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Research Article

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Differential Effects of the Various Isoforms of Platelet-derived Growth Factor on Chemotaxis of Fibroblasts, Monocytes, and Granulocytes

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Abstract

The chemotactic activities of three different isoforms of platelet-derived growth factor (PDGF) on fibroblasts, monocytes, and granulocytes of human origin were investigated. PDGF-AB and PDGF-BB induced strong, dose-dependent responses in both fibroblasts and monocytes, whereas PDGF-AA did not stimulate chemotaxis of these cell types. Instead, PDGF-AA inhibited the chemotactic activity of PDGF-AB and PDGF-BB on fibroblasts and monocytes. However, PDGF-AA was not able to block monocyte chemotaxis induced by FMLP. In contrast, in granulocytes, dose-dependent chemotactic responses were obtained with all three isoforms of PDGF. All isoforms gave maximal responses at concentrations between 5 and 20 ng/ml. At higher concentrations the migration was reduced. Reduction and alkylation of the PDGF molecule, which leads to loss of the mitogenic activity, also caused a loss of the chemotactic activities for all three cell types.

The data suggest that the various isoforms of PDGF stimulate and inhibit chemotaxis in an isoform- and cell type–specific manner. (*J. Clin. Invest.* 1990. 85:916–920.) chemotaxis • fibroblasts • phagocytes • PDGF • FMLP

Introduction

Chemotactic factors are released by various cell types during wound healing and inflammation, and play an important role in the recruitment of different types of cells to these sites. A number of chemoattractants, especially for granulocytes and monocytes, have been identified. These factors bind to specific cell-surface receptors, whereby the molecular events of the chemotactic response are initiated (1, 2). Interestingly, certain growth factors have been found to have chemotactic activity for various cell types. Thus, platelet-derived growth factor (PDGF),[1] a release product of platelet α-granula, is not only a major mitogen for connective tissue cells in vitro (3, 4), but also a potent chemotactic agent for fibroblasts (5, 6) and smooth muscle cells (7).

PDGF is made up as dimers of A and B polypeptide chains that are linked by disulphide bonds. All three possible dimeric forms of PDGF have been identified and purified from platelets and transformed cells. Human platelets contain PDGF-AB and PDGF-BB (8, 9), whereas porcine platelets contain mainly PDGF-BB (10). PDGF-AA is produced, e.g., by human osteosarcoma cells (11). The A chain occurs as two variants due to differential splicing; the COOH-terminal three amino acids in the shorter variant are replaced by 18 different amino acids in the longer variant (12, 13). All isoforms of PDGF are available in recombinant form (14–16).

PDGF binds to two structurally related but distinct receptor types: PDGF α-receptors, also called A-type receptors, bind all three isoforms with high affinities, whereas β-receptors, also called B-type receptors, bind PDGF-BB with high affinity and PDGF-AB with lower affinity, but do not bind PDGF-AA with any appreciable affinity (17, 18).

Previous studies have shown that, in contrast to PDGF purified from human platelets, PDGF-AA has no chemotactic activity or ability to induce actin reorganization and membrane ruffling in human foreskin fibroblasts (19); these effects are therefore likely to be mediated by the β-receptor in these cells. With regard to the chemotactic activity of PDGF on granulocytes and monocytes, conflicting data have been published (20, 21), and no attempts have been made to discriminate between the different isoforms of PDGF. The aim of the present investigation was therefore to analyze the chemotactic response of phagocytic cells and fibroblasts to the different isoforms of PDGF.

Methods

PDGF. PDGF was purified from human platelets, and PDGF-AB and PDGF-BB were separated by immobilized metal-ion affinity chromatography (8). Recombinant PDGF-AA (long and short variant) and PDGF-BB were purified to apparent homogeneity from supernatants of yeast cells transfected with PDGF A and B chain cDNA constructs, respectively (15).

Purified recombinant PDGF-AA (short variant) and PDGF-BB were reduced by 25 mM dithiothreitol in 4 M guanidine-HCl, 1 M Tris, pH 8.0, and 10 mM EDTA for 2 h at 37°C or 25 min at 68°C (PDGF-BB), followed by alkylation with 100 mM iodoacetamide for 1 h at room temperature. The reduced and alkylated factors were then dialyzed extensively against 1 M acetic acid at 4°C in the presence of small amounts of HSA.

FMLP was purchased from Sigma Chemical Co., St. Louis, MO.

