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Production of platelet-derived growth factor-like molecules and reduced expression of platelet-derived growth factor receptors accompany transformation by a wide spectrum of agents

(radioreceptor assay/autocrine/oncogenes)

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ABSTRACT A series of nontransformed human and murine cells and derivative cell lines transformed by methylcholanthrene; by simian virus 40, Kirsten and Moloney murine sarcoma viruses, simian sarcoma virus, and adenovirus; and by a "spontaneous" event in culture were examined for the expression of receptors for the platelet-derived growth factor (PDGF) and for production of substances able to compete with ^{125}I-labeled PDGF for binding to the cell-surface PDGF receptor. In each case, transformation resulted in a 50–100% decrease in available PDGF receptors. All transformed cells except the methylcholanthrene-transformed mouse cells produce a PDGF competitor into the conditioned medium. Levels of PDGF competitor in conditioned medium at the end of a 48-hr collection were as high as 2 ng/ml—high enough to be measured by radioreceptor assay diluted 1:30 and to maximally stimulate [^{3}H]thymidine incorporation by human fibroblasts. The PDGF competitor activity detected in a radioreceptor assay does not reflect irreversible (e.g., proteolytic) damage to the receptor of test cells since its effects are reversed by acetic acid dissociation. Antiserum against human PDGF neutralizes 20–80% of the PDGF competitor found in conditioned medium from different transformed human cells and 100% of the activity from normal human endothelial cells. The possibility that induction of expression of the cellular *PDGF* gene may be involved in the mechanism of transformation of PDGF-responsive mesenchymal cells is discussed.

Oncogenically transformed animal cells often differ from their nontransformed counterparts in two properties that are relatively easy to demonstrate in culture: they often require lower concentrations of serum for optimal growth (1–6) and they often grow when suspended in soft agar (7). One possible explanation for these differences, termed the "autocrine hypothesis" by Sporn and Todaro (8), is that transformed cells produce a factor(s) that stimulates their own growth in monolayer or in suspension culture (9). Such factors, termed "transforming growth factors" (TGFs) (10) have been strongly implicated in mediating transformation by Kirsten and Moloney murine sarcoma viruses (8, 11). TGFs secreted by these cells can compete with epidermal growth factor (EGF) for binding to cell-surface EGF receptors (11, 12). However, not all transformed cells secrete an EGF-like TGF (13). Recently, production of platelet-derived growth factor (PDGF)-like molecules by an osteosarcoma cell line (14), by a cloned glioma cell line (15), and by simian virus 40 (SV40)- and simian sarcoma virus (SSV)-transformed 3T3 cells (16, 17) has been reported. In addition, homology between $p28^{sis}$, the oncogene product of SSV, and PDGF has been demonstrated (18, 19). Because of the possibility that autocrine secretion of PDGF or PDGF-like molecules could be important in decreasing the mitogen requirement of monolayer cultures of transformed cells and in permitting growth in soft agar, we have investigated the expression of PDGF receptors and the production of substances competing for PDGF binding (PDGF-c) using a series of nontransformed cell lines and derivative cell lines transformed by a wide spectrum of transforming agents, as well as two cell lines from naturally occurring human tumors.

MATERIALS AND METHODS

Procedures and Materials. Binding and other procedures are described in the figure legends. Pure PDGF and ^{125}I-labeled PDGF (^{125}I-PDGF) were prepared as described (20–22). Monospecific antiserum to pure PDGF (E. Raines, personal communication) was prepared in a goat. IgG was prepared by sodium sulfate precipitation and DEAE Sephacel chromotography.

