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Polymorphonuclear Leukocytes Induce PDGF Release From IL-1β–Treated Endothelial Cells
Role of Adhesion Molecules and Serine Proteases

L. Totani, A. Cumashi, A. Piccoli, R. Lorenzet

Abstract—Polymorphonuclear leukocytes (PMNs) and endothelial cells interact at sites of vascular injury during inflammatory response and during the development of atherosclerotic lesions. Such close proximity leads to the modulation of several of the biological functions of the 2 cell types. Because we have shown previously that PMNs enhance release of growth factors from resting endothelial cells, we decided to evaluate whether coincubation of PMNs with interleukin-1β (IL-1β)–stimulated human umbilical vein endothelial cells (HUVEC) could further modulate mitogen release from HUVEC. We found that PMN-HUVEC coincubation resulted in a 10-fold increase in mitogen release, compared with HUVEC alone (14±6 versus 1.3±0.1). When PMNs were incubated with IL-1β–treated HUVEC, a further increase in mitogen release (up to 35-fold) was observed. The mitogenic activity was immunologically related to platelet-derived growth factor (PDGF) because the activity was abolished by an anti-PDGF antibody. PDGF-AB antigen, detected in low concentrations in conditioned medium from HUVEC alone, was increased 4-fold when IL-1β or PMNs were incubated with HUVEC and dramatically upregulated (up to 40-fold) when PMNs were cocultured with IL-1β–treated HUVEC. The presence of the protease inhibitor eglin C abolished mitogenic activity generation, suggesting a role for PMN-derived elastase and cathepsin G. Indeed, purified elastase and cathepsin G mimicked PMN-induced mitogen release from HUVEC. Because PMNs firmly adhered to IL-1β–treated HUVEC, we investigated the role of cell-cell adhesion in mitogen release. Adhesion and PDGF release were inhibited by ≈60% in the presence of anti-CD11a/CD18 and anti-intercellular adhesion molecule-1 monoclonal antibodies. This study suggests a new role for PMNs and their interaction with endothelium in pathological conditions in which intimal hyperplasia is a common feature. (Arterioscler Thromb Vasc Biol. 1998;18:1534-1540.)

Key Words: polymorphonuclear leukocytes ■ endothelial cells ■ adhesion molecules ■ serine proteases ■ PDGF

Leukocyte adhesion to cytokine-activated endothelial cells is a key event in inflammatory reactions. A well-regulated sequence of events controls adhesion of polymorphonuclear leukocytes (PMNs) to endothelial cells. The initial step involves adhesion molecules that belong to the selectin family: L-selectin on PMN membrane and P-selectin or E-selectin on activated endothelial cells. The selectin-dependent step is followed by affinity modulation of β2-integrins (CD11/CD18) that, in turn, bind to counter-receptors on the endothelial cell membrane, of which intercellular adhesion molecule–1 (ICAM-1), constitutively expressed but upregulated several-fold by agonists such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), plays a predominant role. Tight adhesion of PMN to the endothelial cell favors functional cell-cell cross-talk. Engagement of CD11b/CD18 (Mac-1), responsible for PMN adhesion to cytokine-activated endothelial cells, results in calcium influx and content release of secondary and azurophilic granules. CD11/CD18 integrins, together with ICAM-1 and E-selectin, also have been implicated in the enhanced LTB₄ production that is observed during PMN/TNF-α–activated endothelial cells cocultures. Very recently, PMN adherence to vascular endothelial cells has been shown to be responsible for triggering intracellular signals in endothelial cells that regulate cadherin/catenin complex disorganization. The binding of PMN through β2-integrins appears to be required.

Activated endothelial cells undergo enhanced detachment when exposed to unstimulated PMNs. The effect requires cell-cell contact and expression of PMN-derived proteases, because a filter insertion and/or specific inhibitors prevent endothelial damage. A role for membrane-bound proteases has also been suggested in PMN-induced microvascular endothelial cell killing. Among the several PMN-derived products, the proteolytic enzymes cathepsin G and elastase seem to play a prominent role in PMN-endothelial cell cross-talk. Cathepsin G has been shown to increase albumin flux across the endothelial monolayer. In addition, cathepsin G, as well as elastase, suppresses thrombin-induced prostacyclin production in human endothelial cells. Finally, cathepsin G stimulates calcium
increase and inositol phosphate production in endothelial cells.\textsuperscript{15} 

