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Hypoxia-mediated induction of acidic/basic fibroblast growth factor and platelet-derived growth factor in mononuclear phagocytes stimulates growth of hypoxic endothelial cells

(endothelium/angiogenesis)

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ABSTRACT Wound repair and tumor vascularization depend upon blood vessel growth into hypoxic tissue. Although hypoxia slows endothelial cell (EC) proliferation and suppresses EC basic fibroblast growth factor (bFGF) expression, we report that macrophages (MPs) exposed to $PO_2 \approx 12$ – 14 torr (1 torr = 133.3 Pa) synthesize and release in a time-dependent manner platelet-derived growth factor (PDGF) and acidic/basic FGFs (a/bFGFs), which stimulate the growth of hypoxic ECs. Chromatography of hypoxic MP-conditioned medium on immobilized heparin with an ascending NaCl gradient resolved three peaks of mitogenic activity: activity of the first peak was neutralized by antibody to PDGF; activity of the second peak was neutralized by antibody to aFGF; and activity of the third peak was neutralized by antibody to bFGF. Metabolically labeled lysates and supernatants from MPs exposed to hypoxia showed increased synthesis and release of immunoprecipitable PDGF and a/bFGF in the absence of changes in cell viability. Possible involvement of a heme-containing oxygen sensor in MP elaboration of growth factors was suggested by the induction of bFGF and PDGF by normoxic MPs exposed to nickel or cobalt, although metabolic inhibitors such as sodium azide were without effect. These results suggest a paracrine model in which hypoxia stimulates MP release of PDGF and a/bFGF, inducing EC proliferation and potentially promoting angiogenesis in hypoxic environments.

As the cells lining the vascular lumen, endothelial cells (ECs) are rapidly and directly subject to alterations in blood oxygen tension. Cultured ECs exposed to hypoxic conditions demonstrate decreased proliferation and migration: a confluent EC monolayer wounded by removing a swath of cells and then placed in hypoxia exhibits a considerably delayed reparative response compared with normoxic controls (1). This situation poses a pathophysiologic paradox, since EC proliferation and migration, necessary for angiogenesis in repair of injury or in vascularization of tumors (2), usually occur in the setting of hypoxemia and tissue hypoxia.

One way to resolve this apparent contradiction was suggested by the observations of Knighton *et al.* (3) that hypoxic macrophages (MPs) secrete angiogenic activity. In this context, we have found that exogenous basic fibroblast growth factor (bFGF) enhances proliferation and migration of hypoxic ECs (1). Taken together, these data showed the importance of identifying which cells present in the vascular wall produce mitogens under hypoxic conditions and determining whether these mitogens stimulate the growth of hypoxic ECs. The

results of the current study indicate that, on exposure to hypoxia, human MPs, but not smooth muscle cells or fibroblasts, synthesize and secrete acidic and basic FGF (a/bFGF) and platelet-derived growth factor (PDGF) that stimulate the growth of hypoxic ECs. These results support a paracrine model for the regulation of EC proliferation in wound repair/ischemia in which hypoxic MPs produce a/bFGF and PDGF, leading to EC proliferation and migration toward sites of MP accumulation.

MATERIALS AND METHODS

Cell Culture. MPs, adherent cells from the mononuclear fraction of human peripheral blood, were obtained as described previously (4). Human vascular smooth muscle cells were obtained from the aortic arch of cardiac transplant donors by the method of Colucci *et al.* (5). Human fibroblasts were obtained after extensive scraping of vessels following isolation of ECs and smooth muscle cells. Bovine aortic and adrenal capillary ECs were obtained and grown as described previously (1).

Exposure of Cells to Hypoxia. Cells were cultured at the following densities: MPs, $\approx 3 \times 10^4$ cells per cm^2 ; smooth muscle cells, $\approx 5 \times 10^5$ cells per cm^2 ; fibroblasts and ECs, $\approx 10^5$ cells per cm^2 . Fresh serum-free medium [equilibrated to a $PO_2 \approx 14$ torr (1 torr = 133.3 Pa)] was added as cultures were placed in a chamber which maintained a humidified atmosphere with low oxygen concentrations (Coy Laboratory Products, Ann Arbor, MI). During the course of these experiments, the PO_2 of the culture medium remained ≈ 14 torr, the pH of the medium remained constant, and by 48 h the glucose concentration fell from 5 mM to ≈ 3 mM. This degree of glucose depletion by itself did not induce expression of mitogens in any of the cells studied under normoxic conditions.

