Chemotaxis of Monocytes and Neutrophils to Platelet-derived Growth Factor

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ABSTRACT The platelet-derived growth factor (PDGF) is shown to be chemotactic for monocytes and neutrophils. Maximum monocyte chemotaxis to PDGF is fully equal to that achieved with C5a and occurs at ≃20 ng/ml (≃0.7 nM). Maximum neutrophil chemotaxis is ≃60% that achieved with C5a but occurs at ≃1 ng/ml (≃32 pM). The chemotactic activity of PDGF is blocked by specific antisera to PDGF and by protamine sulfate, a competitive inhibitor of PDGF binding to cell surfaces. In contrast to PDGF, epidermal growth factor shows no chemotactic activity for inflammatory cells at 0.5–100 ng/ml. The high level of chemotactic activity of PDGF suggests that in addition to its role as a mitogen for smooth muscle cells and fibroblasts, PDGF may be involved in attracting inflammatory cells to sites of platelet release.

INTRODUCTION

Platelet α-granules contain a potent mitogenic protein, the platelet-derived growth factor (PDGF),[1] which is released during normal coagulation and when platelets contact injured blood vessel walls (1–4).

Recently, serum from platelet-rich plasma was shown to increase random motion of aortic smooth muscle cells in culture (5). Subsequently, it was reported that purified PDGF induces migration of smooth muscle cells into collagen-coated filters (6). We now show that apparently homogenous preparations of human PDGF are chemotactic for human monocytes and neutrophils. PDGF, therefore, may act in concert with other platelet derived substances, such as platelet factor 4 (PF4) (7) and products of the platelet lipoxygenase pathway (8), in attracting inflammatory cells to the site of blood vessel injury.

METHODS

PDGF was purified from outdated, frozen human platelet packs into two active, electrophoretically homogenous peptide fractions of molecular weight 31,000 (PDGF I) and 28,000 (PDGF II) (9). PDGF I and PDGF II have essentially identical amino acid compositions, equal mitogenic activity, and apparently equal binding affinity for 3T3 cells, but differ in the amounts of covalently bound carbohydrate.[2] To insure consistency, however, only PDGF II has been used in these experiments. PDGF II is estimated to be ≃99% pure by SDS polyacrylamide gel electrophoresis and by isoelectric focusing. Immunodiffusion analysis of the IgG fraction of rabbit antisera to PDGF results in a single precipitin band of identity against purified PDGF and against concentrated platelet extracts.[3] PDGF II stimulated Swiss mouse 3T3 cells to induce DNA synthesis optimally at 20 ng/ml, as reported previously (9).

Epidermal growth factor (10) was obtained from Collaborative Research, Waltham, MA, and is estimated to be ≃99% pure. C5a, the chemotactic activity derived from the fifth component of complement, was obtained from serum by incubation with zymosan and ε-aminocaproic acid (1 M) at 37°C, followed by gel filtration over Sephadex G-100 (11). PF4 was purified to apparent homogeneity by previously reported methods (12).

Isolation of blood cells. Preparations of human blood mononuclear cells and neutrophils were obtained using Ficoll/Hypaque gradients and suspended in modified Eagle's medium supplemented with 2% human albumin (American Red Cross Blood Services, Washington, D. C.) at densities of 2.5×10^6 and 1.5×10^6 cells/ml, respectively (7). Direct microscopic examination confirmed >99% purity of the respective cell populations.

Chemotaxis assays. Chemotaxis was measured in triplicate in modified Boyden chambers using 5-μm pore (monocyte) or 2-μm pore (neutrophil) filters (Nucleopore Corp., Pleasanton, CA), overlying 0.45-μm pore filters (Millipore Corp., Bedford, MA) (7). C5a, at twice the concentration for

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[1] *Abbreviations used in this paper*: C5a, chemotactic activity derived from C5; HPG, high power grid; PDGF, platelet-derived growth factor; PF4, platelet factor 4.

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[3] Huang, J. S., and T. F. Deuel. Manuscript in preparation.

50% maximal chemotaxis, was used as the positive control. Five high power (×400) grids (HPG) were counted per filter. Cell migration was corrected by subtraction of blanks in which the lower compartment contained medium only. These blank values averaged 47±2.7, HPG, standard error of the mean (SEM), (monocyte migration) and 62±7.7, HPG (neutrophil migration).

In some experiments PDGF was incubated for 1 h (37°C) with 20 ng rabbit anti-PDGF IgG (600 ng protein) and then tested for chemotactic activity.

RESULTS

Fig. 1 depicts monocyte migration to increasing concentrations of PDGF. Migration activity was detectable at 5 ng/ml PDGF and maximum cell migration was found at 20 ng/ml. At >20 ng/ml, cell migration was markedly reduced. Essentially identical results have been found in three separate experiments. Maximum cell migration to PDGF was similar to that found with C5a in the same experiment. Protamine sulfate (100 μg/ml), a competitive inhibitor of PDGF binding to 3T3 cells, effectively blocked monocyte migration induced by PDGF.