Cells. Adenovirus-transformed rat embryo cells were obtained from J. Lewis (University of Washington). Mouse Swiss/3T3 and their SV40 transformants (Swiss/3T3 SV 101) were obtained from R. Pollack (Columbia University). Swiss/3T3 D1 is a clone from the above 3T3 selected in our laboratory for quiescence in PDGF-deficient medium and for responsiveness to added PDGF. Swiss/3T3 TRD1 is a "spontaneously" transformed variant of Swiss/3T3 D1 found as a colony of rounded actively growing cells in a culture of quiescent cells and has been cloned and recloned by dilute plating. Human bone marrow fibroblasts (HBM) and their SV40 transformants (HBM 5A) were obtained from H. Ozer (Hunter College). Rat fibroblastoid cells (NRK), their simian sarcoma virus transformants (NRK-SSV), and mouse NIH 3T3 cells (NIH) and their SSV transformants (NIH-SSV) were obtained from S. Aaronson (National Cancer Institute) and T. Hunter (Salk Institute). The following cells were obtained from American Type Culture: mouse BALB/c 3T3 clone A31 (BALB/3T3 A31), and their SV40 (BALB/3T3 SV-T2) and Moloney murine sarcoma virus (BALB/3T3 M-MSV) transformants; mouse C3H/10 T1/2 clone 8 (C3H/10 T1/2 C18) and their methylcholanthrene transformants (C3H/MCA C1 15); human fetal lung fibroblasts (WI-38) and their SV40 transformants (WI-38 VA 13); and human transitional cell bladder carcinoma (T24). The human hepatoma line Hep G2 was obtained from D. Aden and B. Knowles (Wistar Institute).

RESULTS

The Number of PDGF Receptors Is Decreased in Transformed Cells. Figs. 1 and 2 show that ^{125}I-PDGF binding to cells transformed by many different agents is greatly de-

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Abbreviations: TGF, transforming growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; PDGF-c, substance competing for binding to the PDGF receptor; ^{125}I-PDGF, ^{125}I-labeled PDGF; ^{125}I-EGF, ^{125}I-labeled EGF; SV40, simian virus 40; SSV, simian sarcoma virus.

creased compared with ^{125}I-PDGF binding to the nontransformed parental cells. The transforming agents included methylcholanthrene (Fig. 1*A*), SV40 (Fig. 1 *B*, *D*, *E*, and *F*), adenovirus (data not shown), Kirsten and Moloney murine sarcoma viruses (Figs. 1*B* and 2*A*), SSV (Fig. 2), and a "spontaneous" transformation event (Fig. 1*C*). For these comparisons an attempt was made to assay ^{125}I-PDGF binding to parental and transformed cell lines under comparable conditions of cell density and culture history, including a two day incubation period in medium containing 2% PDGF-deficient calf serum (23) to allow maximal expression of receptors. Rat NRK and mouse NIH/3T3 cells transformed by SSV or by Kirsten murine sarcoma virus were poorly attached to the cell culture surface and often detached when ^{125}I-PDGF binding was carried out at 4°C as described for Fig. 1. For this reason binding to these lines, and to the corresponding parental lines, was measured at 37°C for 45 min (Fig. 2). In cases where we have compared ^{125}I-PDGF binding to diploid human fibroblasts or mouse 3T3 cells at 4°C and at 37°C we have found that the apparent number of receptors detected per cell is essentially identical but that the apparent K_d is 2- to 3-fold higher under the conditions used for 37°C binding (data not shown). We observed no general effect of transformation on the affinity of the receptors for ^{125}I-PDGF as determined by the concentration of ^{125}I-PDGF giving half-maximal ^{125}I-PDGF binding (Figs. 1 and 2). We have found (21, 22) that the apparent affinity of the PDGF receptor can vary significantly depending on the conditions of assay and of the cells (especially on the receptor densities) so that the significance of the small differences in apparent K_d observed between parental and transformants and between different parental lines is not clear at this time.

