Abstract. A specific radioimmunoassay for type beta transforming growth factor (TGF-β) was developed and used to show that human platelets treated with thrombin release TGF-β as a consequence of degranulation. The thrombin concentrations required to induce release of TGF-β parallel those concentrations that release the alpha-granule marker, beta-thromboglobulin. Related studies showed that TGF-β acts on early passage, explant cultures of bovine aortic smooth muscle cells by inhibiting the effect of mitogens on proliferation of subconfluent cell monolayers yet synergizing with mitogens to stimulate growth of the same cells when cultured in soft agar. The results show that primary cultures of bovine aortic smooth muscle cells and established normal rat kidney cells behave similarly with regard to TGF-β action. Moreover, the data suggest that platelet-mediated proliferation of aortic smooth muscle cells in vivo may not result solely from the stimulatory effect of platelet-derived growth factor (PDGF), but rather from an interaction of platelet factors which has the intrinsic ability to limit as well as stimulate mitosis.

The rate of fibroblast and smooth muscle cell division is controlled, in large part, by the action of peptide growth factors present in platelets. Physiologically, these peptides are released as a consequence of platelet degranulation at sites of injury; together with growth factors in plasma, they stimulate mitosis of connective tissue cells (36, 37, 47). The best studied of these platelet peptides is platelet-derived growth factor (PDGF), a component of alpha-granules (16, 17) that is a chemoattractant (13, 41, 42) and mitogen (36, 37, 47) for fibroblasts and vascular smooth muscle cells. It is important that PDGF has also been implicated in the etiology of proliferative diseases. Expression of PDGF-like proteins is a common feature of many transformed cells (7) and the hallmark of transformation by simian sarcoma virus (11, 52). Similarly, exposure of vascular smooth muscle cells to PDGF (35) or PDGF-like proteins (10, 40, 43) may be an important component in the proliferation of these cells during atherogenesis.

We and others have shown that PDGF is only one of several growth factors in human platelets (4-6, 9, 14, 26); type beta transforming growth factor (TGF-β) can be purified (5) from platelets with yields similar to that of PDGF. TGF-β is a widely distributed growth factor in animal tissues (27), but platelets contain ~100-fold more TGF-β than do the other cells or tissues examined to date (5). Consistent with the role of platelet factors as participants in wound repair, TGF-β can stimulate cell proliferation, protein synthesis, and collagen production in an in vivo wound healing system (46). A functional, epidermal growth factor (EGF)-like peptide is also present in human platelets (4, 6, 26), but lack of the purified protein has precluded detailed studies of its structure and action.

The initial in vitro studies with TGF-β focused on its role in cellular transformation (28, 30); this peptide stimulates growth of non-neoplastic fibroblastic cells suspended in medium with 10% serum and agar (conditions of anchorage-independent growth). In particular, NRK fibroblasts grow efficiently in soft agar when both TGF-β and EGF (or TGF-alpha, an EGF-like peptide) are added to the culture medium (1, 28, 30). AKR-2B fibroblasts require only the addition of TGF-β (30). Recent studies with TGF-β have demonstrated that this growth factor is actually a bifunctional regulator of cell proliferation; in addition to its stimulatory effects on anchorage-independent growth of mesenchymal cells, TGF-β can inhibit the soft agar growth of many epithelial tumor cells and several fibroblastic cell lines (29, 49). In NRK cells, TGF-β inhibits monolayer growth of subconfluent cultures yet stimulates the growth of confluent monolayers and cells suspended in agar (29).

The inhibitory effects of TGF-β on monolayer cultures of NRK cells have not been observed in all studies (21), suggesting that culture conditions can alter the cellular response to TGF-β. The widespread use of subcloned cell lines has further complicated the interpretation of TGF-β action. Moses et al. (24) have recently studied the effects of TGF-β on early...
passage skin fibroblasts in an attempt to examine the action of this growth factor in uncloned cell cultures. The results show that the growth factor requirement for colony formation of these primary fibroblasts resembles that of established AKR-2B cells; TGF-β stimulates colony formation in the absence of a detectable synergism with EGF.

The experiments reported here were designed to examine the potential role of TGF-β as a mediator of cell proliferation during wound repair and atherogenesis. We show that immunoreactive TGF-β is released from platelets during thrombin-induced degranulation and that the same concentrations of thrombin release TGF-β and the alpha-granule marker (16, 17) beta-thromboglobulin. We also show that TGF-β acts on explant cultures of bovine aortic smooth muscle cells by inhibiting growth when the cells are cultured as subconfluent monolayers and synergizing with EGF and PDGF to stimulate growth when the cells are cultured in soft agar. The results suggest that release of TGF-β during platelet degranulation may be an important element in the regulation of smooth muscle cell division.