We have previously shown that serine proteases of PMN origin induce porcine aortic endothelial cells to synthesize and release platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), 2 mitogens endowed with chemotactic and mitogenic properties for mesenchymal-derived cells.\textsuperscript{16} This observation suggests that PMN-endothelial cell interaction may contribute to pathogenesis of intimal proliferation in the vascular wall and to cell proliferation and tissue fibrosis, which accompany inflammatory diseases. In animal models in which vascular injury was induced by balloon angioplasty, perivascular manipulation, or electrical injury, an early wave of PMN adhesion and infiltration preceding smooth muscle cells proliferation was observed.\textsuperscript{17,18} 

In the present study we have used a homologous, human, in vitro model to determine whether interaction of PMNs with cytokine-activated human umbilical vein endothelial cells (HUVEC) could modulate mitogen generation. Our results indicate that PMNs induce the secretion of a mitogen immunologically related to PDGF from HUVEC and that elastase and cathepsin G, released from adhering PMNs, are the mediators responsible for mitogen release. A novel role for CD11a/CD18 (LFA-1)-ICAM-1 recognition is suggested.

**Methods**

**Chemicals**

Medium 199, F-12 medium, and FCS were purchased from Eurobio (Les Ulis), DMEM, CS, penicillin, streptomycin, glutamine, and trypsin-EDTA were purchased from Gibco BRL. HEPES and citrate were obtained from Merck. Nutridoma-NS was purchased from Boehringer-Mannheim. Dextran T500 and Ficoll-Hypaque came from Pharmacia Fine Chemicals. Endothelial cell growth factor (ECGF) was prepared according to Maciag et al.\textsuperscript{20} Peprotec and Dr D. Boraschi (Dompe Research Center, L’Aquila, Italy) were sources of recombinant IL-1α. Heparin, SBTI (1 mg protein inhibits 3 to 5 mg trypsin with activity of approximately 10 000 benzoylarginine ethyl ester units per milligram of protein), α1-antitrypsin (α1-AT) (2 to 4 mg inhibit 1.0 mg of trypsin with activity of 10 000 benzoylarginine ethyl ester units per milligram of protein), SOD (activity, 2.5 to 5.0 U/mg protein), catalse (activity, 2.0 to 5.0 U/mg protein), BSA collagenase, and BCECF-AM were purchased from Sigma Chemical Co. Eglin C, cathepsin G, and elastase from human neutrophils were from Calbiochem Biochemicals. The anti-CD11a and anti-CD11b subunits of the CD11/CD18 integrin were from the hybridoma cells (clones TS1/22.1.13 and LM2/1.6.11, respectively) purchased from ATCC. The anti–ICAM-1 monoclonal antibody was kindly provided by Dr Nancy Hogg. The antibodies were from the hybridoma cells (clones TS1/22.1.13 and LM2/1.6.11, respectively) purchased from ATCC. The anti–ICAM-1 monoclonal antibody was kindly provided by Dr Nancy Hogg. The antibodies were used as purified IgG1 at 30 μg/mL. This concentration was found saturable. The polyclonal goat anti-PDGF antibody was from R & D Systems. Methyl-[3H]-thymidine ([3H]-TdR) (2 Ci/mmol) was purchased from Du Pont de Nemours, Biotechnology Systems Division. Tissue culture dishes were from Falcon Labware Division, Becton Dickinson Co and Costar Data Packing Corp.

**Cell Culture**

Endothelial cells were obtained from human umbilical vein as previously described.\textsuperscript{21} For experiments, HUVEC were plated in 12-well plates at a concentration of 8×10⁴/well and grown to confluence in a humidified atmosphere of 93% air, 7% CO₂ at 37°C in 50% 199 medium, 50% DMEM supplemented with 15% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1% glutamine. The number of HUVEC at confluence was 0.25×10⁶/well. HUVEC were always used at the second passage, and cells were shown to be von Willebrand factor-positive as judged by immunofluorescent staining.

**PMN Preparation**

Human blood was collected from healthy donors who had not taken any drugs during the 2 weeks preceding the study, using citrate as anticoagulant. PMNs were isolated by dextran sedimentation and density-gradient centrifugation using the Ficoll-Hypaque technique according to Bøyum.\textsuperscript{22} The cells were resuspended in F-12 medium supplemented with 1% Nutridoma-NS at the desired concentration.