EC Proliferation Assays. Bovine aortic or capillary ECs were plated (2×10^4 cells per cm^2) and incubated for 24 h in hypoxia. EC growth under hypoxia was assessed during the subsequent 24 h (total time in hypoxia of 48 h) by incorporation of [*methyl*- 3H]thymidine added during the last 7 h of the experiment. The effect of culture medium conditioned by various cultured cells or samples from the heparin column (see below) on EC growth in hypoxia was studied by adding them 24 h after plating the ECs. Where indicated, these test samples were first preincubated for 8 h at 4°C with the IgG fraction of rabbit anti-bFGF (at the indicated concentration; ref. 6; R&D Systems), rabbit anti-aFGF (at the indicated concentration;

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Abbreviations: EC, endothelial cell; MP, mononuclear phagocyte/macrophage; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; VPF/VEGF, vascular permeability factor/vascular endothelial growth factor.

R&D Systems), rabbit anti-PDGF (at the indicated concentration; R&D Systems; this antiserum recognizes PDGF A and B chains), rabbit anti-hst/K-FGF (20 $\mu\text{g/ml}$; ref. 7; generously provided by C. Basilico, New York University School of Medicine, NY), or rabbit anti-human vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) (40 $\mu\text{g/ml}$; generously provided by J. Olander, Monsanto). In each case, controls with nonimmune rabbit IgG had no effect on EC proliferation in the presence or absence of test samples.

Chromatography of MP-Conditioned Medium on Immobilized Heparin. Conditioned medium (50 ml) from MP cultures ($\approx 10^7$ cells) exposed to hypoxia ($pO_2 \approx 14$ torr) for 48 h was applied directly to heparin Ultragel (bed volume, 2.5 ml; IBF Biotechnics, Columbia, MD). Material was eluted from the column with an ascending salt gradient (0.02–2 M NaCl). Collected fractions (1 ml) were dialyzed against Hanks' balanced salt solution, filtered (0.2- μm pore size), and equilibrated with the hypoxic gas mixture, and then 0.1 ml was added to the hypoxic EC cultures (total volume of medium, 2 ml) to assess its effect on cell proliferation.

Immunoprecipitation of Hypoxic MP Supernatants and Lysates for PDGF and a/bFGF. MPs were incubated in methionine- and cysteine-poor minimal essential medium (GIBCO) for 8 h under hypoxia, [^{35}S]methionine and [^{35}S]cysteine were added, and metabolic labeling was continued for another 8 h under hypoxic conditions. Normoxic cultures were treated in an identical fashion and labeled for 8 h, as for hypoxic cells. Culture supernatants of hypoxic MPs were subjected to immunoprecipitation with rabbit anti-aFGF IgG (30 $\mu\text{g/ml}$), anti-bFGF IgG (30 $\mu\text{g/ml}$), or anti-PDGF IgG (30 $\mu\text{g/ml}$) by adding the antibody to 15 ml of conditioned medium (derived from $\approx 4 \times 10^6$ cells) and incubating the mixture for 12 h at 4°C. Then, a suspension of *Staphylococcus aureus* protein A (0.4 ml per tube; 10%; IgGSorb, The Enzyme Center, Malden, MA) was added, the mixture was incubated for 30 min at 4°C, and the precipitate was collected by centrifugation (5000 rpm for 10 min). The pellet was washed three times in Tris-buffered saline (20 mM Tris-HCl, pH 7.4/0.1 M NaCl) containing 0.05% Triton X-100 and 1 mM EDTA. Samples were then boiled in reducing Laemmli sample buffer (8) and electrophoresed through SDS/15% polyacrylamide. Gels were fixed, soaked in Enhance (New England

Nuclear), dried, and autoradiographed. Immunoprecipitation of growth factors from metabolically labeled cell lysates was performed according to the same method described above, except that cell pellets were washed three times with Tris-buffered saline, frozen at -80°C , thawed, and lysed in the same buffer also containing 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 100 units of Trasylol per ml.