Materials and Methods

Growth Factors

TGF-β was purified from freshly drawn human platelets as described (5) except that ura was removed from the purified protein by desalting with high pressure liquid chromatography (2). EGF was purified from mouse submaxillary glands similarly to described procedures (39). Final preparations migrated as single bands during SDS PAGE, eluted at single peaks during analytical C-18 high pressure liquid chromatography, and had amino acid compositions in agreement with published values. Porcine PDGF (Bethesda Research Laboratories, Inc., Rockville, MD) was ~95% pure as determined by SDS gels and lacked TGF-β as determined by specific receptor binding assays. Human PDGF, purified to homogeneity, was generously provided by Gary Crotendorst and Russell Ross. TGF-β, EGF, and albumin-free samples of PDGF were quantitated by amino acid analysis. Growth factor-enriched extracts of human platelets were prepared as described (5), freeze-dried, redissolved in 4 mM HCl, and neutralized with protamine-free culture medium. Protein concentration of the extract was determined by the Coomassie binding procedure (Bio-Rad Laboratories, Richmond, CA) using bovine plasma albumin as reference. All growth factor solutions were diluted into culture medium (see below) containing 1 mg/ml bovine serum albumin before use. Neutralized solutions of PDGF and platelet extract were heated (100°C, 5–10 min) before use.

Radioimmunoassay for TGF-β

Rabbit antiserum (N-113), prepared against keyhole limpet hemocyanin-coupled TGF-β from human platelets, was titrated by its ability to bind 125I-labeled TGF-β (prepared as described in reference 12) and used for radioimmunoassay in an amount (0.5 μl/tube) that precipitated 50% of the bindable tracer. The titration was performed in siliconized Eppendorf tubes (1.5-ml capacity) by incubating (2 h at room temperature) selected volumes of antiserum or normal rabbit serum (both diluted to 125 μl with buffer) with 125I-labeled TGF-β (104 cpm in 25 μl) and precipitating the bound label (1 h at room temperature with gentle rocking) with fixed Staphylococcus aureus cells, (Boehringer-Mannheim Diagnostics, Houston, TX; 25 μl diluted with 1 ml buffer). Precipitated radioactivity was collected by high-speed centrifugation (1 min, table-top centrifuge, Brinkmann Instruments Co., Westbury, NY), the supernatant was carefully precipitated with 0.5 μl of the unfractionated, heat-inactivated serum. After 3 min with thrombin, the supernatant (containing released platelet factors) was collected by centrifugation. Careful collection of the top 80–90% of the supernatant prevented contamination of the sample with platelets.

Cell Cultures

NRK fibroblasts (clone 49F) were maintained in Dulbecco's modified Eagle's medium containing gentamicin (DME) and 5% fetal bovine serum (Bio-Fluids Inc., Rockville, MD). Bovine vascular smooth muscle cells were obtained by explant culture from the medial layer of fetal bovine aorta as described (13, 34). In brief, the explants were cultured in T-25 flasks with DME, 10% in fetal calf serum. The cultures were fed biweekly and trypsinized after 3–4 wk. The trypsinized cultures were suspended in medium and filtered through four layers of sterile gauze to remove the small pieces of medial aorta. The resulting preparations of smooth muscle cells (defined as passage 1) were placed in two T-75 tissue culture flasks, maintained in medium with 5% fetal bovine serum, and thereafter split in a 2:1 ratio. Smooth muscle cells used in the experiments reported here were at passages 2–4.

Assays for Cell Growth

Cell growth was measured in both soft agar and monolayer culture. The soft agar assay was performed as described (5, 31) with 3,000 cells seeded in DME containing 10% calf (whole blood-derived) serum (Gibco, Grand Island, NY) or 10% bovine plasma-derived serum. (Bovine plasma was prepared at the National Institutes of Health-Poolesville Animal Center by plasmaphoresis of 4–12-mo-old calves. The plasma was sterilized by filtration and converted to 10% plasma-derived serum in DME as described in reference 4.) To measure mitosis of NRK and smooth muscle cells in monolayer culture, 1.4-ml portions of a cell suspension (3,000 cells/ml) were added to tissue culture dishes (35 × 100 mm; resulting monolayers were ~10% confluent) and incubated for 8 d. At selected intervals the cultures were trypsinized, and the number of cells was determined with a Coulter counter. The medium for monolayer cultures was DME with 5% plasma-derived serum; 5% fetal calf serum; or 5% plasma-derived serum containing 0.1 nM EGF. In some experiments, TGF-β (20 pM) was added to the cultures 4–6 h after seeding. Stimulation of DNA synthesis by PDGF was determined with both smooth muscle and NRK cells cultured in 1% plasma-derived serum as described (3) except that 3H-deoxyuridine (Amersham Corp., Arlington Heights, IL, 1 μCi/ml) was used instead of [3H]-thymidine. In some experiments, the assay was modified to examine stimulation of DNA synthesis by EGF and TGF-β (see the legend to Table 1 for details).

