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# Type β transforming growth factor is the primary differentiation-inducing serum factor for normal human bronchial epithelial cells

(terminal squamous differentiation/human lung carcinomas/type β transforming growth factor receptors/epinephrine/cyclic AMP)

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**ABSTRACT** **Type β transforming growth factor (TGF-β) was shown to be the serum factor responsible for inducing normal human bronchial epithelial (NHBE) cells to undergo squamous differentiation. NHBE cells were shown to have high-affinity receptors for TGF-β. TGF-β induced the following markers of terminal squamous differentiation in NHBE cells: (*i*) increase in Ca ionophore-induced formation of cross-linked envelopes; (*ii*) increase in extracellular activity of plasminogen activator; (*iii*) irreversible inhibition of DNA synthesis; (*iv*) decrease in clonal growth rate; and (*v*) increase in cell surface area. The IgG fraction of anti-TGF-β antiserum prevented both the inhibition of DNA synthesis and the induction of differentiation by either TGF-β or whole blood-derived serum. Therefore, TGF-β is the primary differentiation-inducing factor in serum for NHBE cells. In contrast, TGF-β did not inhibit DNA synthesis of human lung carcinoma cells even though the cells possess comparable numbers of TGF-β receptors with similar affinities for the factor. Epinephrine antagonized the TGF-β-induced inhibition of DNA synthesis and squamous differentiation of NHBE cells. Although epinephrine increased the cyclic AMP levels in NHBE cells, TGF-β did not alter the intracellular level in NHBE cells in either the presence or absence of epinephrine. Therefore, epinephrine and TGF-β appear to affect different intracellular pathways that control growth and differentiation processes of NHBE cells.**

Understanding the processes that control growth and differentiation of normal human epithelial cells and elucidating how these controlling mechanisms differ in carcinoma cells is critical to an understanding of carcinogenesis. Many malignant cell types have a decreased dependency for peptide mitogens (1–3). This reduced growth-factor requirement has been commonly observed for the fibroblast cell model systems. Unlike fibroblasts, however, the majority of human cancers are derived from epithelial cells that normally terminally differentiate (4), and a diminished response to terminal differentiation inducers is a hallmark *in vitro* phenotypic marker of carcinoma cells (5–9). To investigate why malignant cells lose sensitivity to agents that induce terminal differentiation of their normal counterparts, a controlled culture system is indispensable. Using a serum-free culture system for normal human bronchial epithelial (NHBE) cells, we have shown that human and bovine whole blood-derived serum (BDS), specifically the platelet fraction, induced terminal squamous differentiation of NHBE cells, while malignant human lung carcinomas did not respond to this differentiation-inducing effect of BDS (9–11). We have now found that type β transforming growth factor (TGF-β), one of the defined constituents of human platelets, is a potent growth inhibitor and an inducer of squamous differentiation for NHBE cells but not for lung carcinoma cells when cultured in monolayer under serum-free conditions.

TGFs are operationally defined as peptides that reversibly confer the transformed phenotype on normal indicator cells (12, 13). Two distinct classes of TGFs have been identified. Type α TGFs bind to epidermal growth-factor (EGF) receptors and show considerable sequence homology, although no immunological crossreactivity, to EGF (14, 15). Type β TGF is a 25-kDa disulfide-linked dimer that binds to a unique cellular receptor (16–19). TGF-β is found in both neoplastic and normal tissues (20–23) and the platelet is quantitatively the major non-neoplastic source of the peptide (24). The phenotypic transformation of normal rat kidney (NRK) indicator cells requires the concerted action of both classes of TGFs and platelet-derived growth factor (25). Recently, TGF-β was shown to have a bifunctional action on a number of cell types, either causing a stimulation or an inhibition of growth depending on the culture conditions and the spectrum of other growth factors acting on the cells (26, 27).