Northern Analysis for bFGF and FGF Receptors. Northern blots to assess levels of bFGF and the FGF receptors flg and bek (9) in hypoxic ECs were carried out by extracting total RNA from cells (10), performing electrophoretic fractionation of the RNA on a 1.2% agarose gel (11), and transferring this fractionated RNA to nitrocellulose. cDNA probes for bFGF, flg, and bek, generously provided by C. Dionne (Rhone-Poulenc Rorer, Horsham, PA), were labeled by random hexamer labeling (Random Primed DNA Labeling Kit, Boehringer Mannheim). Hybridization of cDNA probes to normoxic and hypoxic cell RNA was carried out at 42°C. The intensity of hybridization bands on autoradiograms was quantitated by densitometry (Microcomputer Imaging Device, Imaging Research, St. Catherine's, ON, Canada).

Radioreceptor Assays for PDGF and bFGF. Quantification of the bFGF and PDGF content of macrophage supernatants from cultures exposed to either hypoxia or metabolic inhibitors was accomplished by using radioreceptor assays and concentrated conditioned medium (concentrated 20-fold by using the Centricon system; 3000 molecular weight cutoff; Amicon). The radioreceptor assay for bFGF was adapted from the methods of Moscatelli (12) and Olwin and Hauschka (13). The limit of detection in this assay was 0.5 ng. The radioreceptor assay for PDGF was performed according to the method of Witte *et al.* (14). The limit of detection in this assay was 0.3 ng.

Statistical Analysis. Statistical analysis was performed by the Neuman-Keuls method for multiple comparisons by using analysis of variance.

RESULTS AND DISCUSSION

Hypoxic ECs and Cell Growth: Stimulation of Proliferation by Conditioned Medium from Hypoxic MPs. Growth of bovine aortic and capillary ECs slows under hypoxic ($pO_2 \approx 14$ torr) conditions (1). Recent findings linking decreased levels of EC

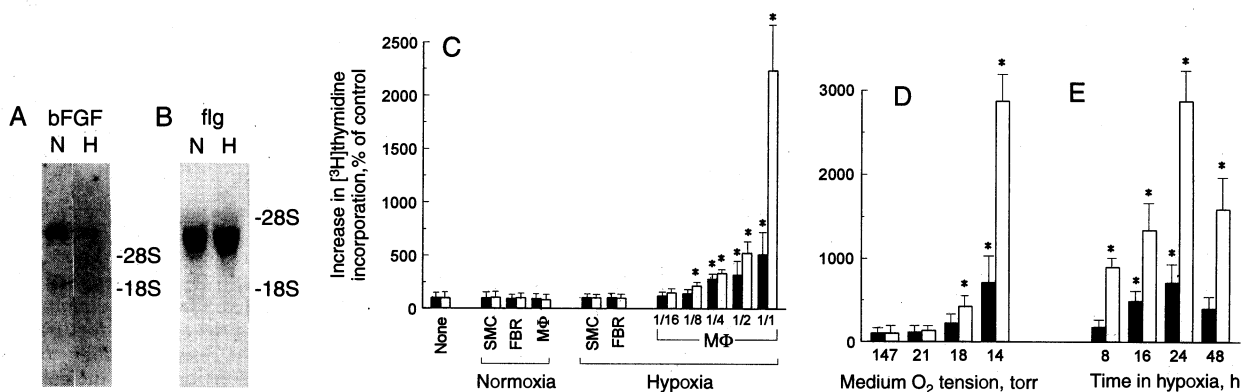


FIG. 1. Effect of hypoxia on EC growth: expression of mRNA for bFGF and the FGF receptor flg and modulation of EC proliferation by medium conditioned by smooth muscle cells, fibroblasts, or macrophages. (A and B) Effect of hypoxia on mRNA levels of bFGF and flg. Subconfluent bovine capillary ECs (2×10^4 cells per cm^2 ; total of 2×10^7 cells) were incubated for 24 h in hypoxia (H) or normoxia (N). Total RNA was subjected to agarose gel electrophoresis (25 μg of RNA per lane), transferred to nitrocellulose, and hybridized with ^{32}P -labeled cDNA probes for bFGF (A) or flg (B). The positions of 28S and 18S rRNA are noted. (C) Effect of conditioned medium from hypoxic fibroblasts (FBR), smooth muscle cells (SMC), or MPs (M Φ) on the growth of hypoxic aortic (dark bars) or capillary (open bars) ECs. Fibroblasts (10^5 cells per cm^2 , total of 2×10^7 cells), smooth muscle cells (5×10^5 cells per cm^2 ; total of 2×10^7 cells), and MPs (3×10^4 cells per cm^2) were incubated in serum-free medium for 24 h at a $pO_2 \approx 14$ torr. Aliquots of conditioned medium were then added to cultures of hypoxic aortic or capillary ECs (the fractions refer to the dilution of medium used; SMC and FBR conditioned media were utilized only at 1:1), and proliferation was assessed by the incorporation of [^3H]thymidine. (D and E) MPs were incubated for 24 h at the indicated oxygen tension (D) or for the indicated time at a $pO_2 \approx 14$ torr (E). The effect of aliquots of MP-conditioned medium on the proliferation of hypoxic ECs was then tested as described above. In each case the mean \pm SEM of triplicate determinations is shown. * in C–E denotes $P < 0.01$ compared with normoxic controls.

