Platelet-derived growth factor (PDGF) is encoded by separate genes for two possible subunit chains (A-chain and B-chain) which can form three possible dimers (AA, AB, and BB). We have recently presented evidence that multiple forms of PDGF receptor exist which distinguish between these isoforms (Hart, C. H., Forstrom, J. W., Kelley, J. D., Smith, R. A., Ross, R., Murray, M. J., and Bowen-Pope, D. F. (1988) Science 240, 1529-1531). We used this specificity to determine the amount of PDGF from different sources which is able to bind to each class of receptor and found that each source has a characteristic isoform composition. Levels of total PDGF activity in sera from different species ranged more than 15-fold, from less than 1 ng/ml in dog, chicken, pig, and calf, to greater than 13 ng/ml in mouse and human. Despite these differences in PDGF content, the total mitogenic activities of the sera were comparable indicating that the relative importance of PDGF as a serum mitogen may vary considerably between species. Analysis of the total PDGF into the amounts of each isoform revealed great differences in composition. PDGF-BB constitutes only about 15% of the total binding activity in human PDGF purified by the method of Raines and Ross (Raines, E. W., and Ross, R. (1982) J. Biol. Chem. 257, 5154-5160) but is the predominant isoform in whole blood serum from all other species. In contrast to serum, medium conditioned by cultured PDGF-secreting cell types contained no detectable PDGF-BB except in two cases: medium conditioned by vascular endothelial cells and by cells transformed by simian sarcoma virus. The existence of isoform-specific PDGF receptors and the large variation in PDGF isoform composition dependent upon source may provide an important mechanism through which the effects of PDGF can be targeted to different cell types and/or toward eliciting different cell responses.

Platelet-derived growth factor (PDGF) was first recognized

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The abbreviation used is: PDGF, platelet-derived growth factor.
PDGF-BB. Nister et al. (1988) have also reported that PDGF-BB, but not PDGF-AA, is chemotactic for fibroblasts. In order to understand the involvement of PDGF in cell behavior, it will therefore be necessary to determine which isoforms of PDGF are present and which types of PDGF receptor are expressed by the potential responding cell. In this report, we use the pattern of expression of PDGF receptors on diploid human fibroblasts, which express 20-fold more β-subunit than α-subunits (Hart et al., 1988), to determine the concentrations of two functional classes of ligand: PDGF-BB, which can bind to all forms of the receptor, and PDGF-AA or PDGF-AB, whose binding is limited by the relatively small number of receptor α-subunits. Using these assays, we found a remarkable range in the amounts of total PDGF activity as well as in the relative abundance of different isoforms in different sources of PDGF. Serum from different species usually contains a predominance of PDGF-BB. Human serum is the exception, with the great majority being PDGF-AA or PDGF-AB. By contrast, medium conditioned by most PDGF-secreting cell types contains largely, or exclusively, PDGF-AA or PDGF-AB. Medium conditioned by vascular endothelial cells contains both isoforms, and 3T3 cells transformed by simian sarcoma virus oncogene isogenic to the B-chain of PDGF, secrete exclusively PDGF-BB. These results indicate that regulate of the isoform of PDGF produced under different circumstances may be used as a mechanism through which the effects of PDGF can be targeted to different cell types and/or toward eliciting different cell responses.

**Materials and Methods**

Ligands and Antibodies—Monoclonal antibody PR7212 is a mouse IgG, which recognizes the human PDGF receptor (Hart et al., 1987) through its β-subunit (Gronwald et al., 1988). It was purified from ascites fluid using protein A-Sepharose.

PDGF-AB was purified from outdated human platelets as described by Raines and Ross (1992) up to the CM-Sephadex step. Further purification, and separation of isoforms, was accomplished by affinity chromatography. Monoclonal 121.6.1.1.1, which binds the B-chain of PDGF, was coupled to Sephadex and used to immunoaffinity purify PDGF-AB and PDGF-BB. PDGF-BB was then removed by passage over a second monoclonal 120.1.2.1.1 affinity column which binds PDGF-BB but not PDGF-AB. The breakthrough was purified by reverse phase high performance liquid chromatography on C-18 resin. This PDGF-AB is at least 95% pure, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and amino acid sequence analysis, and is at least 99.5% free of PDGF-BB as determined by partial amino acid sequence analysis and by the assays described in this report. The concentration of PDGF-AB as determined by amino acid analysis agrees well with the concentration determined by radioreceptor assay using a preparation of platelet PDGF which we have been using as a standard since 1982.