In this report, we tested the possibility that TGF-β is the major differentiation-inducing serum factor for NHBE cells in a serum-free culture system. Receptor assays revealed that NHBE cells have high-affinity receptors for TGF-β and that a decrease in sensitivity to growth inhibitory activity of TGF-β shown in carcinoma cell lines was not because of lack of receptors on these cells. Since epinephrine and other cAMP enhancers stimulate growth of NHBE cells and cholera toxin neutralizes the inhibition of growth of NHBE cells caused by BDS (11, 28), we examined whether epinephrine could affect the action of TGF-β on NHBE cells in the serum-free system. The experiments revealed that epinephrine showed antagonistic interactions with TGF-β. However, additional investigation suggested that TGF-β did not induce NHBE cells to undergo squamous differentiation by altering cAMP levels in the cells.

## MATERIALS AND METHODS

**Cell Culture Methods.** NHBE cells were obtained from outgrowths of normal human bronchial tissue explants in LHC-9 medium (LHC-8 medium supplemented with 0.3 nM retinoic acid and 1.6 μM epinephrine) as described in detail (9, 10, 29, 30). NHBE cells were cultured in surface-coated dishes in serum-free LHC-8 medium (29). LHC-8 medium was based on modified MCDB 151 (31) with the following added supplements: insulin (5 μg/ml), EGF (5 ng/ml), transferrin (10 μg/ml), hydrocortisone (0.2 μM), gentamicin

Abbreviations: TGF-β, type β transforming growth factor; NHBE cells, normal human bronchial epithelial cells; PA, plasminogen activator; CLE, cross-linked envelope; BDS, whole blood-derived serum; BPE, bovine pituitary extract; EGF, epidermal growth factor; PMA, phorbol 12-myristate 13-acetate; NRK cells, normal rat kidney cells.

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(50 $\mu$g/ml), bovine pituitary extract (BPE; 35 $\mu$g of protein per ml).

**Growth and Nucleic Acid Synthesis Assays.** The clonal growth and double-labeling assays for DNA or RNA synthesis were performed as described (9, 32). Also, a single-labeling assay for DNA synthesis was used. For this assay, NHBE cells were seeded in 24-well cluster plates (Costar, Cambridge, MA) at 5000 cells per well in LHC-8 medium. Medium was changed to test medium after 1 day. Plates were incubated for 2 days and then [$^3$H]thymidine (81.7 Ci/mmol, 0.5 $\mu$Ci per 50 $\mu$l in LHC-8 medium per well; 1 Ci = 37 GBq) was added, and plates were incubated another day. Acid-precipitable radioactivity was measured. Although the double-labeling assay has the advantage of an internal control (for equalizing colony-forming efficiencies among individual experiments), the single-labeling DNA synthesis assay showed statistically similar results when compared to those obtained by the double-labeling protocol. Thus, in this study we primarily used the single [$^3$H]thymidine-labeling DNA synthesis assay. The single-labeling assay was done in triplicate and results were expressed as mean values ± SEM (see Figs. 1 and 3).

**Differentiation Markers.** Cell surface areas were determined after a 3-day incubation with test compounds in 24-well plates seeded at 5000 cells per well as described (9). The percentage of cells capable of forming cross-linked envelopes (CLEs) in the presence of calcium ionophore (A23187, Sigma) (33, 34) was determined by the modified method of Rice and Green (34). Plasminogen activator (PA)-mediated conversion of plasminogen to plasmin was determined by measuring the plasmin catalyzed release of [$^{14}$C]anilide from benzylcarbonylglycylprolylarginyl-[$^{14}$C]anilide (New England Nuclear) based on a described method (35). Protein concentrations were determined with the BIORAD protein assay.

**Binding of $^{125}$I-labeled TGF-$\beta$ to NHBE Cells.** Human platelet TGF-$\beta$ was iodinated to a specific activity of 2–4 mCi/nmol using a modified chloramine T method (18), and binding of $^{125}$I-labeled TGF-$\beta$ to NHBE cells was determined essentially as described (18), but with the modification that prior to adding the labeled growth factor, the cell monolayers were washed with fresh serum-free medium (without BPE) and incubated in two changes of this medium for 2 hr at 37°C to allow dissociation or internalization of endogenous receptor-bound growth factor.