Results

Initial experiments were designed to study the release of TGF-β during platelet degranulation. A radioimmunoassay, rather than a bioassay, was used to examine the released products for TGF-β, thus minimizing the likelihood that other released platelet factors, such as PDGF (16, 17), might interfere with TGF-β quantitation. Fig. 1 shows the characteristics of the radioimmunoassay developed for TGF-β. Antiserum (N-113), generated in rabbits against keyhole limpet hemocyanin-coupled TGF-β from human platelets, effectively bound the radioiodinated growth factor; 50% of maximal immunoprecipitation occurred with 0.5 μl of the unfraccionated, heat-inactivated serum (Fig. 1, inset, solid circles). This amount of antiserum was used under conditions of radioimmunoassay (Fig. 1) and detected ~0.5 ng unlabeled TGF-β. PDGF (0.1-
Figure 1. Radioimmunoassay for TGF-β. The figure shows specific binding of radiolabeled TGF-β to antiserum N-113 and use of the antiserum under conditions of radioimmunoassay. ©, antiserum. O, normal rabbit serum. See Materials and Methods for protocols.

Figure 2. Release of TGF-β and beta-thromboglobulin by treatment of human platelets with thrombin. Gel-filtered human platelets were degranulated by exposure to thrombin (see Materials and Methods). The supernatants were tested for presence of beta-thromboglobulin (©) and TGF-β (●) using a commercial radioimmunoassay kit (Amersham Corp.) and the assay described in Fig. 1, respectively. TGF-β detection required concentration of the supernatant (0.5-ml portions of the supernatants were dialyzed against 1 M acetic acid in a microdialysis unit [Bethesda Research Laboratories], freeze-dried, and dissolved in 0.1 ml of radioimmunoassay buffer (see legend to Fig. 1) modified such that the final albumin concentration was 20 mg/ml). When this entire sample was assayed (corresponding to released products from 0.5 × 10⁸ platelets), immunoreactive TGF-β was detected at 0.8–5 ng (for 0.01–0.3 U of thrombin, respectively). Immunoreactive beta-thromboglobulin was detected at 20 to 100 ng after a 10- to 20-fold dilution of the supernatants into radioimmunoassay buffer. The basis for the apparent decrease in release of TGF-β with 1 U/ml thrombin is not clear at present.

100 ng), EGF (0.1–1,000 ng), and insulin (0.1–1,000 ng) were not detected at all by the immunoassay (data not shown). Others have shown that this antiserum also neutralizes the activity of TGF-β in bioassays (23).

Fig. 2 shows the use of the immunoassay to examine release of TGF-β from fresh gel-filtered human platelets after their degranulation with thrombin. The results show that thrombin treatment efficiently releases immunoreactive TGF-β (solid circles). Moreover, the concentrations of thrombin that release the growth factor agree well with those concentrations that release the alpha-granule marker (16, 17) beta-thromboglobulin (open circles). Quantitative analysis of the data in Fig. 2 indicates that a single human platelet releases 1,200–2,400 molecules of TGF-β. A similar value (2,500 molecules TGF-β per platelet) can be derived from the yield of the growth factor after purification (assuming 20% recovery of TGF-β [2, 5]), demonstrating that platelet degranulation releases a large part of intracellular TGF-β. These results support other studies showing that TGF-β stimulates protein synthesis and cell division in a rat wound healing system (46).

In addition to the physiological release of PDGFs during wound repair, these peptides may have an important role in atherogenesis; other investigators have suggested that exposure to PDGF, or PDGF-like molecules, may be an important component in the proliferation of aortic smooth muscle cells (35, 43). We have examined the effect of TGF-β on monolayer and soft agar growth of explant cultures from bovine aortic smooth muscle.