**Release Studies**

Confluent HUVEC were washed 3 times with 199-medium containing 2 mg/mL of BSA before incubation with or without IL-1b (10 ng/mL) in F-12 medium containing 1% Nutridoma-NS. After 90 minutes incubation, PMNs were added to HUVEC in a final volume of 0.5 mL at 37°C in 7% CO₂ over a period of 15 hours, unless otherwise specified. When SBTI (100 μg/mL), α1-AT (1 mg/mL), eglin C (100 μg/mL), SOD (50 μg/mL) + Catalase (50 μg/mL) or monoclonal antibody (MoAb) to ICAM-1 were used, they were added to HUVEC at the beginning of incubation. MoAbs directed against the adhesive molecules CD11a and CD11b were added to PMNs 30 minutes before coincubation with the endothelial monolayer. At the desired time, the conditioned medium was collected, centrifuged for 5 minutes at 12 000 rpm to remove cellular debris, and stored at −20°C until mitogen assay.

**Mitogenic Activity Assays**

To determine the level of the total mitogenic activity of endothelial cell–conditioned medium, BALB/c 3T3 mouse fibroblasts (clone A31, American type Culture Collection) were used as previously described.\textsuperscript{23} The cells were grown to near confluence in 96-well microtiter plates and then induced to quiescence by 48-hour exposure to DMEM supplemented with 5% heat inactivated plasma–derived human serum. Aliquots (3 to 80 μL) of endothelial cell–conditioned medium samples were added to the 3T3 cell cultures and incubated for 15 hours at 37°C. Medium, 7.5% humidified incubator. The resulting mitogenic stimulation was then measured by pulsing with [3H]-TdR (0.25 μCi/well) for 3 hours. At the end of incubation, the cells were washed and harvested on a filter paper by a Cell Harvester (Inotech). The filter was then placed in an Automatic Filter Counting System (Inotech), and the activity was determined by evaluation of the [3H]-TdR incorporated into DNA by ionizing scintillation. For each experiment, a standard curve using increasing concentrations of calf serum was generated. One unit of mitogenic activity was defined as the activity conferred by aliquots of the conditioned medium equivalent in magnitude to 50% of the maximal mitogenic effect observed with calf serum in the concomitant standard curve.

**Measurement of Elastase and Cathepsin G Release**

Confluent HUVEC were incubated in phenol red-free RPMI medium, 1% BSA, with or without IL-1b (10 ng/mL), 90 minutes before PMN (2.5×10⁶/well) addition. Conditioned medium, collected at different time intervals (from 30 minutes to 15 hours) was analyzed for elastase and cathepsin G activity. The elastase- and cathepsin G–specific substrates MEO-succinyl-Ala-Ala-Pro-Val-p-nitroanilide and N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, respectively, were added to conditioned medium (final concentration, 100 μmol/L) and the hydrolysis of p-nitroanilide was measured as variation of optical density per minute, at 410 nm.\textsuperscript{24} 

**PDGF Determination**

Conditioned medium from PMNs and HUVEC, alone or together, was sedimented by centrifugation to eliminate cellular debris, and the presence of PDGF was assessed by enzyme-linked immunosorbent assay (ELISA) as specified by the manufacturers (Amer sham). The assay quantitates human PDGF-AB and its sensitivity is 1.68 pg/well (8.4 pg/mL) in tissue culture supernatants.