**TGF-$\beta$ and Antibodies to TGF-$\beta$.** Human platelet TGF-$\beta$ was prepared according to Assoian *et al.* (24) and used throughout these experiments. For immunization, the TGF-$\beta$ was coupled to keyhole limpet hemocyanin (KLH). Rabbits were immunized with three doses of KLH–TGF-$\beta$ (100 $\mu$g of TGF-$\beta$ per dose per rabbit) in Freund's adjuvant at 3-week intervals. The IgG fraction was purified from the resulting antiserum by affinity chromatography on protein A-Sepharose according to the method of Goding (36). Anti-TGF-$\beta$ antibodies prepared in this way inhibited the binding of TGF-$\beta$ to its specific receptors on NRK cells, and also suppressed the growth of NRK cells in soft agar in response to exogenous TGF-$\beta$ (L.M.W., unpublished observation). Although TGF-$\beta$ from human platelets was used as the antigen, the antiserum reacted equally well with bovine TGF-$\beta$. The IgG fraction of normal rabbit preimmune serum prepared in the same way served as a control. Both of the IgG fractions were extensively dialyzed against phosphate-buffered saline and contained 4–5 mg of protein per ml.

**cAMP Assay.** For the measurement of cellular cAMP levels, 5 × 10$^5$ NHBE cells were seeded in 60-mm culture dishes in LHC-8 medium. After 2 days, the medium was removed and replaced with LHC-8 medium containing test compounds. After a 4-hr incubation, media were removed and the cells were exposed to 0.5 ml of ice-cold 10% trichloroacetic acid. Then samples were subjected to femtomole-sensitive radioimmunoassay (37). Since our previous observation showed that cAMP levels in NHBE cells reach a stable plateau after 4 hr of incubation (28), we used a 4-hr incubation for this assay.

## RESULTS

**Effects of TGF-$\beta$ on Growth and Differentiation of NHBE Cells.** TGF-$\beta$ in LHC-8 medium inhibited both clonal growth and DNA synthesis of NHBE cells in a dose-dependent manner (Table 1). The responses of NHBE cells from four patients were similar. The $ID_{50}$ of TGF-$\beta$ on DNA synthesis was 0.4 ± 0.1 pM, with >95% inhibition at 4 pM within 72 hr after addition to the medium. TGF-$\beta$ at 4 pM only moderately inhibited RNA synthesis up to a maximum 70% at 72 hr.

Since NHBE cells became squamous when exposed to TGF-$\beta$ (Table 1), the effects of TGF-$\beta$ on several markers of terminal squamous differentiation were examined. These were increases in Ca ionophore-induced formation of CLEs, extracellular activity of PA, and cell surface area. All markers increased significantly ($P < 0.05$) in the presence of TGF-$\beta$ (Table 1).

The inhibition of DNA synthesis induced by TGF-$\beta$ was examined for reversibility as a marker of terminal differentiation. When NHBE cells were exposed to 0.4–4 pM TGF-$\beta$ for 1 day and the medium was replaced with TGF-$\beta$-free medium, DNA synthesis failed to resume. To eliminate the possibility that TGF-$\beta$ nonspecifically bound to the cell surface subsequently dissociated and inhibited DNA synthesis, the IgG fraction of anti-TGF-$\beta$ antiserum was added to the fresh TGF-$\beta$-free medium after 1 day of exposure to TGF-$\beta$. No difference was observed in the irreversible inhibition of DNA synthesis between the antibody-supplemented medium and the control.

**Effects of Anti-TGF-$\beta$ Antiserum on Activities of TGF-$\beta$ or BDS.** If TGF-$\beta$ is the primary inducer of differentiation found in BDS, anti-TGF-$\beta$ antibody should neutralize the effects of BDS on NHBE cells. The IgG fraction of rabbit anti-TGF-$\beta$ antiserum prevented inhibition of DNA synthesis caused by either TGF-$\beta$ or BDS in a dose-dependent manner (Fig. 1). Morphologically, there was little difference between NHBE cells in 8% BDS-containing medium supplemented by a 1:40 dilution of the IgG fraction of anti-TGF-$\beta$ antiserum and the cells maintained in LHC-8 control medium. In control experiments, the IgG fractions of anti-TGF-$\beta$ antiserum and

