid treatment indicated that changes in protein synthesis were not involved, and that only the receptors detected at the cell surface were diminished. It is not clear from our experiments whether the quantity of protein was reduced or if the receptors were in a form undetectable by the antibody and the ligand. Our results suggested that TGase was involved in the decrease, since addition of cystamine, a TGase inhibitor, restored the receptor levels (Fig. 6). TGase is an enzyme associated with the plasma membrane, and catalyzes the formation of an amide bond between the γ-carboxamide group of peptide-bound glutamine residues and the primary amino groups such as the ε-amino group of lysine residues [Folk, 1980]. TGase can therefore crosslink the receptors to each other or to other membrane proteins in a way that reduces the binding of the ligand and the antibody. Contrary to REL cells, in CCL 64 cells, TGase activity was not increased by retinoids, and the receptor levels were not modified by RA and RET. This observation is consistent

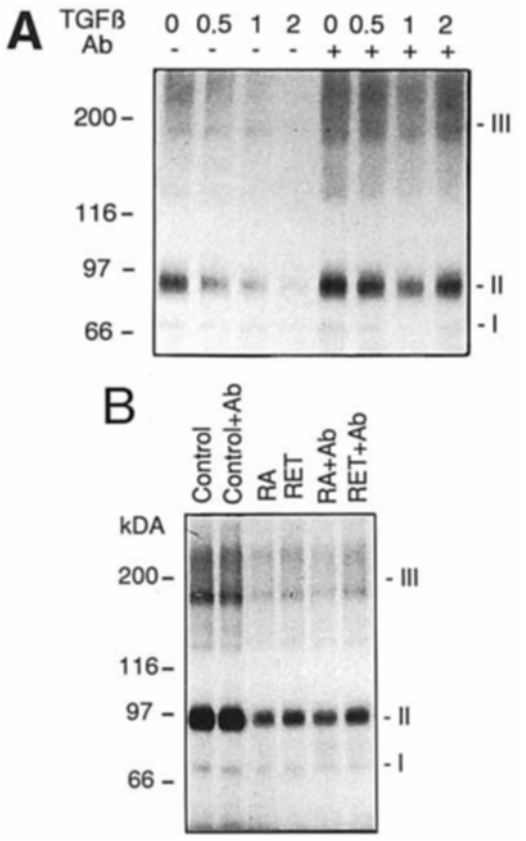

Fig. 5. Effect of the addition of a TGF-β neutralizing antibody in the incubation medium on receptor levels. **A:** Confluent cells were incubated for 24 hr with TGF-β1 at different concentrations, in the presence or absence of 50 μg/ml anti-TGF-β1,2,3 neutralizing antibody (Ab). The incubations were realized at 37°C, and ^{125}I-TGF-β crosslinking was performed as described in Materials and Methods. **B:** Confluent cells were incubated in the same conditions as indicated in Figure 1, and in the presence or absence of 50 μg/ml neutralizing antibody.