Cell culture. Human foreskin fibroblasts, AG 1523, were grown to confluence in Eagle's MEM supplemented with 10% newborn calf serum (NCS). Before use, the cells were detached by trypsinization (2.5 mg/ml for 10 min at 37°C), washed in Hank's balanced salt solution, and resuspended in Eagle's MEM with 10% NCS to a cell concentration of 2×10^8 cells/liter.

Isolation of human monocytes and granulocytes. Blood from apparently healthy donors was collected in glass tubes containing heparin (Venoject, Terumo, Belgium). The blood samples were mixed with an equal volume of dextran solution (Pharmacia AB, Uppsala, Sweden)

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1. *Abbreviations used in this paper:* NCS, newborn calf serum; PDGF, platelet-derived growth factor.

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(20 g/liter; dissolved in 0.15 M NaCl); the cells were then allowed to sediment at room temperature for 30 min. The leukocyte-rich plasma was removed, centrifuged, and the cell pellet suspended in 2 ml 0.15 M NaCl. Purification of monocytes was then done according to the method described by Vadas et al. (22), using a stepwise gradient of 16, 18, 20, 22, and 23% (wt/vol) metrizamide (Nycomed, Oslo, Norway). The leukocytes were applied on top of the gradient and centrifuged 1,200 *g* for 45 min at 20°C. The cells from the 16% metrizamide fractions were used. The purity of monocytes in these fractions was > 80%. Lymphocytes were the only contaminating cells as estimated by light microscopy and staining for unspecific esterase (23). Finally, the monocytes were suspended in Gey's solution (24), supplemented with 0.2% HSA, and adjusted to a cell concentration of 1×10^9 cells/liter.

Granulocytes were obtained as previously described (25) and suspended in Gey's solution with 0.2% HSA to a concentration of 1.5×10^9 cells/liter. The preparation consisted of ~ 90% granulocytes and 10% mononuclear cells.

Chemotaxis assay. The migration of fibroblasts, monocytes, and granulocytes was assayed by means of the leading front technique using a modified Boyden chamber (26, 27).

For monocyte and granulocyte migration, micropore filters (Millipore/Continental Water Systems, Bedford, MA) with pore sizes of 5 and 3 μm, respectively, were used. 100 μl of the cell suspension was added above the filter of the Boyden chamber; attractants to be tested were diluted in Gey's solution with 0.2% HSA and added below the filter. After 60 min at 37°C, the filters were fixed, stained, and mounted. Cell migration was assayed as the migration distance of the two furthest migrating granulocytes or monocytes visible in focus of one high-power field (12.5 × 24). The migration distance in each filter was calculated as the mean of the readings of three to five different areas of the filter. All experiments were performed with duplicate or quadruplicate filters for each concentration of the different isoforms of PDGF. For each set of experiments, the migration of granulocytes or monocytes in Gey's solution with 0.2% HSA and with the same medium below the filter was used as control, and referred to as 100% migration.

For fibroblast migration, micropore filters (pore size 8 μm) were coated with a solution of type-I collagen (100 μg/ml; a kind gift of Kristofer Rubin, Dept. of Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden) at room temperature overnight. The filters were air dried for 30 min immediately before use. The assay was performed as previously described (19). The migration of fibroblasts suspended in Eagle's MEM with 10% NCS, and with the same medium below the filter in the Boyden chamber, served as control, and was referred to as 100% migration.

Results

Chemotactic response of human foreskin fibroblasts to the various isoforms of PDGF. A strong dose-dependent chemotactic response for fibroblasts was obtained with PDGF-AB, as well as with PDGF-BB from two different sources, i.e., human platelets and recombinant material produced by transfected yeast cells (Fig. 1). The maximal response was obtained with 5 ng/ml of PDGF-AB or 10–20 ng/ml of PDGF-BB. At higher concentrations, the chemotactic response was reduced. In contrast, recombinant PDGF-AA did not induce migration of human foreskin fibroblasts (Fig. 1).

We recently found that PDGF-AA secreted by the clonal malignant glioma cell line U-343 MGa Cl2:6 inhibited the chemotactic activity of PDGF purified from human platelets on human fibroblasts (19). We therefore investigated recombinant forms of the two variants of PDGF-AA, with regard to their ability to reduce the chemotactic activities of optimal concentrations of PDGF-AB (5 ng/ml) or PDGF-BB (10 ng/ml). Both the short (Fig. 2) and the long (data not shown)