Production of Binding Competitor(s) by Transformed Cells. To determine whether the decrease in binding of exogenous ^{125}I-PDGF would reflect the production of substances (PDGF-c) able to occupy the PDGF receptor, we determined the ability of conditioned medium to block subsequent binding of ^{125}I-PDGF to monolayers of diploid human fibroblasts. Fig. 3 shows that media conditioned by different test cells vary widely in their content of PDGF-c. To correct for differences in the number of cells present during preparation of conditioned medium the concentration of medium is expressed as units/ml, where 1 unit is the production by 10^6 cells over a period of 48 hr. All of the transformed lines tested except the methylcholanthrene-transformed mouse line (C3H/MCA Cl 15) produce detectable PDGF-c. High producers, such as T24 bladder carcinoma, secrete activity detectable at a 1:100 dilution of conditioned medium, while low producers such as Molony murine sarcoma virus-transformed BALB/c 3T3 cells produce levels that can be reliably assayed only after a 10-fold concentration by ultrafiltration. Of the nontransformed cells tested only the conditioned medium from human umbilical vein endothelial cells contained high levels of PDGF-c (see also ref. 24), while no PDGF-c was detected even after a 10-fold concentration in medium conditioned by adult diploid human foreskin fibroblasts, WI-38 diploid human fetal lung fibroblasts, diploid human bone marrow fibroblasts, chicken embryo fibroblasts, rat NRK cells, and the mouse cell lines C3H/10T$_{1/2}$ Cl 18, BALB/c 3T3, NIH 3T3, and Swiss 3T3.

Fig. 3 also shows the effect of pure PDGF standards (ng/ml), on subsequent ^{125}I-PDGF binding. The competition