**Immunoinhibition Studies**

The postculture medium from HUVEC/PMN coincubation was preincubated with goat anti-human PDGF-AB neutralizing antibody or with nonimmune goat immunoglobulin G for 15 minutes at 37°C.
pmns (106/mL) for 1, 3, 6, and 15 hours. Conditioned medium steadily increased with time. When PMNs were added to activity became detectable between 1 and 3 hours and reached a 35-fold increase within 15 hours when compared with HUVEC alone. The effect was time-dependent and could be detected after 3-hour incubation (Figure 1). Little if any activity could be detected in conditioned medium of HUVEC cultured in the absence of stimulation (Figure 1). A strong mitogenic activity was already detectable after 3 hours and reached a 35-fold increase within 15 hours when compared with HUVEC alone. A strong mitogenic activity was already detectable after 3 hours and reached a 35-fold increase within 15 hours when compared with HUVEC alone. PMNs, was incubated with HUVEC, in agreement with similar results were observed when IL-1β–treated HUVEC was incubated in the presence of antibodies directed against several adhesion molecules. The effect of the antibodies is shown in Figure 2. The mitogenic activity expressed in conditioned medium from IL-1β–treated HUVEC incubated with PMNs in the absence of antibodies was assigned a value of 100%. The presence of MoAb 15.2, directed against the endothelial ICAM-1 adhesion molecule, prevented mitogen release by 60%. Because PMN binding to endothelial ICAM-1 is mediated by the integrins CD11a and CD11b, we tested whether these adhesion molecules were involved. MoAb TS1/22, directed against CD11a, could prevent PDGF release to the same extent as anti-ICAM-1. By contrast, MoAb LM2/1, which specifically binds to CD11b, had no effect. Interestingly, the MoAbs reduced binding of PMNs to HUVEC by 47% and 51%, respectively. No effect on either mitogenic activity or adhesion was observed with a matched control antibody.

**Adhesion Assay**
PMN/HUVEC adhesion was determined as previously described. Confluent HUVEC were incubated in F-12 medium with or without IL-1β (10 ng/mL for 2 hours). PMNs were prelabeled with the fluorescent probe bis-carboxyethyl-carboxyfluorescein acetoxymethyl ester for 30 minutes at 37°C. Fluorescent PMNs (105/well) were incubated with HUVEC for 30 minutes at 37°C. At the end of coincubation, the wells were rinsed 3 times to remove nonadherent PMNs, and adherent cells were lysed in 0.2% Triton-X-100. The number of adherent PMNs was quantified by fluorometric assay. All reagents used in this study were dissolved in sterile solvents and filtered through nonpyrogenic sterile 0.22-μm Millex filters (Millipore). To avoid endotoxin contamination, sterile, pyrogen-free working conditions were observed.

**Statistical Analysis**
All results represent mean±SEM at least 3 separate experiments. Statistical analysis was performed by ANOVA (randomized complete block), using Dunnett’s test for multiple comparisons.

**Results**

**Effect of PMNs on Growth Factor Release from Resting and IL-1β-Stimulated HUVEC**
Coincubation of PMNs with HUVEC (ratio, 4:1) resulted in release of mitogenic activity into the surrounding medium, as assessed by [3H]-TdR incorporation into 3T3 cells. The effect was time-dependent and could be detected after 3-hour incubation (Figure 1). Little if any activity could be detected in conditioned medium of HUVEC cultured in the absence of PMNs within 15 hours. Similar results were observed when IL-1β, instead of PMNs, was incubated with HUVEC, in agreement with earlier observations. The effect was time-dependent: the activity became detectable between 1 and 3 hours and steadily increased with time. When PMNs were added to IL-1β–treated HUVEC, an increase in growth factor release could be observed. A strong mitogenic activity was already detectable after 3 hours and reached a 35-fold increase within 15 hours when compared with HUVEC alone. The effect was synergistic, because conditioned medium collected after 15 hours’ incubation of HUVEC with PMNs or IL-1β was responsible for 7- and 10-fold increases in mitogenic activity, respectively, compared with HUVEC cultured alone.

Supernatants from PMNs incubated with or without IL-1β in gelatin-coated wells did not induce [3H]-TdR incorporation into 3T3 cells, indicating that either resting or stimulated PMNs are not the source of the mitogenic activity observed in our experimental system; this is in agreement with previous observations. To exclude the possibility that the observed effect on the 3T3 cells could be due to the cooperation of IL-1β and components of conditioned medium, IL-1β, at the maximal concentration used in our experiments, to postculture medium from HUVEC or HUVEC/PMN coincubation before the mitogenic assay was added. [3H]-TdR incorporation into 3T3 cells induced by medium conditioned by HUVEC or HUVEC/PMN coincubation was not modified by IL-1β addition (not shown).