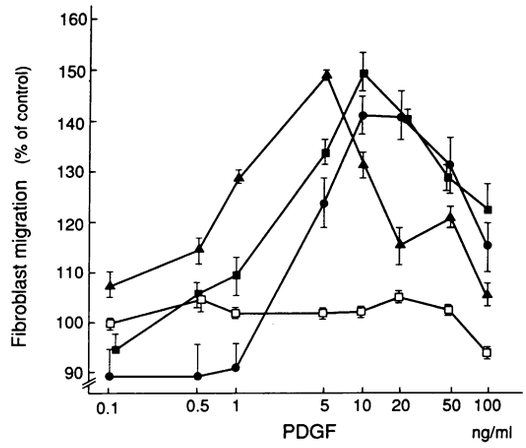

Figure 1. Chemotactic response of fibroblasts to PDGF-AB (▲), platelet PDGF-BB (●), recombinant PDGF-BB (■), and the short variant of PDGF-AA (□). The results are means and SEM of three experiments. A significant ($P < 0.001$, *t* test) chemotactic response was obtained with 1–50 ng/ml of PDGF-AB, 5–50 ng/ml of platelet PDGF-BB, and 1–100 ng/ml of recombinant PDGF-BB.

variant of PDGF-AA at concentrations > 10 ng/ml significantly ($P < 0.05$) reduced the chemotactic activity of PDGF-AB as well as PDGF-BB.

Stimulation of monocyte chemotaxis by the various isoforms of PDGF. Monocytes were found to migrate towards a concentration gradient of PDGF-AB, as well as platelet or recombinant PDGF-BB (Fig. 3). A significant ($P < 0.01$) response was noticed at concentrations above 1 ng/ml of PDGF-AB or recombinant PDGF-BB. PDGF-BB purified from human platelets induced monocyte chemotaxis at a somewhat higher concentration (5 ng/ml). The maximal chemotactic activity was obtained at concentrations between 10 and 20 ng/ml. Higher concentrations gave reduced response of

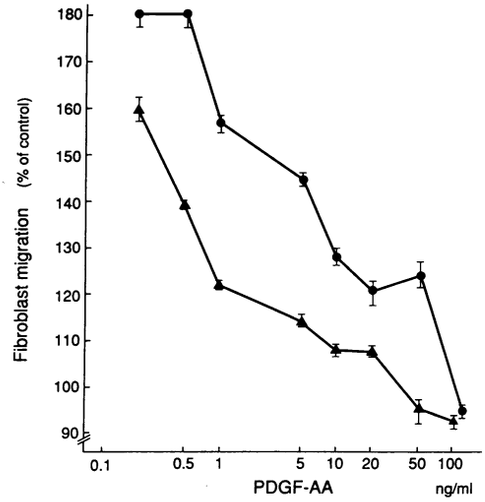

Figure 2. Inhibition by PDGF-AA of chemotaxis induced by PDGF-AB (▲) and PDGF-BB (●). Fibroblasts migrated toward a solution of 5 ng/ml of PDGF-AB or 10 ng/ml PDGF-BB, and various concentrations of the short variant of PDGF-AA. Results are means and SEM of two experiments. A significant reduction of PDGF-AB– or PDGF-BB–induced chemotaxis was obtained with > 5 ng/ml or > 10 ng/ml of PDGF-AA, respectively.

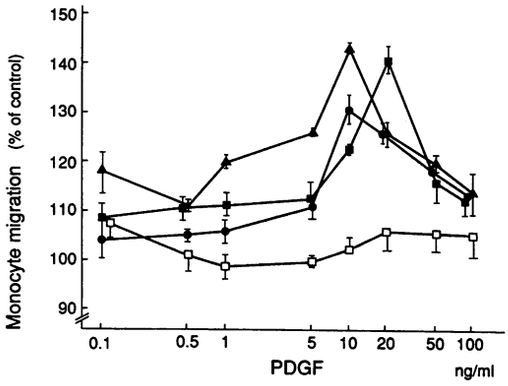

Figure 3. Chemotactic response of monocytes to PDGF-AB (▲), platelet PDGF-BB (●), recombinant PDGF-BB (■), and the short variant of PDGF-AA (□). A significant ($P < 0.01$, t test) response was obtained with 1–100 ng/ml of PDGF-AB, 5–100 ng/ml of platelet PDGF-BB, and 0.5–100 ng/ml of recombinant PDGF-BB (means and SEM of three experiments).