Both PDGF-AA (110 amino acid “endothelial form”) (Collins et al., 1987b; Tong et al., 1987) and PDGF-BB (109 amino acids) were recombinant molecules produced by yeast using the expression system previously used to express v-sis (Kelly et al., 1988). The recombinant forms were at least 95% pure, as evaluated by silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis and amino acid sequence analysis. The concentrations of the recombinant forms was based on amino acid composition analysis.

PDGF-AB was radioiodinated using either iodine monochloride (Bowen-Pope et al., 1982) or Iodobeads (Pierce Chemical Co.) to a specific activity of 5.5 × 10^6 cpm/μg. Monoclonal anti-PDGF receptor antibody PR7212 (Hart et al., 1987) was radioiodinated using Iodobeads to a specific activity of 8.6 × 10^6 cpm/μg. Radioiodinated PDGF-BB (3.97 × 10^6 cpm/ng) was prepared using Iodobeads to radiolabel a mutant version of PDGF-BB expressed in yeast, in which the phenylalanine at position 23 was replaced by tyrosine.

**Reverse Phase HPLC**—Human serum was drawn from normal adult male volunteers. Baboon and macaque blood was drawn by the veterinary staff of the Northwest Regional Primate Center at the University of Washington. Rat and mouse blood was obtained by cardiac puncture. Serum was prepared by allowing the blood to clot in centrifuge tubes at room temperature for 5 h, followed by clot retraction overnight at 4°C and centrifugation at 10,000 × g for 20 min to remove clot and cells. Unless noted otherwise, sera from at least five individuals were pooled. The sera from the other species were purchased from Gibco. Conditioned media were collected over 48 h from confluent monolayers in 150-mm dishes incubated in Dulbecco's modified Eagle's medium containing 1% calf serum.

**Down-regulation Assay**—Normal human dermal skin fibroblasts (SK-6) were plated in Dulbecco's modified Eagle's medium with 1% calf serum at 2 × 10^5 cells/well in 24-well trays and used within 10 days. Two identical sets of standards and test substances diluted in binding medium (HEPES-buffered Ham's F-12 medium with 0.25% bovine serum albumin) were incubated on two parallel sets of SK-5 test cells. After 2 h at 37°C in an air incubator, the medium was aspirated and the dishes rinsed once with cold binding rinse (phosphate-buffered saline with 0.25% bovine serum albumin). One set of dishes was then incubated with 125I-PDGF-AB at 0.5 ng/ml and the other set incubated with 125I-PR7212 at 0.0025 μg/ml. After incubating 1.5 h at 4°C on an oscillating table, the dishes were rinsed four times with binding rinse, and the bound label was solubilized and counted as described previously (Bowen-Pope and Ross, 1985).

**Specific binding was determined using 500 ng/ml pure PDGF-BB (for 125I-PDGF-AB binding) or 20 μg/ml monoclonal PDGF-212 (for 125I-PR7212 binding) and has been subtracted.**

**Double Radioreceptor Assay**—This protocol is as described above except that the first incubation was performed at 4°C for 3 h, and the second incubation used 0.5 ng/ml. 125I-PDGF-AB was used in place of 125I-PR7212.

**Results**

**Binding Assays Which Discriminate between Different Isoforms of PDGF**

**A Down-regulation Assay Using Anti-receptor Monoclonal Antibody**—The existence of subsets of receptor with specificity for different isoforms of PDGF suggested that a binding assay could be developed that would make use of this specificity to distinguish between the presence of the different isoforms. The relative abundances of these different classes of receptors varies between different cell types and correlates with their relative responsiveness to the different isoforms. Adult human dermal fibroblasts express 20-fold fewer α- than β-subunits (Hart et al., 1988). Since binding of PDGF-AB requires at least one α-subunit (to bind the A-chain of the PDGF), the cells will run out of α-subunits before more than a twentieth of the β-subunits are occupied. PDGF-AA will occupy no β-subunits, while PDGF-BB will be able to occupy all β-subunits. As described below, this makes it possible to analyze PDGF activity into that contributed by PDGF-BB and that contributed by PDGF-AA and/or PDGF-AB.