Table 1. Effect of TGF-$\beta$ on squamous differentiation of NHBE cells

| Condition | Clonal growth rate | DNA synthesis | RNA synthesis | CLEs | PA | Cell area |
|---|---|---|---|---|---|---|
| TGF-$\beta$ | | | | | | |
| 0.12 pM | 98 | 71* | 52* | 150* | 110 | 110 |
| 0.4 pM | 54* | 24‡ | 63 | 200‡ | 140* | 113 |
| 1.2 pM | NCG | 4‡ | 36* | 140* | 100 | 146† |
| 4.0 pM | NCG | 4‡ | 29* | ND | ND | 159* |
| BDS (8%) | NCG | 9‡ | 48* | 200† | ND§ | ND |

Results are given as percentage of the control values in LHC-8 medium. DNA and RNA synthesis were determined in duplicate by the double-labeling assay. The control values (100%) of the assays are as follows: clonal growth rate, 0.79 population doublings per day ($n$ = 18); CLE formation, 17.5% ($n$ = 3); PA activity, 5.3 nmol [$^{14}$C]anilide release per hr per mg of protein ($n$ = 2); cell area, 2100 $\mu m^2$ ($n$ = 20). ND, test not done; NCG, no clonal growth. Significantly different from control by Student's $t$ test: *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.005$.

§PA activity cannot be measured with BDS-supplemented medium because BDS contains plasmin.

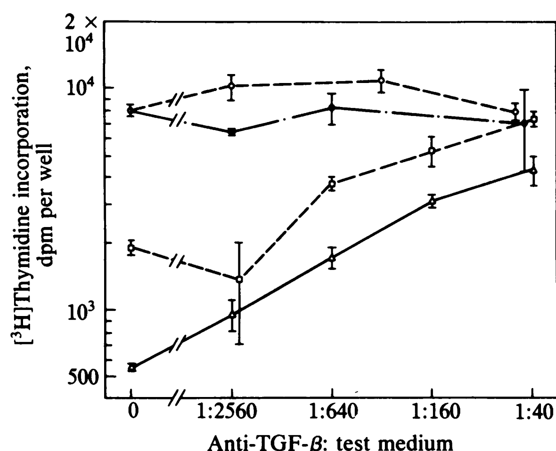


FIG. 1. Effect of IgG fraction of anti-TGF-$\beta$ antiserum on inhibition of DNA synthesis by TGF-$\beta$ or BDS. The effects of the IgG fraction of anti-TGF-$\beta$ antiserum on 1.2 pM TGF-$\beta$ (□) and on 8% BDS (△). The inhibitory effects of TGF-$\beta$ or BDS were neutralized by the IgG fraction of anti-TGF-$\beta$ antiserum. The IgG fractions of anti-TGF-$\beta$ antiserum (●) or normal preimmune rabbit serum (○) alone did not affect the DNA synthesis of NHBE cells. Abscissa shows the dilution of IgG fraction with test medium.

preimmune rabbit serum did not affect DNA synthesis (Fig. 1) and the IgG fraction of a normal rabbit preimmune serum did not significantly affect the inhibition of DNA synthesis caused either by BDS or by TGF-$\beta$ (data not shown).

**Specific Receptor for TGF-$\beta$ on NHBE Cells.** Since TGF-$\beta$ has specific saturable membrane receptors in several cell types (18, 19), NHBE cells were assayed for the presence of specific receptors for TGF-$\beta$. The ligand binding was saturable (data not shown), and a typical Scatchard analysis of TGF-$\beta$ receptors on NHBE cells is shown in Fig. 2. Three sets of experiments provided the following results: a $K_d$ of 13 ± 3 pM (mean ± SD) with 10,000 ± 3000 binding sites per cell. The presence or absence of epinephrine (1.6 $\mu$M; see below) had no significant effect on the TGF-$\beta$ receptors of NHBE cells (data not shown). In one of three trials, there was some indication of low-affinity receptors (Fig. 2), but their affinity was too low to be physiologically significant.