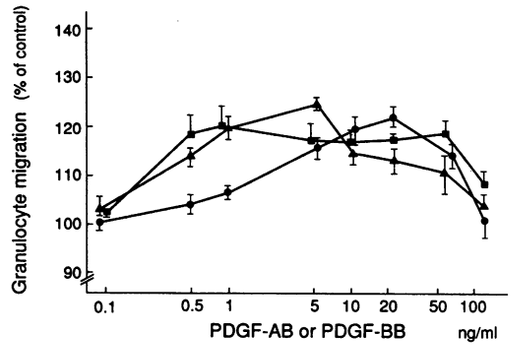

Figure 5. Chemotactic response of granulocytes to PDGF-AB (▲), platelet PDGF-BB (●), and recombinant PDGF-BB (■). A significant chemotactic response was achieved with 0.5–50 ng/ml of PDGF-AB or recombinant PDGF-BB. 5–50 ng/ml of PDGF-BB purified from platelets gave a significant response (means and SEM of three experiments).

the cells. There was no chemotactic response to the short (Fig. 3) or long (data not shown) variant of PDGF-AA.

The short variant of PDGF-AA inhibited monocyte chemotaxis induced by 10 ng/ml of PDGF-AB or 20 ng/ml of PDGF-BB (Fig. 4). PDGF-AA at concentrations > 0.5 ng/ml significantly ($P < 0.01$) decreased the chemotactic activity of PDGF-AB and of recombinant PDGF-BB. We also tested the ability of PDGF-AA to block monocyte chemotaxis induced by an unrelated chemotactic agent. The short variant of PDGF-AA was not able to inhibit monocyte chemotaxis to 10 nM of FMLP (Fig. 4).

Chemotactic response of granulocytes to the various isoforms of PDGF. In contrast to the results obtained with fibroblasts and monocytes, granulocytes were attracted in a dose-dependent way to all three isoforms of PDGF (Figs. 5 and 6).

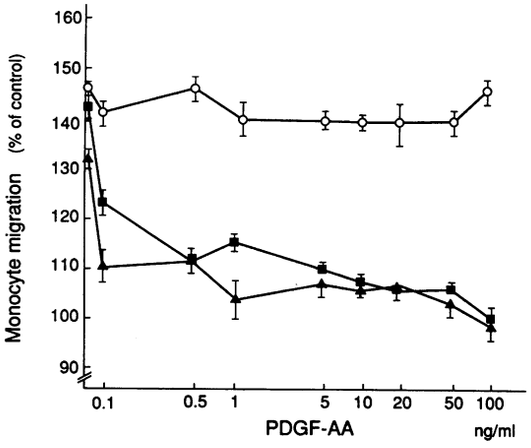

Figure 4. The influence of PDGF-AA on chemotaxis induced by PDGF-AB (▲), recombinant PDGF-BB (■), and FMLP (○). Monocytes migrated toward a solution of 10 ng/ml PDGF-AB, 20 ng/ml PDGF-BB, or 10 nM FMLP, and various concentrations of the short variant of PDGF-AA. Results are means and SEM of four experiments with PDGF-BB, three with PDGF-AB, and two with FMLP. A significant ($P < 0.01$) reduction of PDGF-AB– or PDGF-BB–induced chemotaxis was obtained with > 0.5 ng/ml of PDGF-AA. FMLP-induced chemotaxis was not inhibited by PDGF-AA.

PDGF-AB and -BB induced chemotaxis over a broad concentration interval ranging from 0.5 to 50 ng/ml (Fig. 5); PDGF-AA induced a significant response at 10–50 ng/ml (Fig. 6). The responses to the PDGF preparations were somewhat lower than the response obtained with 10 nM of FMLP (130–135%; data not shown).

Reduced and alkylated PDGF has no chemotactic activity. The mitogenic activity of PDGF is lost when the disulphide bonds in the molecule are reduced. Similarly, reduced and alkylated PDGF-AA was without chemotactic effect on granulocytes (Fig. 6), as well as on fibroblasts and monocytes (data not shown). PDGF-BB was also found to lose its chemotactic activity for all three cell types after reduction and alkylation (data not shown).

Discussion

In the present study we show that PDGF-AB and -BB are strong chemotactic factors for fibroblasts and for phagocytic cells. In contrast, PDGF-AA was found to be chemotactic only for granulocytes. Some of our findings are at variance with previous reports. Graves et al. (21) reported that PDGF puri-

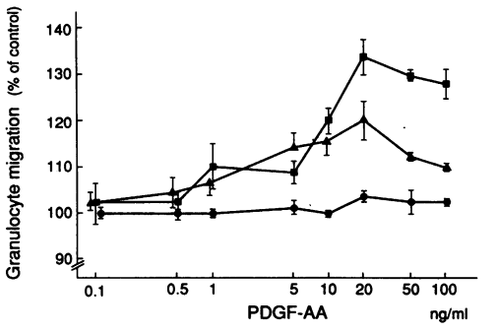

Figure 6. Chemotactic response of granulocytes to the short (■) and long (▲) variant of PDGF-AA and to reduced and alkylated PDGF-AA (short variant; ●). > 10 ng/ml of the short variant of PDGF-AA (■) induced a significant ($P < 0.001$) response. Concentrations between 1 and 50 ng/ml of the long version of PDGF-AA gave significant response ($P < 0.01$). The results are means and SEM of three experiments.