As expected from the work of others (36, 37), sparse monolayers of these cells (Fig. 3A) became quiescent when maintained in medium containing 5% plasma-derived serum (solid squares) and divided regularly when the medium contained 5% whole blood-derived serum (data not shown) or 5% plasma-derived serum, 0.1 nM in EGF (solid circles). The addition of TGF-β did not stimulate growth of the quiescent cells (open squares) and antagonized the effect of EGF in the growing cultures (open circles). Control studies (data not shown) demonstrated that TGF-β also inhibited the effect of 5% calf serum on smooth muscle cell growth in monolayer. Interestingly, these effects of TGF-β on vascular smooth muscle cells are strikingly similar to those observed for the monolayer growth of established NRK fibroblasts studied in parallel and under identical conditions (Fig. 3B and reference 29).

The similar effects of TGF-β on monolayer growth of vascular smooth muscle and NRK cells was confirmed in a mitogen assay measuring [³H]thymidine incorporation after incubation of growth factors for 22 h with confluent serum-starved cells (Table I). TGF-β did not stimulate incorporation
Table I. Effect of EGF, Serum, and TGF-β on [3H]-Thymidine Incorporation in Monolayer Cell Cultures

| Growth factor | Smooth muscle | NRK |
|---------------|---------------|-----|
|               | 22 h          | 42 h| 22 h | 42 h |
| None          | 4             | 4   | 50   | 40   |
| EGF (0.1 nM)  | ND            | 190 | ND   | 45   |
| Calf serum (5%)| 100           | 70  | 250  | 166  |
| TGF-β         | 1 pM          | 5   | 3    | 47   | 45   |
|               | 10 pM         | 4   | 2    | 33   | 29   |
|               | 100 pM        | 4   | 3    | 14   | 42   |

The table shows the ability of EGF, calf serum, and TGF-β to stimulate [3H]-thymidine incorporation into DNA of serum-starved confluent cells. The experiment was performed similarly to the procedure described (5). In brief, cells were seeded in 96-well plates in DME, 5% calf serum. The next day, the medium was changed to DME, 0.2% calf serum. Cells were incubated in this low-serum medium for 3 d, during which they became confluent and quiescent. Growth factors were incubated with the quiescent cells for 15 to 35 h before the addition of [3H]-thymidine. After a 7-h pulse with the radiolabeled (22 or 42 h total incubation with growth factor), the cells were fixed with 5% trichloroacetic acid, washed, extracted, and counted for radioactivity. EGF and TGF-β were tested at concentrations active in both monolayer (see Fig. 3) and soft agar (see Fig. 4) cultures. EGF was tested under standard mitogen assay conditions for mesenchymal cells (about 22-h prereplicative phase), whereas TGF-β was tested under both standard and prolonged (42 h prereplicative phase) assay conditions. Calf serum (5%) was considered the positive control under both conditions. ND, not determined.

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the addition of PDGF.

The experiments shown in Fig. 5 examined the PDGF requirement of the smooth muscle cells in detail. For the results shown in Fig. 5A, cells were grown in soft agar containing 10% plasma-derived serum (rather than whole blood-derived serum) in order to decrease the background levels of platelet-derived peptides. Under these conditions, overall colony formation (colonies of >40 µm diam, ~10 cells, solid squares) is induced (in the presence of saturating amounts of TGF-β and EGF) with concentrations of PDGF that are just slightly greater than those that stimulate cell growth in monolayer (Fig. 5, open circles). Efficient cell division in soft agar (formation of colonies greater than 60 microns in diameter, solid circles) requires about 10-fold more PDGF than does division in monolayer. Identical results were obtained with each of the three PDGF preparations described in Materials and Methods. Note that stimulation of smooth muscle cell division in monolayer (open circles) is induced by the same concentrations of PDGF that are active in other systems (4, 15, 38, 53), and that smooth muscle and NRK cells respond equivalently to PDGF in a monolayer assay measuring stimulation of DNA synthesis (Fig. 5B). Thus, the high concentration of PDGF required to induce colony formation of smooth muscle cells does not reflect an overall insensitivity to this growth factor.