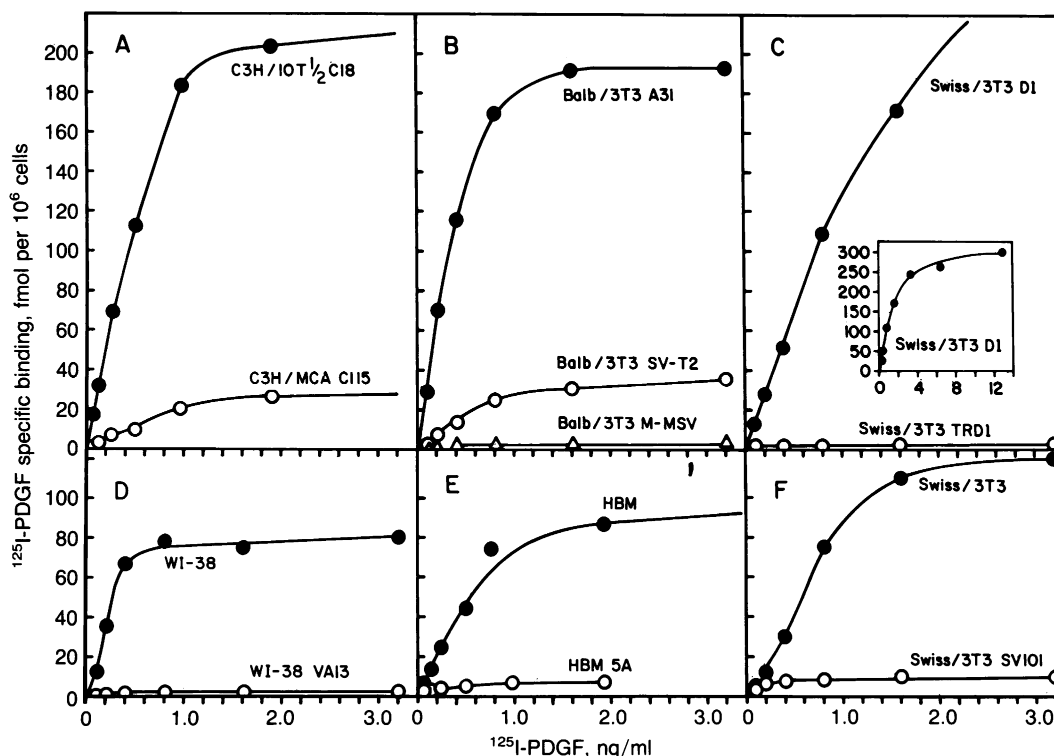

FIG. 1. Specific ^{125}I-PDGF binding to matched parental and transformed cell lines. Cultures were plated in 2.4-cm^2 wells in Dulbecco's modified Eagle's medium containing 5% calf serum. When slightly subconfluent, the plating medium was replaced with Dulbecco's modified Eagle's medium containing 2% PDGF-deficient calf serum and incubated for an additional 2 days. Specific binding was measured by incubation for 3 hr at 4°C with gentle shaking in binding medium (1.0 ml per well) containing the indicated concentration of ^{125}I-PDGF (about 20,000 cpm/ng) with or without partially purified PDGF (20 μg/ml; the equivalent of ≈400 ng of pure PDGF per ml) to determine nonspecific binding (all values plotted have been corrected for nonspecific binding) or in binding medium without ^{125}I-PDGF to determine the number of cells per well at the conclusion of the assay. Binding was terminated by rinsing 4 times with binding rinse and cell-bound ^{125}I-PDGF was determined by extracting each culture with 1 ml of 0.1% Triton X-100 with 0.1% bovine serum albumin and gamma counting the entire extract. All measurements were made using triplicate wells and are plotted as the mean. Nonspecific binding was relatively independent of cell type and averaged ≈0.7% of input cpm.

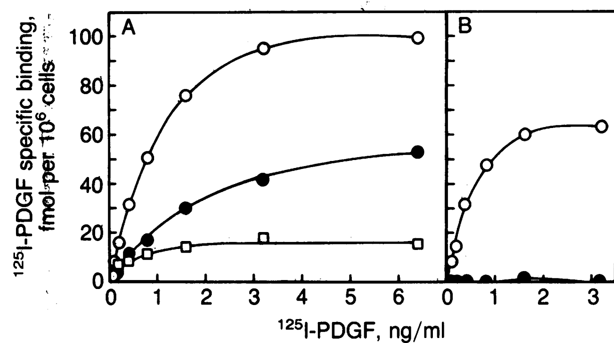

FIG. 2. Specific ^{125}I-PDGF binding to matched parental and transformed cell lines. Binding and analysis were carried out as described for Fig. 1 except that binding was at 37°C for 45 min without shaking and all rinse solutions were used at 37°C to minimize cell detachment. (*A*) ○, NIH cells; ●, NIH-K cells; □, NIH-SSV cells. (*B*) ○, NRK cells; ●, NRK-SSV cells.

curve is approximately parallel to the curves generated by the different PDGF-c preparations, suggesting that human PDGF and PDGF-c interact with the PDGF receptor in a comparable fashion.

PDGF-c Is Not a Receptor-Degrading Protease. The data presented so far could be explained by the production or activation of a protease that damages the PDGF receptor,