fied from human platelets did not induce chemotaxis of monocytes, whereas we (Fig. 3), as well as Deuel et al. (20), found a potent effect. The explanation for the discrepancy is not known, but it should be noted that we observed chemotaxis in response to both PDGF-AB and -BB, purified from human platelets as well as from recombinant sources; any possible interference by contaminating platelet factors can therefore be excluded. Williams et al. (28) reported that reduced PDGF retained its chemotactic activity for human neutrophils and monocytes, and suggested that PDGF mediated mitogenesis and chemotaxis through different structural determinants. In contrast, we found that reduced and alkylated recombinant PDGF-AA and -BB had no chemotactic effect on any of the cell types tested.

It was recently reported that fibroblasts display two distinct PDGF receptor classes, denoted α-receptors and β-receptors (17, 18). The human foreskin fibroblasts used in this study have about fourfold more PDGF β-receptors than α-receptors (15); the amounts of α- and β-receptors on monocytes and granulocytes have not been determined. Cells that lack PDGF β-receptors, e.g., endothelial cells isolated from large vessels (29, 30), do not respond chemotactically to PDGF (31, 32). This, and the finding that PDGF-AA, which does not bind to PDGF β-receptors, does not stimulate chemotaxis of human fibroblasts (Fig. 1), suggests the chemotactic activity of PDGF is mediated by the β-receptor class. The recent finding that after transfection of PDGF β-receptor cDNA, endothelial cells become able to migrate toward a concentration gradient of PDGF-BB (33), supports this conclusion.

It cannot be excluded that the lack of chemotactic effect of PDGF-AA in human fibroblasts and monocytes is due to too few α-receptors on the target cells. However, the fact that human foreskin fibroblasts have a fairly large number of α-receptors ($\sim$ 30,000/cell,) which is sufficient to induce a mitogenic response (15), is consistent with the conclusion that the α-receptor does not transduce a chemotactic signal in human fibroblasts. Likewise, the α-receptor seems incapable of mediating actin reorganization and induction of circular membrane ruffles in human fibroblasts (19). The data presented in this communication thus extend previous studies and indicate that there are functional differences between the two PDGF receptor types.

That different signals arise from activation of the two PDGF receptor classes is furthermore illustrated by the finding that PDGF-AA inhibited the chemotaxis induced by PDGF-AB or -BB in human fibroblasts (Fig. 2) and monocytes (Fig. 4). Inasmuch as PDGF-AA did not block FMLP-induced monocyte chemotaxis, the inhibitory effect is not explained by a general deactivation process or by a toxic effect. It has been suggested that the FMLP receptor, after binding of the ligand, interacts with a G protein and the chemotactic signal is mediated by phospatidylinositol turnover (1, 2). Our results do not exclude the possibility that chemotaxis induced by FMLP or PDGF-BB is transmitted via the same signal system. In such case, however, the PDGF route seems to be blocked by PDGF-AA at a point proximal to the convergence of the two signal pathways.

In contrast to the inhibitory effects on fibroblasts and monocytes, PDGF-AA stimulated chemotaxis of granulocytes (Fig. 6). The explanation for the different effects of PDGF-AA on the different cell types remains to be elucidated. The possibility that they are mediated by as yet unidentified receptor classes has not been excluded. Alternatively, the two cell types might differ in the intracellular signalling systems that are activated after stimulation by PDGF-AA. This possibility is illustrated by the fact that leukocytes differ from fibroblasts in that they can respond to chemoattractants and migrate in the presence of RNA and protein synthesis inhibitors (34, 35).

The present work shows that PDGF-AB and -BB stimulate chemotaxis of fibroblasts and monocytes, as well as granulocytes, and that PDGF-AA has agonistic or antagonistic effects depending on cell type. These findings are significant for the understanding of the role of PDGF in stimulation of chemotaxis in vivo. Thus, depending on which isoform of PDGF that is secreted by cells at a site of injury or inflammation, different cell types will be recruited to the area. The possible involvement of PDGF in pathological growth reactions such as atherosclerosis and fibrosis (3), which are associated with the accumulation of inflammatory cells, warrants studies on PDGF-induced chemotaxis in vivo.

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