Despite the fact that the PDGF preparations used in these studies were purified to homogeneity by distinct methodologies (32, 48) and exhaustively heated (100°C, 5–10 min) before use, we have considered the possibility that stimulation of smooth muscle cell growth in soft agar by high concentrations of PDGF might be due to heat-stable contaminants in the preparations rather than to PDGF itself. If small amounts of contaminating platelet factors were responsible for induction of colony formation in these preparations of purified PDGF, then crude PDGF should stimulate soft agar growth of smooth muscle cells more efficiently. We have used a heated platelet extract as a source of highly impure PDGF and show (Fig. 6) that colony formation of vascular smooth muscle cells (assayed in the presence of saturating concentrations of TGF-β and EGF, solid symbols) still requires more PDGF than does stimulation of DNA synthesis in monolayer (open circles). Moreover, the quantitative relationship between the amount of PDGF that stimulates DNA synthesis in monolayer, small colony formation, and large colony formation is identical for both pure and crude PDGF (compare Figs. 5 and 6; open circles, filled squares, and closed circles, respectively). We conclude that high concentrations of PDGF itself are required to synergize with TGF-β and EGF and stimulate colony formation of bovine aortic smooth muscle cells.

**Discussion**

We have developed a specific radioimmunoassay for TGF-β and shown that this growth factor is released from platelets degranulated with thrombin. Note that the concentrations of thrombin that release TGF-β also release the alpha-granule marker beta-thromboglobulin. These results suggest that TGF-β, like PDGF (16, 17), is contained within the platelet alpha-granule, but a direct analysis of TGF-β content in isolated alpha-granules remains an important matter for further study. Similarly, recent studies have shown that certain cell lines secrete TGF-β in a biologically inactive state (8, 20); the activity can be expressed in vitro by exposure of the growth factor to acid (pH 2). We are currently examining the biological state of TGF-β in platelets.

In addition to the generally accepted role of PDGFs in wound repair, Ross and Glomset have suggested that exposure of aortic smooth muscle cells to PDGF may stimulate proliferation of smooth muscle cells in atherogenesis (35). In vivo model systems examining the effect of platelet adhesion and degranulation on proliferation of aortic smooth muscle cells have yielded conflicting results (34, 45), but it is clear that aortic smooth muscle cells in culture do proliferate in response to PDGF. The data reported here show that platelet-derived TGF-β also acts on cultures of aortic smooth muscle cells. The effect of TGF-β on these cells depends on conditions of cell morphology and culture; TGF-β inhibits proliferation of subconfluent monolayers yet stimulates EGF-dependent growth of the cells when suspended in soft agar. These effects are qualitatively identical to those observed with NRK cells (reference 29 and this report) and thereby identify a primary cell that behaves similarly to established NRK cells with regard to TGF-β action. Of particular interest is the important role of EGF in colony formation of smooth muscle cells (Fig. 4) despite its rather weak effect on stimulating mitosis of these cells in monolayer (Table I). An appropriate interpretation of these results will require further study.

Two differences between the effects of TGF-β on growth of smooth muscle and NRK cells are that colony formation of NRK cells is more efficient and requires less PDGF than does that of smooth muscle cells. Both of these differences may well be due to the nature of the NRK cells (line 49F) used in these studies; the cells were specifically selected for efficient growth in soft agar (Joseph De Larco, personal communication). For an unselected, early passage cell, the growth of explant cultures of bovine smooth muscle cells in soft agar is reasonably efficient: at least half of the seeded cells will divide in soft agar in response to optimum concentrations of growth factors, and ~15% of the cells will divide many times to form relatively large colonies (of >60 µm diam). The high amounts of PDGF needed to stimulate anchorage-independent growth of smooth muscle cells, in fact, complements other studies (1, 19, 22, 50) showing that cells of mesenchymal origin can have quantitatively different growth factor requirements for colony formation. When suspended in soft agar containing 10% calf

**Figure 6.** Ability of a crude platelet extract to stimulate monolayer and soft agar growth of smooth muscle cells. The figure shows the concentrations of a heated platelet extract (see Materials and Methods) that stimulates 3H-deoxyuridine incorporation in monolayer (C) and colony formation in soft agar (●, ●). See the legend to Fig. 5 for details of the soft agar and monolayer assays.
serum, colony formation of mesenchymal cells may require the addition of EGF (Rat-1 cells); TGF-β (AKR-2B cells); EGF and TGF-β (NRK cells); EGF, TGF-β, and insulin-like growth factor (BALB/c 3T3 cells); or EGF, TGF-β, and PDGF (smooth muscle cells; this report).

Studies by Seifert et al. (40) have shown that explant cultures of smooth muscle cells from pup, but not adult, rat aorta produce mitogenic concentrations of a PDGF-like peptide. Nilsson et al. (25) have reported that adult rat smooth muscle cells, isolated by collagenase digestion of aortic media, secrete a PDGF-like peptide upon their "modulation" to a mitotic phenotype. In contrast to these reports, the explant cultures of fetal bovine aortic smooth muscle cells used throughout this study did not appear to secrete large amounts of a PDGF-like peptide as determined by stimulation of mitosis with exogenous PDGF (Fig. 5) and induction of quiescence in subconfluent cultures with plasma-derived serum (Fig. 3A).