"Checkerboard analysis" established that the cellular migration of monocytes toward PDGF represented chemotaxis, not enhanced random cell migration (chemokinesis). Table I shows that monocyte migration occurred only when the concentration of PDGF in the lower compartments exceeded that in the upper compartment, i.e., only in response to a gradient of PDGF with the higher concentration in the lower compartment. When equal concentrations of PDGF were present in lower and upper compartments, no migration above background was found.

TABLE I
*Monocyte Chemotactic Activity to PDGF**

		PDGF, Upper compartment, ng/ml			
		0	1	5	20
PDGF, lower compartment, ng/ml	1	35±2.6	5±2.0	3±3.1	−1±2.8
	5	54±2.8	7±2.3	2±3.1	1±2.8
	20	86±2.8	34±3.1	25±2.6	2±3.1

* SEM, n = 15

PDGF also stimulated PMN migration (Fig. 2). The peak PMN response was found at a PDGF concentration of ≃1 ng/ml and represented a response ≃60% that achieved with C5a. As PDGF concentrations were increased, cell migration decreased. At 20 ng/ml PDGF, cell migration was nearly back to base line. The results of duplicate experiments were in close agreement.

Experiments using rabbit anti-PDGF IgG supported the specificity of monocyte migration to PDGF (Fig. 3). PDGF-induced monocyte chemotaxis was blocked by preincubation of PDGF with the IgG. The chemotactic activities of C5a or PF4 were not influenced by incubation with anti-PDGF IgG. These results help establish that neither contaminating C5a nor PF4 is responsible for the monocyte chemotaxis induced by PDGF preparations, and exclude the possibility that contaminating PDGF may have been responsible for the chemotactic activity previously reported with PF4 (7).

To determine whether another well-defined growth factor has monocyte chemotactic activity, epidermal growth factor was tested at 10 concentrations between 0.5 and 100 ng/ml. This substance had no effect on monocyte migration (data not shown).

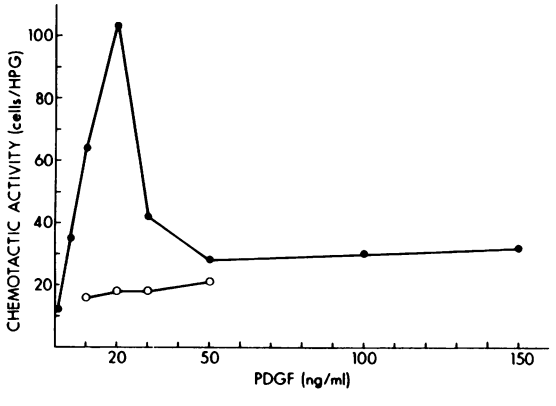

FIGURE 1 Monocyte migration to increasing concentrations of PDGF. PDGF at the concentrations shown was added to the lower compartment of modified Boyden chambers and cell migration measured as described in Methods. (●) Monocyte migration in response to PDGF alone. (○) Monocyte migration in response to PDGF in the presence of protamine sulfate 100 μg/ml. SEM were determined for each point and ranged from ±0.9 to ±2.3 cells/HPG.

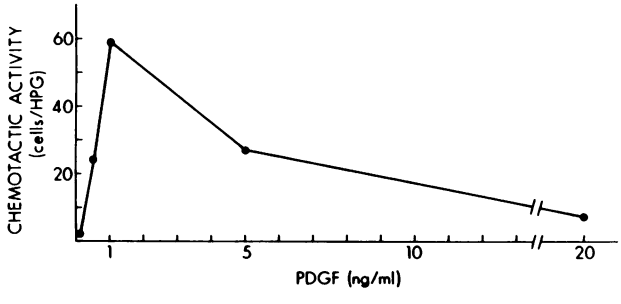

FIGURE 2 Polymorphonuclear leucocyte migration in response to increasing concentrations of PDGF. PDGF at the concentrations shown was added to the lower compartment of a modified Boyden chamber and cell migration measured as described in Methods. SEM for individual points ranged from ±1.0 to ±1.9 cells/HPG.