The operation of β-subunits can be measured using the subunit-specific monoclonal antibody PR7212 or using 125I-PDGF-BB. Monoclonal PR7212 recognizes the β-subunit of the PDGF receptor via an epitope which is outside of the ligand-binding domain, since 125I-PR7212 will bind to a receptor which is already occupied by PDGF. In order to be able to detect the binding of ligand to PDGF receptors recognized by monoclonal PR7212, we performed the initial binding at 37°C to permit internalization and degradation of occupied cell surface receptors. In this assay format, which we refer to as a “down-regulation assay,” PDGF-BB causes a concentration-dependent decrease in subsequent binding of both 125I-PR7212 and 125I-PDGF-AB (see Fig. 1A). Binding of the two labels is decreased with exactly the same concentration dependence and to the same extent. By contrast, preincubation with PDGF-AB (Fig. 1B) or PDGF-AA (Fig. 1D) results in a concentration-dependent decrease in 125I-PDGF-AB binding but only a very slight decrease in 125I-PR7212 binding. In this assay system, the ability of the test ligand to down-regulate
more than about 10% of I25I-PR7212 binding is thus diagnostic of the presence of PDGF-BB. Since I25I-PDGF-AB binding is down-regulated by all three ligands, the proportion of this activity which due to PDGF-AB can be calculated from the difference between the ability to down-regulate I25I-PDGF-AB binding (which all isoforms do) and to down-regulate I25I-PR7212 binding (which only PDGF-BB can do by more than 10%).

We used this assay to analyze the isoform content of fluids known to contain PDGF. We found that PDGF from different cell types or sera was composed of a characteristic ratio of isoforms and that this ratio, as well as the total amount of PDGF activity, could vary dramatically. Thus, PDGF-BB could represent anywhere from 0 to 100% of total PDGF activity. For example, Fig. 1C shows that increasing concentrations of pig whole blood serum down-regulate I25I-PR7212 and I25I-PDGF-AB binding to the same extent. This would indicate that all of the PDGF-competing activity consists of PDGF-BB, consistent with the report by Stroobant and Waterfield (1984) that PDGF purified from pig platelets by Bolton-Hunter technique is more cumbersome, expensive, and changes the charge of the iodinated reactive group. By contrast, monoclonal PR7212 is easily radiiodinated and shows very low nonspecific binding (usually less than 5% of total). As an alternative to Bolton-Hunter iodination of the native B-chain, we have used iodobeads to iodinate a mutant version of the B-chain expressed in yeast in which the phenylalanine at position 23 is replaced by an iodinatable tyrosine. This form of recombinant PDGF-BB, designated “PDGF-BBtyr,” is indistinguishable from PDGF-BB in binding competition assays (not shown) and has the same EDso and gives the same maximal stimulation in [3H]thymidine incorporation assays (Fig. 2). Concentrations of PDGF-BB and PDGF-BBtyr are based on amino acid composition analysis because this seems to make the fewest a priori assumptions about how the different isoforms of PDGF will bind.

The use of this assay to measure total PDGF activity and to determine the proportion of PDGF-BB. Human dermal fibroblasts were incubated at 37 °C for 2 h with test medium, followed by determination, at 4 °C, of binding of I25I-PR7212 (open symbols) or I125I-PDGF-AB (closed symbols). See "Materials and Methods" for details. Results are displayed as percent of specific binding seen in the absence of PDGF. Data points represent the mean of determinations using duplicate cultures. A, effect of PDGF-BB, B, PDGF-AB, C, human whole blood serum (circles), pig whole blood serum (squares), and medium conditioned (condit. med.) by U20S cells (triangles). D, PDGF-AA.

Fig. 1. Use of the down-regulation assay to measure total PDGF activity and to determine the proportion of PDGF-BB. An alternative to using I25I-PR7212 after a 37 °C incubation would be to look down-regulation assay described above employs down-regulation of I25I-PDGF-AB binding to detect all isoforms and down-regulation of I25I-PR7212 binding to detect PDGF-BB binding to the more abundant B-receptors. An alternative to this that the B-chain of PDGF does not contain a tyrosine residue (Chiu et al., 1984) and hence cannot be conveniently radiiodinated by most of the usual iodination procedures. Radiiodination of primary amines by the Bolton-Hunter technique is more cumbersome, expensive, and changes the charge of the iodinated reactive group. By contrast, monoclonal PR7212 is easily radiiodinated and shows very low nonspecific binding (usually less than 5% of total). As an alternative to Bolton-Hunter iodination of the native B-chain, we have used iodobeads to iodinate a mutant version of the B-chain expressed in yeast in which the phenylalanine at position 23 is replaced by an iodinatable tyrosine. This form of recombinant PDGF-BB, designated “PDGF-BBtyr,” is indistinguishable from PDGF-BB in binding competition assays (not shown) and has the same EDso and gives the same maximal stimulation in [3H]thymidine incorporation assays (Fig. 2). Concentrations of PDGF-BB and PDGF-BBtyr are based on amino acid composition analysis because this seems to make the fewest a priori assumptions about how the different isoforms of PDGF will bind.