**Effect of TGF-$\beta$ and TGF-$\beta$ Receptors on Human Lung Carcinoma Cells.** As noted, BDS does not inhibit growth of human lung carcinoma cells (9). Thus, growth-inhibitory effects of TGF-$\beta$ and the properties of specific receptors on

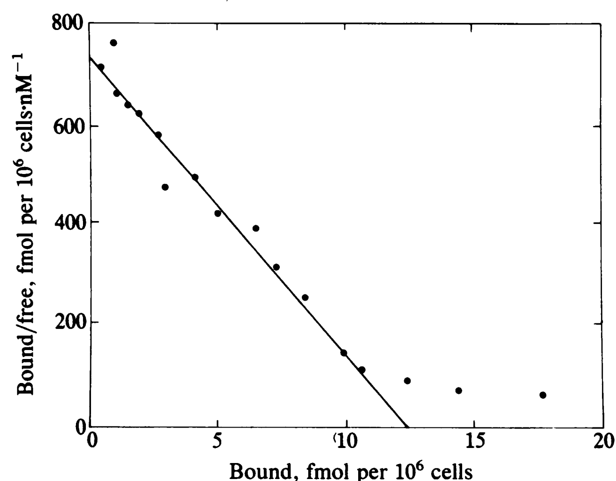


FIG. 2. Scatchard analysis of TGF-$\beta$ binding to NHBE cells. Each data point is the mean of two determinations.

Table 2. Effects of TGF-$\beta$ on the growth of human lung carcinoma cells and their specific receptors for TGF-$\beta$

| Cell type | Histopathology | % cell growth* | TGF-$\beta$ receptors: $K_d$, pM | TGF-$\beta$ receptors: Binding sites per cell |
|---|---|---|---|---|
| NHBE | Normal | 0 | 13 | 10,000 |
| A549 | Adenocarcinoma | 106 | 15 | 10,400 |
| CaLu-1 | Squamous cell carcinoma | 76 | 8 | 7,700 |
| HuT292 | Mucoepidermoid carcinoma | 120 | 1 | 1,800 |

*Growth of cells in the presence of 4 pM TGF-$\beta$ was examined according to the single-labeling assay in the same protocol as described for NHBE cells. Growth expressed as percentage of control value in LHC-8.

carcinoma cells were examined (Table 2). Three representative carcinoma cell lines of different histopathology were examined. TGF-$\beta$ did not show growth inhibitory activity on these malignant counterparts. Receptor assays revealed that TGF-$\beta$-specific receptors on A549 and CaLu-1 showed almost the same characteristics as those on NHBE cells. On the other hand, the receptors on HuT292 showed few binding sites with an extremely high affinity.

**Effects of Epinephrine on TGF-$\beta$ Activity.** To investigate whether the TGF-$\beta$ effect might be modulated by the cAMP system, the effect of epinephrine on TGF-$\beta$ action was examined (Fig. 3), since, of a series of cAMP enhancers, epinephrine gave the most potent mitogenic stimulation to NHBE cells (28). Epinephrine stimulated growth of NHBE cells in the absence of TGF-$\beta$ and neutralized the inhibitory effect of TGF-$\beta$ on DNA synthesis. NHBE cells did not become squamous in the presence of epinephrine in TGF-$\beta$-supplemented medium. On the other hand, small amounts of TGF-$\beta$ (0.12 pM) that only weakly inhibited DNA synthesis of NHBE cells significantly inhibited the growth enhancement caused by epinephrine. However, direct measurement of cAMP levels revealed that TGF-$\beta$ did not cause changes in cAMP levels in the cells, whether or not epinephrine was present (Table 3).