bFGF to the failure of ECs at the leading edge of a wound to proliferate or to migrate into a denuded area (15) led us to consider whether hypoxia would downregulate bFGF. Compared with levels of bFGF mRNA in normoxic controls, there was a striking reduction in the levels of bFGF mRNA in subconfluent hypoxic capillary ECs analyzed by Northern blotting (Fig. 1A). Comparable reduction in the level of antigen were described previously (1).

To determine whether hypoxia modulated EC expression of surface receptors for FGF, a Northern blot analysis of RNA harvested from hypoxic capillary ECs was done, which dem-

onstrated a 1.8-fold increase in levels of transcripts for the EC FGF receptor flg (11) [Fig. 1B; levels of bek mRNA, another FGF receptor (11), were unchanged; data not shown]. These data are consistent with the observation that hypoxic ECs have ≈ 2 - to 3-fold more high-affinity cell surface binding sites for ^{125}I -bFGF than their normoxic counterparts (1).

Since exogenous bFGF augments proliferation and migration of hypoxic ECs (1), we investigated whether cells prevalent in the vascular wall might secrete mitogens stimulating growth of hypoxic ECs. Medium conditioned by hypoxic cultured human MPs stimulated the growth of hypoxic capillary and

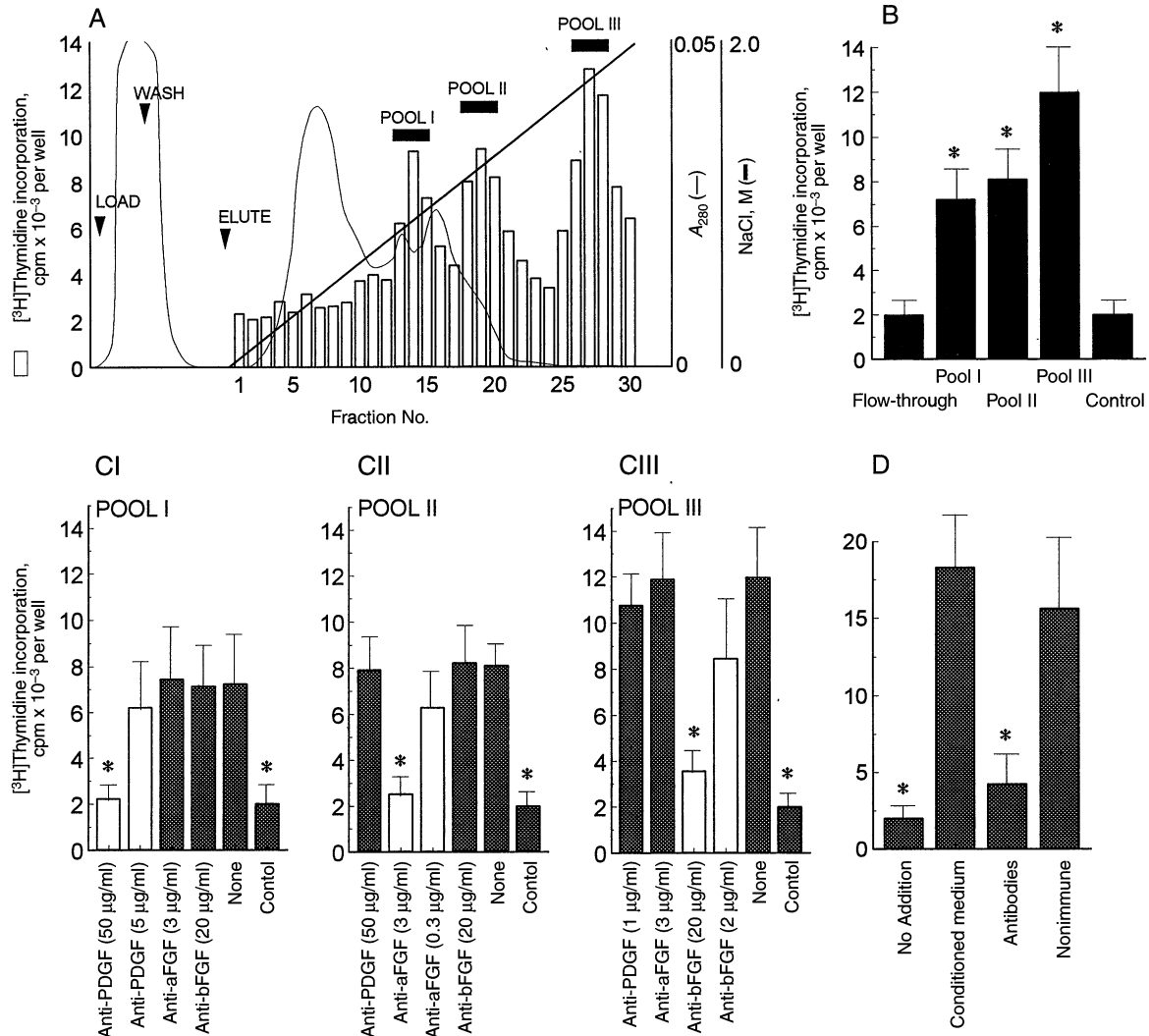


FIG. 2. Chromatography on heparin Ultragel of medium conditioned by hypoxic MPs, and neutralization of mitogenic activity in the eluate by antibodies to PDGF, aFGF, and bFGF. (A) Conditioned medium from MP cultures (10^7 cells) exposed to hypoxia ($pO_2 \approx 14$ torr) for 48 h was applied to heparin Ultragel (load), the column was washed with equilibration buffer (wash), and material was eluted from the column with an ascending salt gradient (thick solid line). The collected fraction number is plotted versus A_{280} (thin solid line), and the effect of that fraction on the growth of hypoxic capillary ECs was quantitated by measuring [^3H]thymidine