Radioiodinated PDGF-BB Confirms the Differences in Isoform Content—We have used I25I-PDGF-BBtyr in 4 °C competition assays which are identical to the down-regulation assay except that all incubations are at 4 °C and I125I-PDGF-BB is used in place of I25I-PR7212. This assay protocol gave the same results as the down-regulation assay when applied to the analysis of test samples. Fig. 3 shows an example of the application of this protocol to a sample which was found to contain both PDGF-BB and other isoforms (medium conditioned by human endothelial vein endothelial cells) and to a sample found to contain I25I-PDGF-AB.

Fig. 2. Mitogenic potency of PDGF isoforms. Confluent monolayers of Swiss 3T3 clone D1 cells were incubated for 48 h in 1% calf serum. The indicated concentration of PDGF was then added, and [3H]thymidine incorporation into trichloroacetic acid-insoluble material was determined between 20 and 22 h later. Open circles, PDGF-AB; closed circles, recombinant PDGF-BB; closed squares, PDGF-BBtyr.
The measurement of PDGF isoforms in sera was performed using direct competition at 4°C. Human dermal fibroblasts were incubated at 4°C for 3 h followed by determination at 4°C of binding of 125I-PDGF-AB (open symbols) or 125I-PDGF-AB (closed symbols). See "Materials and Methods" for details. Results are displayed as percent of specific binding seen in the absence of PDGF. Data points represent the mean of determinations using duplicate cultures. A, effect of PDGF-AB. B, effect of PDGF-AB, medium conditioned by human umbilical vein endothelial cells (circles); medium conditioned by pup rat smooth muscle cells (triangles). C, effect of PDGF-AA.

The Relative Amounts and Proportions of PDGF in Sera from Different Species—Table I summarizes data obtained using the down-regulation assay to determine the type and amount of PDGF present in animal whole blood serum. It is obvious that different animal sera differ widely in their content of total PDGF competing activity, as well as in the relative proportions of different isoforms. As reported previously (Singh et al., 1982; Bowen-Pope et al., 1984b), human whole blood serum contained a very high concentration of total PDGF activity. Of this, PDGF-AB constituted only about 7% of the total competing activity. PDGF purified by the procedure of Raines and Ross (1982) was somewhat enriched in PDGF-AB relative to whole blood serum, but PDGF-AB still accounted for only about 15% of the total competing activity. "Pure" human PDGF-AB isoform was obtained from the total purified PDGF using a series of monoclonal antibodies (see "Materials and Methods"). This pure PDGF-AB reduced 125I-PDGF-AB binding by less than 20% at 20 ng/ml, compared to a 50% reduction of 125I-PDGF-AB binding at 0.15 ng/ml.

Serum from two non-human primates was found to contain a larger proportion of PDGF-AB, about one-half of the total competing activity. Serum from all the other species contained predominantly, or entirely, PDGF-AB. Levels of total PDGF activity in the sera of these species (except mouse) was also lower than in the primates.

The Mitogenic Potencies of Sera—Since sera from different species varied so widely in PDGF content, we examined the mitogenic potency of sera to determine whether the PDGF content of the sera was correlated with the amount of PDGF present. Fig. 4 shows that rat and mouse sera were equally potent as mitogens in this assay, although mouse serum contains 11-fold more PDGF than does rat serum (16 versus 1.05 ng/ml). Human and bovine sera were also comparable in mitogenic potency, although human serum contained 13-fold more PDGF (13.3 versus 1.05 ng/ml). Thus, although PDGF is considered to be the predominant mitogen in human serum, no detectable PDGF-AB (medium conditioned by rat thoracic aortic smooth muscle cells).

![Figure 3](image-url) Use of direct competition at 4°C to measure total PDGF activity and to determine the proportion of PDGF-AB. Human dermal fibroblasts were incubated at 4°C for 3 h followed by determination at 4°C of binding of 125I-PDGF-AB (open symbols) or 125I-PDGF-AB (closed symbols). See "Materials and Methods" for details. Results are displayed as percent of specific binding seen in the absence of PDGF. Data points represent the mean of determinations using duplicate cultures. A, effect of PDGF-AB. B, effect of PDGF-AB, medium conditioned by human umbilical vein endothelial cells (circles); medium conditioned by pup rat smooth muscle cells (triangles). C, effect of PDGF-AA.

![Figure 4](image-url) Mitogenic potency of different sera. Confluent monolayers of Swiss 3T3 clone D1 cells were incubated for 48 h in 1% calf serum. The indicated concentration of PDGF was then added and [3H]thymidine incorporation into trichloroacetic acid-insoluble material was determined between 20 and 24 h later.