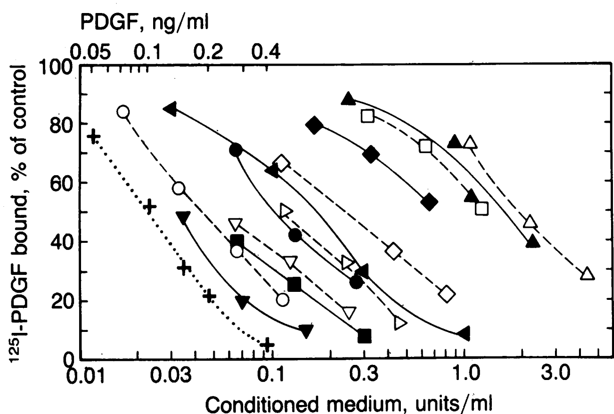

FIG. 3. Competition by conditioned media for ^{125}I-PDGF binding to human fibroblasts. Conditioned media were prepared by incubating confluent cultures in 150-cm^2 dishes in 20 ml of culture medium containing 0.5% PDGF-deficient calf serum. This medium was discarded and replaced with fresh medium and reincubated for 48 hr. This conditioned medium was collected, debris was removed by centrifugation, and medium was concentrated 10-fold by ultrafiltration using an Amicon PM-10 membrane. The number of attached cells present at the end of the collection period was determined using an electronic particle counter. Conditioned media were assayed for PDGF-c content as described (22). Standard concentrations of pure PDGF standards (+) and serial dilutions of conditioned medium in 1 ml of binding medium, were incubated on 2.4-cm^2 cultures of subconfluent human fibroblast cells for 3 hr at 4°C with gentle shaking. The test media were then aspirated, and the cultures were rinsed once with cold binding rinse and incubated for 1 hr at 4°C with gentle shaking and 1 ml of ^{125}I-PDGF per well (0.5 ng/ml). Binding was terminated by rising 4 times with binding rinse and cell-bound ^{125}I-PDGF was determined by gamma counting of Triton extracts. Nonspecific binding (6–15% of total binding) was determined as ^{125}I-PDGF bound after preincubation with partially purified PDGF (20 μg/ml; the equivalent of ≈400 ng of pure PDGF per ml) and has been subtracted. The concentration of conditioned medium is expressed as units/ml. One unit of conditioned medium represents production by 10^6 cells during a 48-hr collection period. Conditioned media tested were as follows: ▼, primary human umbilical vein endothelial cells; ○, T-24; ■, Hep G2; ▽, TRD1; ●, WI-38 VA13; ▷, 5A; ◀, adenovirus-transformed rat embryo cells; ◇, SV101; ◆, NIH-K; □, NIH-SSV; ▲, BALB/3T3 M-MSV; △, NRK-SSV.

Table 1. Use of acetic acid to reveal occupied PDGF receptors

Test substance (amount per ml)	^{125}I-PDGF specific binding, cpm per 10^6 cells	
	Binding rinse	Dissociation rinse
0	8600	8100
PDGF (2 ng)	300	7300
T24 CM (0.1 ml)	1250	7150
WI-38 VA13 CM (0.5 ml)	1625	7700
NIH-SSV CM (0.5 ml)	750	7900

Cultures (2.4 cm^2) of diploid human fibroblasts were incubated at 4°C for 3 hr in conditioned medium (CM) containing the test solutions indicated. The cultures were then rinsed twice with cold binding rinse and incubated for 5 min in either binding rinse or dissociation rinse (20 mM acetic acid/150 mM NaCl/0.25% bovine serum albumin, pH 3.8). The cultures were then incubated for 1 hr at 4°C with ^{125}I-PDGF at 0.5 ng/ml. Nonspecific binding (8% of total binding) and cell number were determined using parallel cultures and were used to calculate specific ^{125}I-PDGF binding per 10^6 cells (mean of triplicate determinations).

thereby interfering with PDGF binding (e.g., see ref. 25). To investigate this possibility, cultures of human fibroblasts were incubated with conditioned medium at 4°C, rinsed with acetic acid, and reincubated with ^{125}I-PDGF. Acetic acid treatment has been shown (26) to dissociate receptor-bound PDGF without damaging the receptor. Table 1 shows that 88–100% of the inhibition produced by the conditioned medium is reversed by acetic acid treatment. Therefore, the conditioned medium contains material that interferes with PDGF binding without damaging the PDGF receptor.

Effect of Conditioned Medium on Expression of PDGF Receptors. In an attempt to reveal receptors on transformed cells that might be blocked by endogenous PDGF-c, we treated cultures of SV40-transformed human fibroblasts and

Table 2. Effect of conditioned medium on expression of PDGF receptors

Cell type	Test substance (amount per ml)	^{125}I-PDGF specific binding, cpm per 10^6 cells: Binding rinse	Dissociation rinse
WI-38 VA13*	0	450	390
Swiss/3T3 TRD1*	0	300	320
Human fibroblast†	0	5,970	4,390
	PDGF (4 ng)	250	360
	T24 CM (1 ml)	642	572
	WI-38 VA13 CM (1 ml)	970	750
	NIH-SSV CM (1 ml)	86	−100
Swiss/3T3†	0	17,100	17,400
	PDGF (4 ng)	510	1,830
	NIH-SSV CM (1 ml)	6,900	6,500
	Swiss/3T3 TRD1 CM (1 ml)	4,900	4,400