## DISCUSSION

TGF activity was first identified by De Larco and Todaro (12), and subsequently TGF-$\beta$ was defined by its ability to induce reversible phenotypic transformation of NRK cells in the presence of EGF (16, 17). More recently it has been shown to inhibit the growth of certain cells (26, 27). The

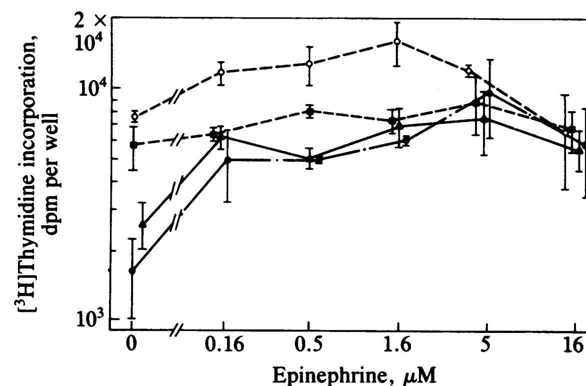


FIG. 3. Effect of epinephrine on DNA synthesis of NHBE cells in the presence or absence of TGF-$\beta$ at the following concentrations: ●, 0.12 pM; ▲, 0.4 pM; ■, 1.2 pM. Control is in LHC-8 (○).

Table 3. TGF-β and epinephrine effects on cellular cAMP and on growth

| | cAMP, pmol per $10^6$ cells | % growth |
|---|---|---|
| Control | 2.9 | 100 |
| + TGF-β | 2.0 | 21‡ |
| + epinephrine | 50* | 200* |
| + epinephrine + TGF-β | 52* | 74† |

The cAMP assay was done in duplicate. Concentrations of TGF-β at 1.2 pM and epinephrine at 1.6 μM were used. In the growth assay, the control value in LHC-8 was 7990 dpm per well ($n$ = 3). Significantly different from control by Student's $t$ test: *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.005$.

present work shows that TGF-β inhibits the growth of NHBE cells and, in addition, is a potent inducer of squamous differentiation in this system. TGF-β induced the following terminal differentiation markers of NHBE cells: (*i*) clonal growth inhibition (9, 38), (*ii*) irreversible inhibition of DNA synthesis, (*iii*) an increase in extracellular PA activity (35, 39), (*iv*) an increase in Ca ionophore-induced CLE formation (33, 34), and (*v*) an increase in cell area (4, 9). BDS also induced terminal differentiation markers of NHBE cells (Table 1; refs. 9–11). Anti-TGF-β antibody was able to neutralize the inhibition of DNA synthesis by TGF-β and BDS in a dose-dependent fashion and prevented the induction of morphological changes by TGF-β or BDS. These data clearly suggest that TGF-β is the primary serum factor that induces terminal squamous differentiation of NHBE cells. In the light of data from a number of other laboratories (refs. 38 and 40; M. E. Kaighn, personal communication) in addition to that presented here, it is reasonable to speculate that TGF-β is also the active factor in serum that inhibits prokeratinocyte growth.

Scott and coworkers showed that there is a distinct complex of $G_1$ arrest state for terminal differentiation in their proadipocyte system (41, 42). In our system, if growth was inhibited by depletion of growth factors (EGF and BPE), formation of CLEs and squamous morphology were significantly inhibited (T.M., unpublished data). Therefore, the correlation between growth inhibition and differentiation is not absolute, suggesting that inhibition of growth *per se* is not sufficient to induce differentiation.

TGF-β showed effects on NHBE cells similar to those previously described for phorbol 12-myristate 13-acetate (PMA) (35). When NHBE cells were exposed to either PMA or TGF-β, the cells were induced to terminally differentiate. Moreover, PMA, BDS, and TGF-β showed little growth-inhibitory activity for human lung carcinoma cell lines and v-Ha-*ras* transfected NHBE cells (refs. 9, 43, and 44; T.M., unpublished data). Thus, in a number of ways the effects of TGF-β were similar to those of PMA on NHBE cells.

In some epithelial cell types, an increase in the cAMP level causes growth stimulation (45, 46). For NHBE cells, increase of cAMP levels by epinephrine alone did not enhance cell growth, but cell growth was enhanced by epinephrine or other cAMP enhancers (3-isobutyl-1-methylxanthine, dibutyryl cAMP, and cholera toxin) in the presence of EGF and BPE (28). Moreover, cholera toxin antagonized the growth-inhibitory effect of BDS on NHBE cells (11). Our results show that epinephrine was able to neutralize the effect of TGF-β in the presence of EGF and BPE. Direct measurement of cAMP showed that TGF-β did not alter the cAMP level of the cells, either with or without epinephrine. TGF-β could prevent the growth enhancement caused by epinephrine without lowering the levels of cAMP in the cells. Therefore, cAMP itself could not be the second messenger of TGF-β action.