**Table I**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total PDGF activity*</th>
<th>PDGF-AB activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant PDGF-AB</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>Human PDGF-AB</td>
<td>&lt;0.05</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Human purified PDGF</td>
<td>12.5 ± 2.5 (2)</td>
<td>12.3 ± 3.5 (4)</td>
</tr>
<tr>
<td>Baboon</td>
<td>17.0 (1)</td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td>(3 individual donors)</td>
<td>13.3 ± 4.5</td>
</tr>
<tr>
<td>Mouse (BALB/c)</td>
<td>16 ± 5 (5)</td>
<td>72 ± 27 (5)</td>
</tr>
<tr>
<td>Monkey (Nemestrina)</td>
<td>4.5 ± 1.8 (3)</td>
<td>63 ± 26 (3)</td>
</tr>
<tr>
<td>Baboon</td>
<td>2.6 ± 0.6 (3)</td>
<td>54 ± 10 (3)</td>
</tr>
<tr>
<td>Rat serum</td>
<td>1.4 ± 0.1 (0)</td>
<td>110 ± 2 (2)</td>
</tr>
<tr>
<td>Bovine (calf)</td>
<td>1.05 ± 0.4 (7)</td>
<td>72 ± 22 (7)</td>
</tr>
<tr>
<td>Bovine (fetal)</td>
<td>0.93 ± 0.3 (5)</td>
<td>82 ± 12</td>
</tr>
<tr>
<td>Lamb</td>
<td>0.92 ± 0.26 (3)</td>
<td>94 ± 20 (3)</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.62 ± 0.21 (3)</td>
<td>113 ± 41 (3)</td>
</tr>
<tr>
<td>Pig</td>
<td>0.6 ± 0.01 (2)</td>
<td>114 ± 19 (2)</td>
</tr>
<tr>
<td>Dog</td>
<td>0.32 ± 0.11 (4)</td>
<td>76 ± 20 (4)</td>
</tr>
</tbody>
</table>

*Total PDGF activity is calculated from the ability of the test solution to down-regulate 125I-PDGF-AB binding using the effect of purified PDGF-AB as a standard. In all cases, at least three dilutions were tested and the concentration of PDGF calculated from the standard curve and corrected for dilution. The values calculated from dilutions giving 20-80% binding inhibition are averaged to give the estimate of PDGF activity. The values recorded in the table are the averages obtained using this procedure repeated the number of times indicated in parentheses.

*The percentage of competing activity represented by PDGF-AB is calculated from the ratio of the amount of test solution needed to reduce 125I-PDGF-AB binding by 50% divided by the amount of test solution needed to reduce binding of 125I-PR7212 by 90%.

*These are standards. Concentrations of PDGF-AB and PDGF-BB were assigned by amino acid analysis. Concentration of PDGF-AA was assigned by competition for 125I-PDGF-AB binding.
is not as significant in bovine or rat sera. The Relative Amounts and Proportions of PDGF Isoforms in Medium Conditioned by Cultured Normal Cells—In most cases, the relative contribution of PDGF-BB to the total competing activity in conditioned medium (Tables II and III) differed dramatically from its relative contribution to the activity in serum (Table I), in that the conditioned medium rarely contained significant proportions of PDGF-BB. We have previously reported that cultured rat pup aortic smooth muscle cells (Seifert et al., 1984) and rat smooth muscle cells from injured carotid arteries (Walker et al., 1986) secrete PDGF into the culture medium. Table II indicates that PDGF secreted by both of these cell types contained no detectable PDGF-BB, although the pup smooth muscle cells expressed elevated levels of B-chain and similar levels of A-chain mRNA compared to adult rat aortic smooth muscle cells (Majesky et al., 1988). By contrast, rat serum contained largely PDGF-BB (Table I).

Both bovine aortic endothelial cells and human umbilical vein endothelial cells were found to secrete a mixture of PDGF-BB and AA/AB (Table II). This is consistent with the reports that both A-chain and B-chain mRNA is expressed (Barrett et al., 1984; Collins et al., 1987a) but contrasts with the conclusion, based on the size of immunoprecipitated, metabolically labeled PDGF, that human endothelial cells do not secrete PDGF-BB (Collins et al., 1987a). We have previously reported that thrombin induces endothelial cells to increase the rate of PDGF secretion (Harlan et al., 1986).