*Cultures (2.4 cm^2) of the cell types indicated in the first column were rinsed twice with cold binding rinse and incubated for 5 min in either binding rinse or dissociation rinse (20 mM acetic acid/150 mM NaCl/0.25% bovine serum albumin, pH 3.8). The cultures were then incubated for 1 hr at 4°C with ^{125}I-PDGF at 0.5 ng/ml. Nonspecific binding (10% of total binding to human fibroblasts and 3% of total binding to 3T3 cells) and cell number were determined using parallel cultures and were used to calculate specific ^{125}I-PDGF binding per 10^6 cells (mean of triplicate determinations).
†Cultures (2.4 cm^2) of the nontransformed cell types indicated in the first column were incubated for 18 hr at 37°C in conditioned medium (CM) containing the test solutions indicated in the second column. The cultures were then rinsed with either binding rinse or dissociation rinse, and ^{125}I-PDGF binding was determined.

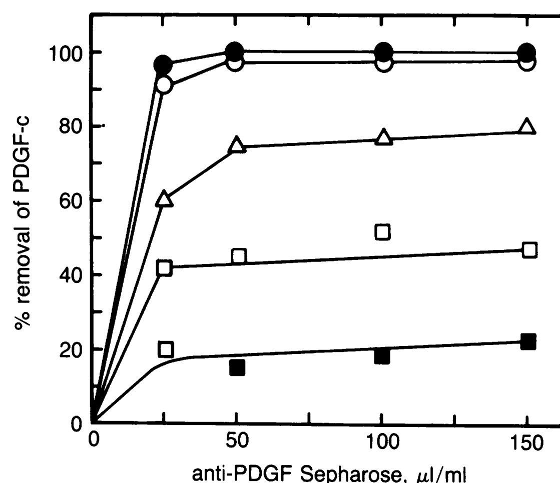

FIG. 4. Removal of PDGF binding competitor activity by anti-PDGF Sepharose. Binding medium containing pure PDGF or conditioned medium at concentrations chosen to give 60–80% inhibition of subsequent specific ^{125}I-PDGF binding was incubated overnight with the indicated amount of a suspension of 1 vol of packed goat anti-PDGF IgG Sepharose to 3 vol of binding medium. The anti-PDGF Sepharose was removed by centrifugation and the supernatant was incubated on test culture of human fibroblast cells followed by incubation with ^{125}I-PDGF and determination of specifically bound ^{125}I-PDGF as described for Fig. 3. A series of concentrations of pure PDGF were assayed in parallel and used to determine the PDGF-c content of each preparation. Results are expressed as % removal of PDGF-c calculated as follows: (PDGF equivalents determined in the absence of antibody minus PDGF equivalents determined after antibody treatment) divided by PDGF equivalents determined in the absence of antibody. The results are plotted as the mean of two experiments, each using triplicate cultures per determination. Standard deviations were usually <7% and always <15%. Test media used were as follows: ●, pure PDGF; ○, human endothelial cell conditioned medium; △, Hep G2 conditioned medium; □, T24 conditioned medium; ■, WI-38 VA 13 conditioned medium.

spontaneously transformed 3T3 cells with acetic acid and then measured ^{125}I-PDGF binding. No significant change in subsequent ^{125}I-PDGF binding was observed (Table 2). This observation is compatible with the possibility that the transformed cells have decreased numbers of available receptors because of autoproduction of PDGF-c and consequent down-regulation. When nontransformed cultures of Swiss 3T3 cells or diploid human fibroblasts were cultured for 24 hr at 37°C in the presence of high concentrations of PDGF or of medium conditioned by transformed cells, their ability to bind exogenous ^{125}I-PDGF is greatly decreased and is not significantly restored by acetic acid treatment (Table 2). Long-term incubation of normal cells in conditioned medium from transformed derivatives thus reproduces the low-receptor phenotype of the transformed cells.