The results presented here raise the possibility that growth of vascular smooth muscle cells in response to platelet degranulation may not be due to PDGF alone but rather to an interaction of platelet-derived peptides that has the intrinsic ability to limit as well as stimulate mitosis. Interestingly, several lines of evidence suggest that the high concentrations of PDGF required to elicit the stimulatory effect of TGF-β in smooth muscle cell culture may be present during atherogenesis. First, as suggested by the response-to-injury hypothesis (35), platelet release may act as a point source for growth factors; the concentrations of TGF-β, PDGF, and the EGF-like peptide (4) may be high for aortic smooth muscle cells near sites of platelet release. Second, endothelial cells in culture produce PDGF-like peptides (10) and may supply medial smooth muscle cells with PDGF in vivo. Third, activated macrophages release PDGF in vitro, and these cells are an important component of the atherosclerotic aorta (43).

Fourth, it is possible to prepare cultures of aortic smooth muscle cells that themselves produce a PDGF-like peptide (25, 51). Taken together with previous studies (11, 35, 36, 46, 52), the results presented here extend the interface of PDGF and TGF-β biology and suggest that both platelet peptides have potential roles in wound repair, transformation, and atherogenesis.

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References

1. Anzano, M. A., A. Roberts, C. Meyers, A. Komoriya, L. Lamb, J. Smith, and M. Sporn. 1982. Synergistic interaction of two classes of transforming growth factors from murine sarcoma cells. Cancer Res. 42:4776–4778.

2. Assoian, R. K. Purification of type beta transforming growth factor from human platelets. Methods Enzymol. In press.

3. Assoian, R. K. 1985. Biphasic effects of type beta transforming growth factor on epidermal growth factor receptors in NRK fibroblasts: functional consequences for epidermal growth factor-stimulated mitosis. J. Biol. Chem. 260:9613–9617.

4. Assoian, R. K., G. R. Grotendorst, D. M. Miller, and M. B. Sporn. 1984. Cellular transformation by coordinated action of three peptide growth factors from human platelets. Nature (Lond.). 309:804–806.

5. Assoian, R. K., A. Komoriya, C. A. Meyers, D. M. Miller, and M. B. Sporn. 1983. Transforming growth factor-beta in human platelets: identification of a major storage site, purification and characterization. J. Biol. Chem. 258:175–1760.

6. Bowen-Pope, D. F., and R. Ross. 1983. Is epidermal growth factor present in human blood? Interference with the radioassay for epidermal growth factor. Biochem. Biophys. Res. Commun. 114:1036–1041.

7. Bowen-Pope, D. F., A. Vogel, and R. Ross. 1984. Production of platelet-derived growth factor-like monomers and reduced expression of platelet-derived growth factor receptors accompany transformation by a wide spectrum of agents. Proc. Natl. Acad. Sci. USA. 81:2396–2400.

8. Brancu, E. L., R. F. Tucker, and H. L. Moses. 1984. Activation of type beta transforming growth factor from conditioned media. J. Biol. Chem. 269 (Pt. 2):273a. (Abstr.)

9. Childs, C. B., J. A. Proper, R. F. Tucker, and H. L. Moses. 1982. Serum contains a platelet-derived transforming growth factor. Proc. Natl. Acad. Sci. USA. 79:5312–5316.

10. DiCorleto, P. E., and D. F. Bowen-Pope. 1983. Cultured endothelial cells produce a platelet-derived growth factor-like protein. Proc. Natl. Acad. Sci. USA 80:1919–1923.

11. Doeldlilte, R. F., M. W. Hunkapiller, L. E. Hood, S. G. Devare, K. C. Robbina, S. A. Aaronson, and H. N. Antroianides. 1983. Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science (Wash. DC). 221:275–277.

12. Frolik, C. A., L. M. Wakefield, D. M. Smith, and M. B. Sporn. 1984. Characterization of a membrane receptor for transforming growth factor-beta in normal rat kidney fibroblasts. J. Biol. Chem. 259:10995–11000.

13. Grotendorst, G. R., H. E. J. Seppa, H. K. Kleinman, and G. R. Martin. 1981. Attachment of smooth muscle cells to collagen towards platelet-derived growth factor. Proc. Natl. Acad. Sci. USA. 78:3669–3673.

14. Heldin, C.-H., B. Westmark, and Á. Wasteson. 1979. Purification of platelet-derived growth factor and partial characterization. Proc. Natl. Acad. Sci. USA. 76:3722–3726.