### Table II

<table>
<thead>
<tr>
<th>Amount and type of PDGF secreted by normal vascular cells</th>
<th>Total PDGF*</th>
<th>PDGF-BB*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human umbilical vein endothelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.13 ± 0.51 (5)</td>
<td>14.8 ± 6.5 (5)</td>
</tr>
<tr>
<td>Thrombin-treated</td>
<td>3.68 ± 2.4 (5)</td>
<td>16.3 ± 7.7 (4)</td>
</tr>
<tr>
<td>Bovine aortic endothelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.8 ± 7.1 (4)</td>
<td>48.2 ± 11.7 (4)</td>
</tr>
<tr>
<td>Thrombin-treated</td>
<td>8.7 ± 2.9 (2)</td>
<td>46 ± 5 (2)</td>
</tr>
<tr>
<td>Rat vascular smooth muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult thoracic</td>
<td>ND b</td>
<td>ND</td>
</tr>
<tr>
<td>Pup thoracic</td>
<td>0.42 ± 0.03 (2)</td>
<td>&lt;5 (2)</td>
</tr>
<tr>
<td>Carotid media</td>
<td>0.86 ± 0.06 (4)</td>
<td>&lt;5 (4)</td>
</tr>
<tr>
<td>Carotid intima</td>
<td>0.89 ± 0.54 (4)</td>
<td>&lt;5 (4)</td>
</tr>
</tbody>
</table>

* Determined as described for Table I.

### Table III

<table>
<thead>
<tr>
<th>Amount and type of PDGF secreted by transformed cells</th>
<th>Total PDGF*</th>
<th>PDGF-BB*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-24 (human carcinoma)</td>
<td>2.03 ± 1.1 (5)</td>
<td>&lt;5 (5)</td>
</tr>
<tr>
<td>HT1080 (human sarcoma)</td>
<td>3.2 ± 0.1 (2)</td>
<td>&lt;5 (2)</td>
</tr>
<tr>
<td>HEP-G2 (human hepatoma)</td>
<td>0.46 (1)</td>
<td>&lt;10 (2)</td>
</tr>
<tr>
<td>VA-13 (SV40-transformed human fibroblasts)</td>
<td>3.2 (1)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>A431 (human carcinoma)</td>
<td>0.11 (1)</td>
<td>&lt;30</td>
</tr>
<tr>
<td>SSVNIH (simian sarcoma virus-transformed mouse 3T3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone 5</td>
<td>2.5 ± 0.2 (2)</td>
<td>95 (2)</td>
</tr>
<tr>
<td>Clone 7</td>
<td>0.75 ± 0.3 (2)</td>
<td>&lt;5 (2)</td>
</tr>
<tr>
<td>KNIH (Kirsten murine sarcoma virus-transformed 3T3)</td>
<td>0.23 (1)</td>
<td>&lt;15</td>
</tr>
<tr>
<td>TRD1 (spontaneously transformed mouse cells)</td>
<td>4.0 ± 2.0 (2)</td>
<td>&lt;3 (2)</td>
</tr>
</tbody>
</table>

* Determined as described for Table I.

Table II confirms this and indicates that thrombin stimulation did not preferentially stimulate secretion of one of the isoforms; i.e. the PDGF secreted in response to thrombin stimulation consisted of the same ratio of isoforms as was secreted by unstimulated endothelial cells.

The Relative Amounts and Proportions of PDGF Isoforms in Medium Conditioned by Cultured, Oncogenically Transformed Cells—Many transformed cell lines, both those derived from naturally occurring tumors and those obtained by intentional transformation of normal cultured cell lines, have been reported to secrete PDGF into their culture medium. We examined conditioned medium from four transformed human lines, and in no case did PDGF-BB account for a detectable proportion of the total PDGF activity (Table III). Of the transformed mouse cell lines, only a subclone (clone 5) of simian sarcoma virus-transformed 3T3 cells accumulated detectable PDGF-BB in the conditioned medium. In this case, PDGF-BB accounted for the great majority of the PDGF activity, as would be expected given that the transforming oncogene of simian sarcoma virus encodes the B-chain of PDGF.

**DISCUSSION**

Multiple Forms of PDGF Receptor—During the last year, we have been led to hypothesize (Hart et al., 1988) that cells can express more than one form of PDGF receptor which differ in their ability to bind the different possible isoforms of PDGF, i.e. PDGF-AA, PDGF-AB, and PDGF-BB. We believe that the pattern of isoform recognition can best be explained by postulating that high affinity PDGF binding sites (receptors) form by association of two subunits, each of which binds one of the subunit chains of the dimeric PDGF ligand. The subunits have the following characteristics: the \( \beta \)-subunit binds only B-chains, and the \( \alpha \)-subunit binds A- or B-chains. Only the \( \beta \)-subunit is recognized by monoclonal PR7212. Depending on how many subunits of each type exists on a given cell type, that cell will have a characteristic pattern of binding (and competition). The physiological consequence of these differences in isoform binding may be to allow different cells to respond differentially to the presence of different isoforms of PDGF. In this report we have made use of the PDGF receptor distribution on fibroblasts to determine, in a modified radioreceptor format, the isoform composition of PDGF derived from different biological sources.