Table 3. Neutralization of mitogenic activity by antibody to PDGF

Test substance	fold stimulation	% neutralization
FGF (40 μg)	13	0
EGF (1 ng)	16	0
PDGF (0.2 ng)	6.7	103
Human endothelial cell CM (0.2 ml)	6.1	25
Hep G2 CM (0.35 ml)	7.5	45
T24 CM (0.35 ml)	6.3	68
VA13 CM (0.1 ml)	4.4	0

Cultures of Swiss 3T3 D1 cells were plated in 2.4-cm^2 wells in Dulbecco's modified Eagle's medium containing 5% calf serum. When confluent, the medium was replaced with 1 ml of Dulbecco's modified Eagle's medium containing 2% PDGF-deficient calf serum. Two days later, test mitogens were added. [^{3}H]Thymidine incorporation was measured 20–22 hr later as described (21). Results are expressed as the ratio of [^{3}H]thymidine incorporation by cultures incubated with test substance to incorporation by untreated cultures. Parallel cultures were incubated with the same test substances to which anti-PDGF IgG had been added to give a final concentration of 100 μg/ml during incubation on the test cultures. Values are calculated from the mean of determinations using triplicate cultures (SD ≤ 10%). CM, conditioned medium.

Antigenic and Functional Similarity Between Human PDGF and PDGF-c. To determine whether PDGF-c produced by transformed cells shared antigenic determinants with human PDGF, we incubated PDGF standards, or preparations of conditioned medium, with increasing concentrations of anti-PDGF IgG conjugated to Sepharose, and then removed the antibody-bound substance by centrifugation. The percentage of the initial PDGF-c removed by the antibody is shown in Fig. 4. Anti-PDGF Sepharose removed essentially 100% of the competitor activity in PDGF standards or in medium conditioned by human endothelial cells but only 80%, 50%, and 20% of the activity present in medium conditioned by Hep G2 hepatoma, T24 carcinoma, or SV40-transformed human fibroblasts, respectively.

The antigenically crossreactive forms of PDGF-c are mitogenic for test 3T3 cells. Table 3 shows that antibodies against PDGF remove significant amounts of mitogenic activity from medium conditioned by transformed cells. The mitogenic activity that is not neutralized may be due to the noncrossreactive forms of PDGF-c (Fig. 4) or to unrelated mitogens. In the case of medium conditioned by human endothelial cells, all PDGF-c is removed by anti-PDGF (ref. 24; Fig. 4) and the remaining mitogenic activity is due to other classes of mitogens (24).

DISCUSSION

Production of PDGF-Like Growth Factors by Transformed Cells. We have shown that production of a substance (PDGF-c) that competes with ^{125}I-PDGF for binding to the PDGF receptor is a common result of transformation by a wide variety of transforming agents, including the retroviruses Kirsten murine sarcoma virus (NIH-K), Moloney murine sarcoma virus (BALB/3T3 M-MSV), and SSV (NIH-SSV, NRK-SSV); the DNA viruses SV40 (WI-38 VA13, HBM 5A, Swiss/3T3 SV101) and adenovirus; a "spontaneous" transformation event (TRD1); and unknown agents for the tumor cell lines T24 (a bladder carcinoma expressing a modified *ras/bas* gene; 27) and Hep G2 (a human hepatoma). The PDGF-competing activity of PDGF-c does not result from damage to the receptors, because its effects at 4°C are reversed by treating the test cultures with acetic acid solutions under conditions that dissociate prebound PDGF (Table 1; refs. 22 and 26). The production of PDGF-c by cells transformed by a broad spectrum of viruses contrasts with the data on the production of EGF-like molecules by transformed cells, where production is restricted to a small subset of transforming agents (13), the best characterized of which are the Kirsten and Moloney murine sarcoma viruses.