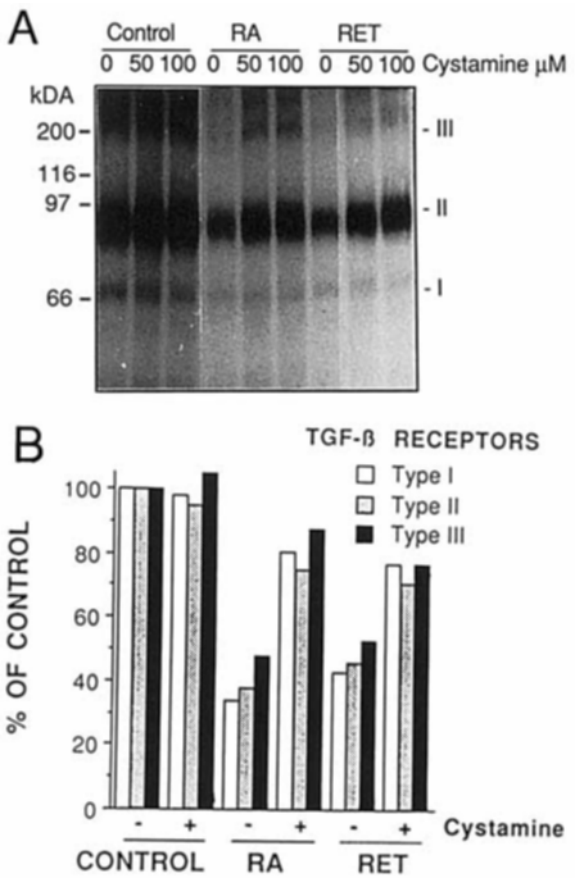

Fig. 6. Effect of the addition of cystamine on binding levels. **A:** Confluent cells were incubated for 24 hr in the absence (controls) or presence of 1 μM RA or RET, together with increasing doses of cystamine. Crosslinking analysis was performed as described in Figure 1. **B:** Densitometric analysis of the bands. Cystamine concentration is 100 μM.

TABLE I. Effect of RA and RET on TGase Activity in REL Cells and CCL 64 Cells*

	REL cells pmole putrescine/hr/mg protein		CCL 64 cells pmole putrescine/hr/mg protein	
Compounds	Cytosol	Membrane extracts	Cytosol	Membrane extracts
None	87 ± 12	15 ± 2	80 ± 28	42 ± 10
RA	83 ± 9	56 ± 6	60 ± 2,5	47 ± 10
RET	79 ± 8	72 ± 12	60 ± 10	38 ± 8

*Confluent cells were exposed to 1 μM RA or 1 μM RET for 24 hr. Cytosol and membrane extracts were prepared, and TGase activity measured as described in Materials and Methods. The background activity measured in the absence of Ca^{2+} (5 mM EGTA) was subtracted from all values. Values are means ± SD from duplicates.

with our hypothesis, linking the decrease of the receptors to the increase in TGase activity.

When the retinoids were removed, the binding levels remained low during several hours: the receptors were not recycled back quickly. Like for all signaling receptors, the trafficking of TGF-β receptors is complex. Signaling receptors are internalized when ligand bound, unlike non-signaling receptors, like receptors for low density lipoproteins or transferrin, the behavior of

which is independent of occupancy [Ajioka and Kaplan, 1986; Watts, 1985]. However, a recent study focusing on the trafficking of epidermal growth factor has shown that its receptor could also be internalized unoccupied. Yet this event was much less frequent than when the receptors were ligand bound, and the free receptors were recycled back to the cell surface very rapidly [Herbst et al., 1994]. The control experiments using the neutralizing antibody (Fig. 5) showed that if the receptors were internalized, that probably was in a free state. Another possibility already mentioned is that the receptors are still at the cell membrane, but not functional because linked to other proteins.

Retinoids are known to modify TGF-β synthesis and activation, and our study shows that they can also modulate the binding of TGF-β to the cell membrane, and thereby probably modify cell responsiveness to TGF-β. Indeed, cell sensitivity to TGF-β action has been correlated with the receptor levels [Laiho et al., 1990; Mulder et al., 1993; Mercier et al., 1995]. It has been recently shown that 1,25 dihydroxyvitamin D_3 could reduce by 40% the expression level of TGF-β type II receptor in osteoblastic cells, and that the reduction correlated well with a diminished cell response to TGF-β [Iimura et al., 1994]. In REL cells, too, the depressed levels at the cell surface could represent, as for all signaling receptors, a strong potential attenuation of the cell responsiveness to TGF-β. It is here of interest to note that in several cell types, retinoids and TGF-β can act additively or synergistically on cell growth [De Benedetti et al., 1991; Fukuda et al., 1994] or even cell death, especially in rat hepatic cells [Fukuda et al., 1994].

Our results thus show that, while retinoids and TGF-βs are two families of compounds presenting many similarities of effects, in REL cells treatment with retinoids tends to reduce and limit the subsequent actions of TGF-βs.