Human diploid fibroblasts (SK-5) express many more \( \alpha \)- than \( \beta \)-subunits and are correspondingly much more mitogenically responsive to PDGF-BB than to PDGF-AA (Kazlauskas et al., 1988). Using these cells, the presence of PDGF-BB can be specifically detected as the only isoform able to down-regulate more than 50% of the binding of \( ^{125}I \)-PR7212 (Fig. 1) or to compete for more than 50% of \( ^{125}I \)-PDGF-BB binding (Fig. 3). Since all three isoforms compete effectively for binding of \( ^{125}I \)-PDGF-AB, we have expressed the fractional representation of the isoforms in terms of their ability to compete for \( ^{125}I \)-PDGF-AB binding, i.e. we have quantitated the isoforms in terms of their activity as competitors.

**Radioreceptor Assays Provide Information About the Content of Biologically Active Ligand**—There are several reasons to using an assay which detects PDGF by its competing activity rather than by some other attribute of PDGF. Unlike amino acid sequence data, the assay does not require purification of the PDGF from the test sample, a process which is laborious and which could result in preferential recovery of one or other of the isoforms. A possible example of differential recovery is the difference between the PDGF-BB content of human whole blood serum and pure PDGF. Table I indicates
that PDGF-BB constitutes only 6% of the activity of freshly drawn human whole blood serum, but accounts for 12 to 17% of the activity of three preparations of purified PDGF. It should be noted, however, that in the example given the whole blood samples were not from the individuals from which the PDGF was purified so that it remains possible that differences in isoform content reflect differences between the two sources of platelets, rather than purification losses. The most important observation about the purified PDGF is that it consists of a mixture of isoforms, with only a minority of the activity being PDGF-BB. Additional advantages of a radioreceptor assay over a radioimmunoassay are that it will not detect antigenic (but biologically inactive) degradation products of PDGF, and is applicable to samples from all species tested (in contrast to the frequent species limitations of radioimmunoassays).

One complicating factor in using the radioreceptor assay is the possible presence of "binding proteins" which are able to bind PDGF and prevent its binding to the PDGF receptors on the test cells (Huang et al., 1983; Raines et al., 1984). Such proteins could complex a significant fraction of the PDGF in those serum samples which contain little PDGF. For this reason, the values presented here should be considered to reflect the concentration of PDGF which is active and available to responsive cells, rather than necessarily equaling the entire amount of PDGF.

The PDGF Content of Sera from Different Species Varies Greatly and Does Not Correlate with Mitogenic Potency—The amount of PDGF activity detected in whole blood sera varied 50-fold among the species surveyed in Table I, from a low of 0.32 ng/ml in dog serum to a high of 16 ng/ml in mouse serum. Although we cannot be certain that PDGF from all species is exactly equal as competitors in this assay, species differences are unlikely to be a major factor for several reasons. Significant species differences in binding affinity seem unlikely given that, in the converse measurement, binding of human 125I-PDGFB to receptors on cells from species as different as fish and chickens indicates a very similar affinity and hence a high degree of conservation of the ligand-receptor interaction (Bowen-Pope et al., 1985b). It is also readily apparent from Table I that the differences in measured serum levels of PDGF do not correspond to phylogenetic relationships. For example, serum levels are high in human but low in baboon, high in mouse but low in rat.

Despite the large range in levels of PDGF in different sera, there was a much smaller range in the mitogenic potencies of those sera for stimulation of 3T3 cells (Fig. 4), suggesting that PDGF is not the major mitogen in these sera, at least as assayed on cultured 3T3 cells. It is interesting that bovine serum (both fetal and calf), by far the most common serum used to culture cells in vitro, is very low in PDGF activity (0.93–1.05 ng/ml). 10% calf serum thus contains only about 0.07 ng/ml PDGF activity, i.e. less than 10% of the ED50 for mitogenic stimulation of 3T3 cells by PDGF (Fig. 2). This might account, in part, for the fact that early attempts to identify and purify mitogens from bovine serum (reviewed in Gospodarowicz and Moran, 1976) did not lead to the discovery of PDGF. By contrast, human serum and platelets, which are very rich in PDGF, were soon recognized as a source of new growth factor activity which is now called PDGF (reviewed in Westermark et al., 1983). The very large range in the relative PDGF content of sera from different species suggests that importance of PDGF from platelets in mediating responses to injury, etc., may be quite species-dependent.