The mitogenic activity released by endothelial cells has been shown to be attributable, at least in part, to a PDGF-like molecule. To determine whether PDGF was released in our experimental conditions, conditioned medium from the different incubations was collected, and PDGF–AB antigen was quantified by ELISA. A small amount of PDGF–AB antigen was detected in conditioned medium from resting HUVEC (53±10 pg/mL, mean±SE; n=3). Addition of PMNs or IL-1β caused an enhancement in PDGF–AB release from HUVEC (231±56 pg/mL and 215±40 pg/mL, respectively; n=3). When PMNs were incubated with IL-1β–treated HUVEC, a dramatic increase in the release of PDGF–AB was observed (2086±404 pg/mL; n=3).

To investigate whether the released PDGF was responsible for the activity, a neutralizing polyclonal antibody anti-PDGF was incubated with conditioned medium from PMN/IL-1β–treated HUVEC before mitogenic assay. The anti-PDGF inhibited the activity by 80±10%, indicating that PDGF was responsible for the activity. Control immunoglobulin G did not significantly modify mitogenic activity.

**Role of Adhesion Molecules**
PMN interactions with endothelium are mediated by adhesion molecules. We explored the possible role of adhesive mechanisms in PMN-induced PDGF release. Coincubation of PMNs with IL-1β–treated HUVEC was carried out in the presence of antibodies directed against several adhesion molecules. The effect of the antibodies is shown in Figure 2. The mitogenic activity expressed in conditioned medium from IL-1β–treated HUVEC incubated with PMNs in the absence of antibodies was assigned a value of 100%. The presence of MoAb 15.2, directed against the endothelial ICAM-1 adhesion molecule, prevented mitogen release by 60%. Because PMN binding to endothelial ICAM-1 is mediated by the integrins CD11a and CD11b, we tested whether these adhesion molecules were involved. MoAb TS1/22, directed against CD11a, could prevent PDGF release to the same extent as anti-ICAM-1. By contrast, MoAb LM2/1, which specifically binds to CD11b, had no effect. Interestingly, the MoAbs reduced binding of PMNs to HUVEC by 47% and 51%, respectively. No effect on either mitogenic activity or adhesion was observed with a matched control antibody.
Effect of Antiproteases on PDGF Release From PMN/IL-1β-Treated HUVEC

To define the mechanism of PMN-induced PDGF release, we investigated the involvement of oxygen-free radicals and proteases. PMNs were coincubated with resting or IL-1β-activated HUVEC monolayer in the presence of various serine protease inhibitors or oxygen-radical scavengers. SOD (50 μg/mL) together with catalase (50 μg/mL), and the serine-protease inhibitors α1-AT (2 mg/mL), SBTI (100 μg/mL) and eglin C (100 μg/mL) were added to IL-1β-treated HUVEC before PMN addition. The serine protease inhibitors α1-AT, SBTI, and eglin C prevented PMN-induced mitogenic activity from IL-1β-activated HUVEC by more than 80% (Figure 3). SOD and catalase however, were not effective, ruling out the involvement of oxygen-free radicals. PMN adhesion to IL-1β-treated HUVEC was not affected by the presence of the different inhibitors. These data suggest that PMN-derived proteases play a role in PDGF release.

Effect of PMN-Derived Proteases on Growth Factors Release From HUVEC

Because eglin C is a specific inhibitor of elastase and cathepsin G,27 we tested the behavior of these proteases in our cultures. Elastase and cathepsin G were incubated for 15 hours with HUVEC instead of PMNs. As can be seen in Figure 4, elastase and cathepsin G induced release of growth factors from HUVEC in a dose-dependent way. The contemporary presence of these proteases resulted in an additive effect. Concentrations of elastase exceeding 50 nmol/L caused endothelial cell detachment and, therefore, could not be used. The enzymatic properties of elastase were essential for the observed activity, because preincubation with PMSF (1 mmol/L) or α1-AT (2 mg/mL) prevented elastase (40 nmol/L)-induced mitogen release from HUVEC (not shown). The addition of these proteases to conditioned medium from IL-1β-treated HUVEC before mitogen assay did not modify mitogenic activity, indicating that postsecretion modification of components released into the conditioned medium was not taking place (not shown).