ACKNOWLEDGMENTS

We are grateful to Mrs. G. Cauneau for typing the manuscript and to Mr. P. Flanzy for the illustration work. This study was supported by the CEC Programme Grant BIOT-CT 91-0261.

REFERENCES

Ajioka RS, Kaplan J (1986): Intracellular pools of transferrin receptors result from constitutive internalization of unoccupied receptors. Proc Natl Acad Sci USA 83:6445–6449.

Attisano L, Wrana JL, Lopez-Casillas F, Massagué J (1994): TGF-β receptors and actions. Biochim Biophys Acta 1222: 71–80.

Blomhoff R, Green MH, Berg T, Norum KR (1990): Transport and storage of vitamin A. Science 250:399–404.

Cai D, Ben T, De Luca LM (1991): Retinoids induce tissue transglutaminase in NIH-3T3 cells. Biochem Biophys Res Commun 175:1119–1124.

Centrella M, McCarthy TL, Canalis E (1991): Glucocorticoid regulation of transforming growth factor β1 activity and binding in osteoblast-enriched cultures from fetal rat bone. Mol Cell Biol 11:4490–4496.

Cheifetz S, Like B, Massagué J (1986): Cellular distribution of type I and type II receptors for transforming growth factor-β. J Biol Chem 261:9972–9978.

Chiocca EA, Davies PJA, Stein JP (1988): The molecular basis of retinoic acid action. Transcriptional regulation of tissue transglutaminase gene expression in macrophages. J Biol Chem 263:11584–11589.

Corbeil J, Rapaport E, Richman DD, Looney DJ (1994): Antiproliferative effect of retinoid compounds on Kaposi's sarcoma cells. J Clin Invest 93:1981–1986.

Davis BH, Kramer RT, Davidson NO (1990): Retinoic acid modulates rat Ito cell proliferation, collagen, and transforming growth factor β production. J Clin Invest 86:2062–2070.

De Benedetti F, Falk L, Ruscetti FW, Colburn NH, Faltynek CR, Oppenheim JJ (1991): Synergistic inhibition of phorbol ester–induced transformation of JB6 cells by transforming growth factor-β and retinoic acid. Cancer Res 51:1158–1164.

Dmitrovsky E, Moy D, Miller WH, Li A, Masui H (1990): Retinoic acid causes a decline in TGF-α expression, cloning efficiency and tumorigenicity in a human embryonal cancer cell line. Oncog Res 5:233–239.

Falk LA, De Benedetti F, Lohrey N, Birchenall-Roberts MC, Ellingsworth LW, Faltynek CR, Ruscetti FW (1991): Induction of transforming growth factor-β1 (TGF-β1) receptor expression and TGF-β1 protein production in retinoic acid–treated HL-60 cells: Possible TGF-β1-mediated autocrine inhibition. Blood 77:1248–1255.

Folk JE (1980): Transglutaminases. Annu Rev Biochem 49:517–531.

Fukuda K, Kojiro M, Chiu JF (1994): Differential regulation of tissue transglutaminase in rat hepatoma cell line McA-RH7777 and McA-RH8994: Relation to growth rate and cell death. J Cell Biochem 54:67–77.

Glick AB, MC Cune BK, Abdulkarem N, Flanders KC, Lumadue JA, Smith JM, Sporn MB (1991): Complex regulation of TGF-β expression by retinoic acid in the vitamin-A deficient rat. Development 111:1081–1086.

Herbst JJ, Opresko LK, Walsh BJ, Lauffenburger DA, Wiley HS (1994): Regulation of postendocytic trafficking of the epidermal growth factor receptor through endosomal retention. J Biol Chem 269:12865–12873.

Iimura T, Oida S, Ichijo H, Goseki M, Maruoka Y, Takeda K, Sasaki S (1994): Modulation of responses to TGF-β by 1,25 dihydroxyvitamin D_3 in MG-63 osteoblastic cells: Possible involvement of regulation of TGF-β type II receptor. Biochem Biophys Res Commun 204:918–923.