Determmants of PDGF-BB Secretion, Accumulation, and Detection—It is apparent from Table I that PDGF-BB is the predominant, and often the only, isoform of PDGF detected in the sera of all species tested, except humans where it represents only 6% of the total activity. This contrasts strikingly with the isoforms found in medium conditioned by cells in culture (Tables II and III). Of the PDGF-secreting cell types tested, only two were found to accumulate PDGF-BB in their conditioned medium: vascular endothelial cells and simian sarcoma virus-transformed 3T3 cells. Since the oncogene of simian sarcoma virus is derived from the B-chain of PDGF, the PDGF-BB detected in the medium is presumably the v-sis product. The amount of PDGF activity detected in media conditioned by many cells does not correlate well with the levels of B-chain mRNA (Betshtoltz et al., 1986). B-chain mRNA can be detected in many of the cell types which have no detectable PDGF-BB in their conditioned medium (Tables I and II). In some cases, as in TRDI or the pup rat smooth muscle cells, it is B-chain mRNA which seems to be preferentially induced as compared with the nonsecreting parental 3T3 or adult rat smooth muscle cells (Fraizer et al., 1988; Majesky et al., 1988). Potential explanations for the low levels of PDGF-BB found in conditioned medium relative to PDGF-AA and PDGF-AB include differences in efficiency of translation (e.g. Rao et al., 1988) or secretion (e.g. Robbins et al., 1985; Collins et al., 1987b), or the preferential formation of PDGF-BB heterodimers within cells expressing both messages. Two additional possibilities are particularly relevant to the approaches and methods used in this report: 1) PDGF-BB is present but less easily detected. This is supported by the observation that it takes up to 10-fold more PDGF-BB than PDGF-AB to compete for 125I-PDGF-AB binding to the test cells. The mass of PDGF-BB would thus be 10-fold greater than the activity of PDGF-BB. 2) PDGF-BB is secreted but is more rapidly bound and degraded. The importance of this mechanism would depend on the level of expression of receptors of the different specificities. Thus, the lack of synthesis or expression of PDGF receptors by vascular endothelial cells (Kazlauskas and DiCorleto, 1985; Hart et al., 1987; Bowen-Pope et al., 1985 and references therein) would allow endothelial cells to accumulate PDGF-BB without losses due to receptor-mediated internalization. Substantial levels of PDGF-BB are found in medium conditioned by endothelial cells derived either from human umbilical vein (15% BB) or from adult bovine aortas (48% BB). The same argument could be applied to explain the recovery of PDGF-BB in whole blood serum, since the cellular components of blood in aggregate can bind very little of the PDGF released from platelets (Bowen-Pope et al., 1984b). However, lack of synthesis of receptors is not sufficient to permit PDGF-BB accumulation, since some of the other cell types in Tables II and III (e.g. T-24, TRDI, HEP-G2) do not accumulate PDGF-BB in their conditioned medium, although they contain easily detectable B-chain mRNA and do not synthesize PDGF receptors.

The Biological Significance of the Existence of Differing Levels of the Different PDGF Isoforms—The existence of multiple forms of PDGF receptors which specifically bind different isoforms of PDGF indicates that cells have the capacity to distinguish between the different isoforms in a mixture. The very wide range in the relative proportions of the isoforms in PDGF from different sources as reported here, as well as a comparatively wide range in the proportions of the receptor classes displayed by different cultured cell types, suggests that there is at least the potential that the type of PDGF isoform present might be at least as important as how much PDGF is present. For example, rat serum was found to contain only PDGF-BB (which binds to all classes of PDGF...
receptor) but cultured neonatal rat smooth muscle cells secrete only PDGF-AA/PDGF-AB which is restricted in action to target cells with PDGF receptor α-subunits. Expression of α-subunits appears to be highly regulated. Rat neonatal smooth muscle cells do not themselves express α-subunits and thus could respond to the PDGF in serum but not to the PDGF which they themselves secrete. Expression of α-subunits can also be regulated acutely. We have found that exposure of 3T3 cells to transforming growth factor-β results in a rapid and virtually complete decrease in expression of receptor α-subunits with an increase in expression of β-subunits. The cells exposed to transforming growth factor-β would thus be unable (or less well able) to respond to the PDGF-AA secreted by neighboring cells but would be perfectly competent to respond to PDGF-BB which could be delivered, along with the transforming growth factor-β, from blood platelets. In ways such as this, the targets and responses to PDGF could be regulated via changes in the isoform composition of the PDGF produced and in the form of PDGF receptor expressed.

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