15. Heldin, C.-H., B. Westmark, and A. Wasteson. 1981. Platelet-derived growth factor: isolation by a large-scale procedure and analysis of subunit composition. Biochem. J. 193:907–913.

16. Kaplan, D. R., F. C. Chao, C. D. Stiles, H. N. Antoianides, and C. D. Scher. 1979. Platelet alpha granules contain a growth factor for fibroblasts. Blood. 53:1043–1052.

17. Kaplan, K. L., M. J. Brockman, A. Chernoff, G. R. Lesznik, and M. Drillings. 1979. Platelet alpha granule proteins: studies on release and subcellular localization. Blood. 53:604–618.

18. Kaplan, K. L., H. L. Nossel, M. Drillings, and G. Lesznik. 1978. Radioimmunoassay of platelet factor 4 and beta-thromboglobulin: development and application to studies of platelet release in relation to fibrinopeptide A generation. Br. J. Haematol. 39:129–144.

19. Kaplan, P. L., and B. Orzanne. 1983. Cellular responsiveness to growth factors correlates with a cell's ability to express the transformed phenotype. Cell. 33:931–938.

20. Kryceve-Martinerie, C., D. A. Lawrence, J. Crochet, P. Jullien, and P. Vigier. 1985. Further study of beta-TGFs released by virally transformed and non-transformed cells. Int. J. Cancer. 35:553–558.

21. Massagué, J. 1984. Type beta transforming growth factor from feline sarcoma virus-transformed rat cells. J. Biol. Chem. 259:9756–9761.

22. Massagué, J., B. Kelly, and C. Mottola. 1985. Stimulation by insulin-like growth factors is required for cellular transformation by type beta transforming growth factor. J. Biol. Chem. 260:4551–4554.

23. Masui, T., L. M. Wakefield, J. F. Lechner, M. A. LaVeck, M. B. Sporn, and C. Harris. Type beta transforming growth factor is the primary differentiation-inducing serum factor for normal human bronchial epithelial cells. Proc. Natl. Acad. Sci. USA. In press.

24. Moses, H. L., R. F. Tucker, E. B. Leof, R. J. Coffey, J. Halpern, and G. D. Stappe. 1985. Type beta transforming growth factor is a growth stimulator and a growth inhibitor. Cancer Cells (Cold Spring Harbor). 3:65–71.

25. Nilsson, J., M. Sjolund, L. Palmberg, J. Thyberg, and C.-H. Heldin. 1985. Arterial smooth muscle cells in primary culture produce a platelet-derived growth factor-like protein. Proc. Natl. Acad. Sci. USA. 82:4818–4822.

26. Oka, Y., and D. N. Orth. 1983. Human plasma epidermal growth factor/ beta-urogastrone is associated with blood platelets. J. Clin. Invest. 72:249–259.

27. Roberts, A. B., M. A. Anzano, C. A. Frolik, and M. B. Sporn. 1982. Transforming growth factors: characterization of two classes of factors from neoplastic and non-neoplastic tissues. Cold Spring Harbor Conf. Cell Prolif. 9:319–323.

28. Roberts, A. B., M. A. Anzano, L. C. Lamb, J. M. Smith, and M. B. Sporn. 1981. New class of transforming growth factor potentiated by epidermal growth factor: isolation from non-tumourous tissues. Proc. Natl. Acad. Sci. USA. 78:5339–5343.

29. Roberts, A. B., M. A. Anzano, L. M. Wakefield, N. S. Roche, D. F. Stern, and M. B. Sporn. 1985. Type beta transforming growth factor: a bifunctional regulator of cellular growth. Proc. Natl. Acad. Sci. USA. 82:119–123.

30. Roberts, A. B., C. A. Frolik, M. A. Anzano, and M. B. Sporn. 1983. Transforming growth factors from neoplastic and non-neoplastic tissues. Fed.
31. Roberts, A. B., L. C. Lamb, D. L. Newton, M. B. Sporn, J. E. De Larco, and G. J. Todaro. 1980. Transforming growth factors: isolation of polypeptides from virally and chemically transformed cells by acid/ethanol extraction. Proc. Natl. Acad. Sci. USA. 77:3494-3498.

32. Raines, E. W., and R. Ross. 1982. Platelet-derived growth factor: high yield purification and evidence for multiple forms. J. Biol. Chem. 257:5154-5160.