Ikura K, Nasu T, Yokota H, Tsuchiya Y, Sasaki R, Chiba H (1988): Amino acid sequence of guinea pig liver transglutaminase from its cDNA sequence. Biochemistry 27:2898–2905.

Jakowlew SB, Cubert J, Danielpour D, Sporn MB, Roberts AB (1992): Differential regulation of the expression of transforming growth factor-β mRNAs by growth factors and retinoic acid in chicken embryo chondrocytes, myocytes, and fibroblasts. J Cell Physiol 150:337–385.

Kojima S, Rifkin DB (1993): Mechanism of retinoid-induced activation of latent transforming growth factor-β in bovine endothelial cells. J Cell Physiol 155:323–332.

Kojima S, Nara K, Rifkin DB (1993): Requirement for transglutaminase in the activation of latent transforming growth factor-β in bovine endothelial cells. J Cell Biol 121:439–448.

Laemmli UK (1974): Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680–685.

Lagarrigue S, Seillan-Heberden C, Martel P, Gaillard-Sanchez I (1992): Altered response to growth factors in rat liver epithelial cells overexpressing human c-Fos protein. FEBS Lett 314:399–403.

Laiho M, Weis FMB, Massagué J (1990): Concomitant loss of transforming growth factor (TGF)-β receptor types I and II in TGF-β-resistant cell mutants implicates both receptor types in signal transduction. J Biol Chem 265: 18518–18524.

Lowry OH, Rosebrough NS, Farr AL, Randall RJ (1951): Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275.

Mercier T, Gaillard-Sanchez I, Martel P, Seillan-Heberden C (1995): Constitutive overexpression of c-fos protein in rat liver epithelial cells decreases TGF-β synthesis and increases TGF-β1 receptors. Biochim Biophys Acta 1266:64–72.

Miller WH, Moy D, Li A, Grippo JF, Dmitrovsky E (1990): Retinoic acid induces down-regulation of several growth factors and proto-oncogenes in a human embryonal cancer cell line. Oncogene 5:511–517.

Mulder KM, Segarini PR, Morris SL, Ziman JM, Ho GC (1993): Role of receptor complexes in resistance or sensitivity to growth inhibition by TGF beta in intestinal epithelial cell clones. J Cell Physiol 154:162–174.

Nakayama H, Ichikawa F, Andres JL, Massagué J, Noda M (1994): Dexamethasone enhancement of betaglycan (TGF-β type III receptor) gene expression in osteoblast-like cells. Exp Cell Res 211:301–306.

Nara K, Nakanishi K, Hagiwara H, Wakita K, Kojima S, Hirose S (1989): Retinoid-induced morphological changes of cultured bovine endothelial cells are accompanied by a marked increase in transglutaminase. J Biol Chem 264: 19308–19312.

Piacentini M, Ceru MP, Dini L, Di Rao M, Piredda L, Thomazy V, Davies PJA, Fesus L (1992): In vivo and in vitro induction of tissue transglutaminase in rat hepatocytes by retinoic acid. Biochim Biophys Acta 1135:171–179.

Piazza GA, Ritter JL (1993): Involvement of transforming growth factor-alpha and its receptor in the growth response of cultured human epidermal cells to retinoic acid. Epith Cell Biol 2:170–175.

Verma AK (1991): Modulation of carcinogenesis by vitamin Laidlaw W., ed. A and its analogs (retinoids). In: "Vitamins and Cancer Prevention." New York: Wiley-Liss, Inc., pp 25–37.

Watts C (1985): Rapid endocytosis of the transferrin receptor in the absence of bound transferrin. J Cell Biol 100: 633–637.

Wolf G (1990): Recent progress in vitamin A research: Nuclear retinoic acid receptors and their interaction with gene elements. J Nutr Biochem 1:284–289.