In a different set of experiments, elastase was incubated with resting or IL-1β-treated HUVEC for 15 hours. At the end of incubation, PDGF-AB antigen was quantified by ELISA in conditioned medium. Elastase-induced PDGF release from resting or IL-1β-treated HUVEC was not significantly different (818 ± 93 and 928 ± 110 pg/mL, respectively, mean ± SEM; n=5). In the same set of experiments, IL-1β-induced PDGF release was 278 ± 96 pg/mL. These results rule out the possibility that the strong effect of IL-1β + PMNs compared with the proteases alone might be explained by IL-1β-triggered PDGF transcription.
PMN-Derived Proteases in PMN/HUVEC Coincubation

The overall picture seems to indicate that PMN activation by IL-1β–treated HUVEC results in PMN degranulation and protease secretion. To test this hypothesis, PMNs were incubated with IL-1β–treated HUVEC for different time intervals, and elastase activity in conditioned medium was then measured as described in Methods. Tests from 5 different experiments failed to detect any activity, raising the question of whether elastase activity could be measured in our experimental conditions.

To answer this question, known amounts of purified elastase (from 10 to 50 nmol/L) were added to HUVEC, and conditioned medium was collected at defined intervals and tested. Elastase activity in conditioned medium from HUVEC, even after brief incubations (5 minutes), was reduced by 97 ± 3.7% (mean ± SEM; n = 5) compared with the activity of elastase incubated in the absence of HUVEC. Similar results were obtained when cathepsin G was tested.

Discussion

In this report, we show for the first time that coincubation of PMNs with resting HUVEC upregulates release of PDGF-AB and that this effect is several-fold more pronounced when PMNs are cultured with IL-1β–treated HUVEC. Although IL-1β per se induces release of mitogens from HUVEC, the contemporary presence of PMNs and IL-1β results in a synergistic effect.

Cultured endothelial cells exposed to IL-1β synthesize and express adhesion molecules on their outer membrane. Direct cell-cell adhesion facilitates PMN cross-talk with different cell types including endothelial cells and platelets. In our experimental system, candidates for this adhesion are the immunoglobulin-like molecule ICAM-1 on the endothelial cell surface and the leukocyte integrins of the CD18 family. As was also found in our experimental conditions, antibodies directed against CD11a and CD11b, members of the CD-18 family, inhibit PMN adherence to stimulated endothelial cells. In the presence of MoAb anti-CD11a or MoAb anti-CD11b, adherence of PMNs to IL-1β–treated HUVEC was reduced by approximately 50%. The inhibition in binding caused by anti-CD11a was accompanied by a marked reduction in mitogen release. Similar inhibition was also observed with a MoAb directed against ICAM-1. Although anti-CD11b was as effective as anti-CD11a in inhibiting PMN binding, no effect on mitogen release could be observed. These results seem to indicate that PMN/HUVEC binding is necessary for the enhanced generation of growth factors and that recognition between CD11a and ICAM-1 is, at least in part, responsible for this effect.

Mitogen release can be inhibited by antiproteases; among these is eglin C, which is a specific inhibitor of elastase and cathepsin G. These proteases, which are present in large amounts in PMN azurophilic granules, have been previously been shown to suppress prostacyclin production, induce release of plasminogen activator inhibitor, increase calcium flux and inositol polyphosphate production in cultured endothelial cells, and cleave the αIIb subunit of the receptor GPIIb/IIa leading to platelet activation. In our experimental system, purified elastase and cathepsin G induced mitogen release from HUVEC in a dose-dependent way, mimicking the effect of PMNs.

This set of experiments, together with the results obtained with the antiadhesion molecules antibodies, is consistent with the hypothesis that adherence of PMNs to IL-1β–activated HUVEC is responsible for PMN degranulation. After elastase and cathepsin G are released, they will, in turn, stimulate PDGF synthesis by HUVEC. The failure to detect these proteases in PMN/HUVEC–conditioned medium does not undermine our hypothesis, as the possibility of a rapid removal and/or inactivation of elastase and cathepsin G by endothelial cells cannot be dismissed.

It could be argued that in a more physiological milieu, α1-AT contained in plasma would inhibit the elastase and cathepsin G released by PMNs. For example, it has been shown that, at sites of inflammation, the close contact between PMNs and endothelial cells creates a protected microenvironment in which the proteases cannot be reached by the inhibitor. It is also possible that accumulation of PMNs at sites of inflammation and the release of high amounts of proteases overcome the protective effect of plasma antiproteinas. Furthermore, it has been reported that plasma α1-AT can be inactivated by PMN-derived oxygen radicals, which are usually generated at sites of inflammation.