33. Reidy, M. A., and S. M. Schwartz. 1981. Endothelial regeneration: time course of intimal changes after small defined injury to rat aortic endothelium. Lab. Invest. 44:301-308.

34. Ross, R. 1971. The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibers. J. Cell Biol. 50:172-186.

35. Ross, R., and J. A. Glomset. 1976. The pathogenesis of atherosclerosis. N. Engl. J. Med. 295:369-377.

36. Ross, R., and A. Vogel. 1978. The platelet-derived growth factor. Cell. 14:203-210.

37. Ross, R., J. Glomset, B. Kariya, and L. Harker. 1974. A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc. Natl. Acad. Sci. USA. 71:1207-1210.

38. Rozengurt, E., P. Stroobant, M. D. Waterfield, T. F. Deuel, and M. Keehan. 1983. Platelet-derived growth factor elicits cyclic AMP accumulation in Swiss 3T3 cells: role of prostaglandin production. Cell. 34:265-272.

39. Savage, C. R., and S. Cohen. 1972. Epidermal growth factor and a new derivative, A(I). Biol. Chem. 247:7609-7611.

40. Seifert, R. A., S. M. Schwartz, and D. F. Bowen-Pope. 1984. Developmentally regulated production of platelet-derived growth factor-like molecules. J. Biol. Chem. 247:7609-7611.

41. Senior, R. M., G. L. Griffin, J. S. Huang, D. A. Waltz, and T. F. Deuel. 1983. Chemotactic activity of platelet alpha granule proteins for fibroblasts. J. Cell Biol. 96:382-385.

42. Seppa, H., G. Grotendorst, S. Seppa, E. Schiffmann, and G. R. Martin. 1982. Platelet-derived growth factor is chemotactic for fibroblasts. J. Cell Biol. 92:584-588.

43. Shimokado, K., E. W. Raines, D. K. Maches, T. B. Barrett, E. P. Benditt, and R. Ross. 1985. A significant part of macrophage-derived growth factor consists of at least two form of PDGF. Cell. 43:277-286.

44. Shipley, G. D., R. F. Tucker, and H. L. Moses. 1985. Type beta transforming growth factor/growth inhibitor stimulates entry of monolayer cultures of AKB-2B cells into S phase after a prolonged prereplicative interval. Proc. Natl. Acad. Sci. USA. 82:4147-4151.

45. Spaet, T. H., M. B. Stemerman, F. J. Veith, and I. Lejnieks. 1975. Intimal injury and regrowth in the rabbit aorta: medial smooth muscle cells as a source of neointima. Circ. Res. 36:58-70.

46. Sporn, M. B., A. B. Roberts, J. H. Shull, J. M. Smith, J. M. Ward, and J. Sodek. 1983. Polypeptide transforming growth factor isolated from bovine sources and used for wound healing in vivo. Science (Wash. DC). 219:1329-1331.

47. Stiles, C. D. 1983. The molecular biology of platelet-derived growth factor. Cell. 33:653-655.

48. Stroobant, P., and M. D. Waterfield. 1984. Purification and properties of porcine platelet-derived growth factor. EMBO (Eur. Mol. Biol. Organ.) 12:2963-2967.

49. Tucker, R. F., G. D. Shipley, H. L. Moses, and R. W. Holley. 1984. Growth inhibitor from BSC-1 cells closely related to platelet type beta transforming growth factor. Science (Wash. DC). 226:705-707.

50. Tucker, R. F., M. E. Volkenant, E. L. Branum, and H. L. Moses. 1983. Comparison of intra- and extracellular transforming growth factors from non-transformed and chemically transformed mouse embryo cells. Cancer Res. 43:1581-1586.

51. Walker, L. N., D. F. Bowen-Pope, and M. A. Reidy. 1984. Secretion of platelet-derived growth factor (PDGF)-like activity by arterial smooth muscle is induced as a response to injury. J. Cell Biol. 99 (4, Pt. 2):416a. (Abstr.)

52. Waterfield, M. D., G. T. Scrace, N. Whittle, P. Stroobant, A. Johnson, A. Wasteson, B. Westmark, C.-H. Heldin, J. S. Huang, and T. F. Deuel. 1983. Platelet-derived growth factor is structurally related to the putative transforming protein p28 of simian sarcoma virus. Nature (Lond.). 304:35-39.

53. Witte, L. D., J. A. Cornicelli, R. W. Miller, and D. S. Goodman. 1982. Effects of platelet-derived and endothelial cell-derived growth factors on the low density lipoprotein receptor pathway in cultured human fibroblasts. J. Biol. Chem. 257:5392-5401.