The PDGF-c activity produced by the transformed cells shares many properties with PDGF in addition to the ability to bind to the PDGF receptor. It has mitogenic activity and is recognized by an anti-PDGF antibody that removes 20–80% of the binding activity (Fig. 4) and also removes mitogenic activity (Table 3). The inability of antibody to PDGF to remove all PDGF-competing activity from conditioned medium may reflect relatively small differences in the biosynthesis of PDGF by transformed cells and by megakaryocytes and endothelial cells—differences that alter antigenic crossreactivity without affecting ability to bind to the PDGF receptor. The ability of the anti-PDGF to recognize some

forms of PDGF-c allowed us to determine that these forms are mitogenic for test cells (Table 3). We do not believe that PDGF-c is necessarily the only mitogen produced by these transformed cells. We have already shown that PDGF-like molecules account for only a fraction of the mitogens produced by vascular endothelial cells (ref. 24; Table 3). The production of mitogens in addition to PDGF-c probably accounts for the differential ability of anti-PDGF antibodies to neutralize PDGF-competing activity and to neutralize the mitogenic potency of conditioned medium. In the former case we are looking only at molecules able to bind to the PDGF receptor. In the latter case we are looking at the combined effects of all mitogens present.

Transformation Decreases the Number of PDGF Receptors. Coincident with the production of PDGF-c by transformed cells, the number of receptors available for binding ^{125}I-PDGF decrease to 0–50% of parental levels (Figs. 1 and 2). This contrasts with the observation that ^{125}I-labeled EGF (^{125}I-EGF) binding decreases only when transformation is effected by certain retroviruses (13). We have measured (data not shown) ^{125}I-EGF to some parental and transformed cell lines and confirm the decrease in ^{125}I-EGF binding to BALB/3T3 M-MSV and the lack of decrease (actually a small increase) in ^{125}I-PDGF binding to SV40-transformed BALB/3T3 T2 and Swiss/3T3 SV101. The decreased binding of both ^{125}I-PDGF (Fig. 1 and 2) and ^{125}I-EGF seems to reflect a decrease in the number of available receptors rather than a shift in the affinity of the receptors.

In the 4°C radioreceptor assay used to measure PDGF-c, conditioned media from some transformed cells can significantly decrease ^{125}I-PDGF binding to diploid human fibroblasts even after substantial dilution (e.g., 1:30 for T24). The low receptor phenotype of transformed cells can be produced under more "physiological" conditions by culturing nontransformed cells at 37°C in medium conditioned by their transformed counterparts or in medium containing PDGF (Table 2). Acetic acid treatment does not reveal cryptic cell surface receptors on either, probably because PDGF receptors occupied by PDGF at 37°C are rapidly internalized and degraded. It is therefore likely that the decreased number of available PDGF receptors on transformed cells results from the prior occupation of PDGF receptors by endogenous PDGF-c.

Mechanism of Expression of PDGF-c. A close homology between PDGF and the putative transforming protein (P28^{sis}) of SSV has been reported (18, 19). If P28^{sis}, or a product of P28^{sis}, can bind to the PDGF receptor, it is possible that the PDGF-c measured in the conditioned medium of SSV-transformed NRK and NIH/3T3 cells (Fig. 3) is translated from a virally encoded message. The number of cellular genes that have been acquired by transforming viruses or that have transforming potential appears to be limited (27, 28). The acquisition of a PDGF-like sequence by SSV supports the hypothesis that PDGF-like molecules are capable of playing a role as effectors of transformation. The PDGF-c produced by the cell lines other than those transformed by SSV is probably encoded by a cellular gene, because neither SV40, Kirsten murine sarcoma virus, Moloney murine sarcoma virus, methylcholanthrene, or the modified *ras/bas* proto-oncogene expressed by T24 have any homology to PDGF (18, 19). This would be analogous to the synthesis of sarcoma growth factor by murine sarcoma virus-transformed cells, in which case the growth factor seems to be synthesized from a cellular gene that has been activated as a consequence of transformation (28). Since PDGF-c from transformed cells differs from PDGF and endothelial cell PDGF-c in being incompletely or less easily neutralized by anti-PDGF antibodies (refs. 14, 15, and 23; Fig. 4) it is possible that PDGF-c from transformed cells is encoded by the same cellular gene that codes for platelet PDGF, but that it is processed somewhat differently (15). It is also possible that PDGF-c is expressed from a different PDGF-like cellular gene—possibly an oncogene (29)—expressed during normal embryological development but whose expression in adult life is inappropriate.

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