Recently, reports have linked the *ras* oncogene product p21 with the GTP-binding protein system, which controls adenylate cyclase activity (47–52). Epinephrine, a β-adrenergic agonist, activates the stimulatory GTP-binding protein system (47) and increases the levels of cAMP in NHBE cells. Moreover, NHBE cells transfected by v-Ha-*ras* oncogene express a decreased sensitivity to differentiation-inducing stimuli, such as PMA, BDS, and TGF-β (ref. 44; T.M., unpublished data). Since the abilities of both epinephrine and Ha-*ras* p21 to block differentiation can be linked to GTP-binding protein pathways (47, 49, 50, 52), a possible role for the *ras* oncogene during epithelial cell carcinogenesis might be to derange the pathways that normally cause the cells to undergo terminal differentiation.

It has been reported that NRK cells and AKR-2B cells have specific receptors for TGF-β with $K_d$ values of 25–30 and 33 pM, and with 17,000 and 10,500 binding sites per cell, respectively (18, 19). TGF-β receptors on NHBE cells have a higher affinity ($K_d$, 13 ± 3 pM) and a comparable number of binding sites (10,000 ± 3000 binding sites per cell). The $ID_{50}$ for TGF-β on DNA synthesis of NHBE cells was ≈0.4 pM. Thus, as shown for NRK cells, the effect of TGF-β on NHBE cells may be mediated by specific receptors for TGF-β, and only a small fraction of these receptors need be occupied for TGF-β to inhibit the growth of NHBE cells. In certain cell types, it has been shown that inhibitory or stimulatory effects of growth factors result from alterations in receptor properties for other growth factors (53, 54). Our results indicate that such interaction does not exist in lung epithelial cells between TGF-β and epinephrine, since normal cells did not show significant differences in TGF-β receptor properties when cultured in medium containing epinephrine or in medium lacking epinephrine. Moreover, the TGF-β binding characteristics of the TGF-β receptors on some carcinoma cells were not significantly different from those on the normal cells, although DNA synthesis of these carcinoma cells was not inhibited by TGF-β.

TGF-β activity has been identified in fetal bovine serum (55), and acid-ethanol extraction of fetal bovine serum yields ≈5 ng of TGF-β per ml as assayed in the soft agar–NRK cell system (M. A. Anzano, personal communication). According to these data, 8% fetal bovine serum-supplemented medium would contain 16 pM TGF-β. However, estimates of the amount of biologically active TGF-β in 8% fetal bovine serum, determined from a TGF-β dose–response curve on NHBE cells, are ≈1 pM TGF-β. Therefore, >90% of the total estimated TGF-β in fetal bovine serum may exist in a biologically unavailable form, or there may be other factors in serum—e.g., epinephrine—that partially neutralize the differentiation-inducing activity of TGF-β. This effect of BDS on TGF-β biological activity could explain the high potency of TGF-β on NHBE cells in the serum-free culture system. Moreover, it also suggests that, in serum-free systems, low levels of contaminants could have effects not seen in serum-supplemented systems. For example, effects formerly attributed to commercially available platelet-derived growth factor (Collaborative Research, Waltham, MA) on NHBE cells in our serum-free system (9) have been shown to be due to contaminating TGF-β (L.M.W. and T.M., unpublished results).

EGF and as yet undefined factors in BPE work in concert with an enhancement of cAMP levels to promote cell division (28), whereas TGF-β stimulates pathways that bring about squamous differentiation. Thus, the interactions between TGF-β and epinephrine are probably indirect. Aberrations in differentiation-inducing pathways (repeatedly noted in carcinomas) could affect the balance between proliferation and terminal differentiation resulting in a permanent tilt toward multiplication.

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