The CD11a-ICAM-1–mediated adhesion might represent a regulatory pathway for PMN activation and protease expression. This hypothesis is consistent with the observation that engagement of members of the adhesion molecule family generates signals activating leukocyte function. Indeed, it has been shown that occupancy of CD11/CD18 sustains LTB4 production and elastase release by PMNs. Antibody-mediated cross-linking of CD18 induced, within seconds, a transient rise of intracellular free Ca2+ concentration, actin polymerization, and exocytosis of azurophilic granules. Coculture of monocytes with HUVEC caused upregulation of IL-1β and TNF-α synthesis, and this phenomenon could be blocked in the presence of antibodies directed against CD18. Moreover, engagement of CD11/CD18 primed neutrophils for activation of the respiratory burst, and this induction was mediated by CD11a and CD11c. Finally, it has been shown that PMN adhesion on plastic surfaces is accompanied by PMN degranulation.

Our results cannot rule out the possibility that CD11a-ICAM-1 binding results in intracellular signals for endothelial cells, leading ultimately to PDGF release. Indeed, purified elastase and cathepsin G were less effective than PMNs in inducing mitogenic activity from HUVEC. The possibility that this difference could be attributed to an IL-1β–triggered PDGF transcription was ruled out by the experiments showing the same amount of PDGF-AB antigen in conditioned medium from resting and IL-1β–treated HUVEC incubated with elastase. These data suggest that other mechanisms and/or molecules may be involved. For example, a role for ICAM-1 in triggering Src activity and phosphorylation of the cytoskeletal protein cortactin has been reported.

Endothelial cells synthesize and release PDGF into the surrounding medium, and modulation of its synthesis has pre-
We have recently shown that PMNs cultured in the presence of porcine aortic endothelial cells induce release of mitogenic activity whose nature is mostly attributable to PDGF and bFGF. In the present study PDGF-AB antigen, detected in low concentrations in conditioned medium from PMN- or IL-1β-treated HUVEC, was highly upregulated when both stimuli were present during incubation with HUVEC. This PDGF was active and responsible for the mitogenic activity, because a specific antibody, anti-PDGF-AB, almost completely inhibited the activity. PDGF is involved in intimal thickening. PDGF mRNA, by in situ hybridization, was found in endothelial cells in human coronary arteries at the site of PTCA injury and in the intimal proliferative lesions of vascular rejection in renal allografts.

If growth factors are considered the final actors in the play of neointimal formation in vivo, the acts leading to cell proliferation are carried out by several other agents. Among these, IL-1β and adhesion molecules play a primary role. In vivo, IL-1β mRNA and protein have been detected on endothelium covering atherosclerotic plaque, as well as on endothelium from coronary arteries in piglets undergoing postcardiac transplant coronary arteriopathy. Similarly, endothelium expression of ICAM-1 and E-selectin has been detected in human atherosclerotic plaque and in transplanted human heart. The expression of adhesion molecules leads to leukocyte accumulation to the site of inflammation; a strong association between expression of adhesion molecules on endothelium from neovasculature and intimal accumulation at inflammatory cells in human atherosclerosis has been demonstrated. Furthermore, PMN vascular infiltration has been described in various animal models of vascular damage evolving toward neointimal formation.

Our results suggest that PMN adherence to IL-1β-treated endothelium is accompanied by release of cathepsin G and elastase. A direct correlation between elastolytic activity and intimal thickening recently has been provided by Cowan and colleagues. These authors described a positive effect of elastase on the neointimal proliferation and intimal thickening after allograft arteriopathy in an in vivo animal model. The overall picture for the role of PMNs in human vascular disease has been corroborated by epidemiological studies in which a positive correlation between increased PMN number and risk of ischemic disease and the severity of coronary artery obstruction was observed.

In conclusion, in the present study we have shown that PMNs induce the release of a molecule immunologically related to PDGF from endothelial cells. PDGF release could be amplified several-fold when PMNs were adhering on IL-1β-treated HUVEC. This effect seems to be related to proteases such as elastase and cathepsin G, which may be released by adhering PMNs. The proposed mechanism is depicted in Figure 5. On the basis of these results, it is conceivable that, in pathological conditions in which PMN infiltration is a predominant feature, the cell-cell functional cross-talk that takes place may support a condition that favors neointimal formation.

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References

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