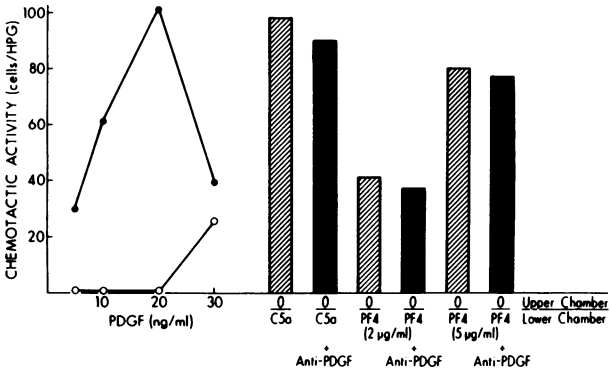

FIGURE 3 Specificity of monocyte migration. Left side: monocyte migration was measured as described in Methods with increasing concentrations of PDGF alone (●) or with increasing concentrations of PDGF and of rabbit anti-human PDGF IgG, (600 ng protein), (○). Right side: monocyte migration to saturating concentrations of C5a, PF4, 2 μg/ml, and PF4, 5 μg/ml in the presence and absence of rabbit anti-human PDGF antisera, (600 ng protein). SEM for individual points ranged from ±0.4 to ±1.5 cells/HPG.

We have found that protamine sulfate competes with PDGF for binding to Swiss mouse 3T3 cells.[2] Fig. 1 demonstrates that 100 μg/ml protamine sulfate effectively abolished monocyte migration to all levels of PDGF tested. The effect of different concentrations of protamine sulfate on PDGF stimulated monocyte migration was then tested. Fig. 4 shows that 10 μg/ml protamine sulfate inhibits monocyte migration ≃65%, and 20 μg/ml essentially abolishes monocyte migration to 20 ng/ml PDGF. Protamine sulfate may be toxic to cells at high concentrations, but no evidence of toxicity is found at 20 μg/ml, as indicated by the strong chemotactic response of monocytes to C5a. Indeed, the chemotactic activity of C5a was only minimally reduced (≃5%) by 20 μg/ml protamine sulfate.

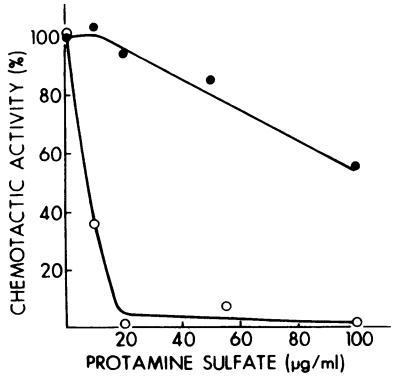

FIGURE 4 Chemotactic activity of C5a (saturating concentrations) and PDGF (20 ng/ml) in the presence of increasing concentrations of protamine sulfate. Chemotactic activity is expressed as a percentage of activity found in the absence of protamine sulfate. (●) C5a. (○) PDGF. SEM for individual points ranged from ±0.5 to ±1.8 cells/HPG.

DISCUSSION

Platelets have been implicated as a source of activity for recruiting inflammatory cells around injured blood vessels (13–17). Recently, we demonstrated that PF4, an ≃7,800-mol wt protein stored in platelet α-granules and released during platelet activation, is chemotactic for human PMN and human monocytes at concentrations found in human serum and likely to be found locally at the sites of injured blood vessels (7). PF4 has an unusual carboxyl terminal region containing two pairs of lysine residues and pairs of leucine and isoleucine residues respectively, suggesting a possible binding site of PF4 to cell surfaces (18). Chemical synthesis of the terminal tridecapeptide of PF4 yields a product even more chemotactically active than intact PF4 (7).

The experiments presented here show that a second platelet α-granule protein, PDGF, is a powerful chemotactic agent for monocytes and PMN at concentrations below those found in human serum after platelet release.[3] PDGF therefore has the capacity for a dual role at sites of platelet release, the recruitment of inflammatory cells and the recruitment and mitogenic stimulation of mesenchymal cells (2), suggesting that PDGF is important in inflammation and in tissue repair. These two activities suggest also that PDGF may be involved in the development of atherosclerosis. Much evidence suggests atherosclerosis begins at sites of endothelial cell loss and platelet deposition (19). Since PDGF is strongly chemotactic and mitogenic for cells localized in the atherosclerotic plaque, PDGF may be an important mediator of the proliferative responses characteristic of atherosclerosis. Models of experimental atherosclerosis have a requirement for circulating platelets (20, 21).

Platelets bind specifically to injured vessels and locally release granule contents. PDGF may thus be specific for sites of vessel injury. PF4 has been demonstrated to penetrate several cell layers deep in vessel walls freshly denuded of endothelium (22). Such experiments with PDGF have not yet been reported, although similar findings are likely since PDGF binds with high affinity to Swiss mouse 3T3 cells in vitro. It is noteworthy that another mitogenic factor, epidermal growth factor, has no effect on monocyte migration, indicating that not all mitogenic proteins are chemotactic for inflammatory cells.

Proteolytic activity cleaving C5 to C5a, arachidonic acid products generated through the lipoxygenase pathway (8), and PF4 (7) relate platelets to inflammatory reactions. The present data show that PDGF

is also a chemotactic agent for monocytes and neutrophils. Although the relative importance and regulation of platelet chemotactic factors remain to be established, it is evident that platelet α-granules are equipped with a multiplicity of factors potentially involved in the attraction of inflammatory cells.

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