incorporation (vertical bars). On the basis of the ability of specific fractions to promote EC proliferation, three pools of activity (I, II, and III) were defined. (B) The flow-through fractions from the column and fractions corresponding to pools I, II, and III were added to cultures of hypoxic capillary ECs, whose growth was assessed by measuring [^3H]thymidine incorporation ("Control" represents culture supernatant from normoxic MPs). (C I–C III) Neutralization of pools of mitogenic activity from the column by antibodies against PDGF, aFGF, or bFGF. Each pool was preincubated for 8 h at 4°C with the IgG fraction of rabbit anti-bFGF, rabbit anti-aFGF, or rabbit anti-PDGF and then added to cultures of hypoxic capillary ECs. EC proliferation was assessed by measuring [^3H]thymidine incorporation (the mean \pm SEM of triplicate determinations is shown). "None" indicates that no antibodies were added, and "control" indicates that neither antibodies nor column eluate was present. Addition of nonimmune IgG ($20 \mu\text{g}/\text{ml}$) had no effect on the proliferative response of hypoxic ECs to material from any pool. (D) Conditioned medium from hypoxic MPs was incubated with hypoxic capillary ECs, and the mitogenic response was determined as above. "No addition" denotes wells with hypoxic ECs alone; "conditioned medium" denotes addition of conditioned medium to hypoxic ECs without further reagents; "antibodies" denotes addition of antibodies against PDGF ($20 \mu\text{g}/\text{ml}$), aFGF ($3 \mu\text{g}/\text{ml}$), and bFGF ($20 \mu\text{g}/\text{ml}$) to conditioned medium (antibodies were preincubated for 8 hr at 4°C with conditioned medium) followed by addition of the reaction mixture to cultures of ECs; and "nonimmune" indicates conditioned medium preincubated with nonimmune rabbit IgG ($50 \mu\text{g}/\text{ml}$). * denotes $P < 0.01$ compared with normoxic controls in B, the "none" group in C, or the group to which conditioned medium was added in D.

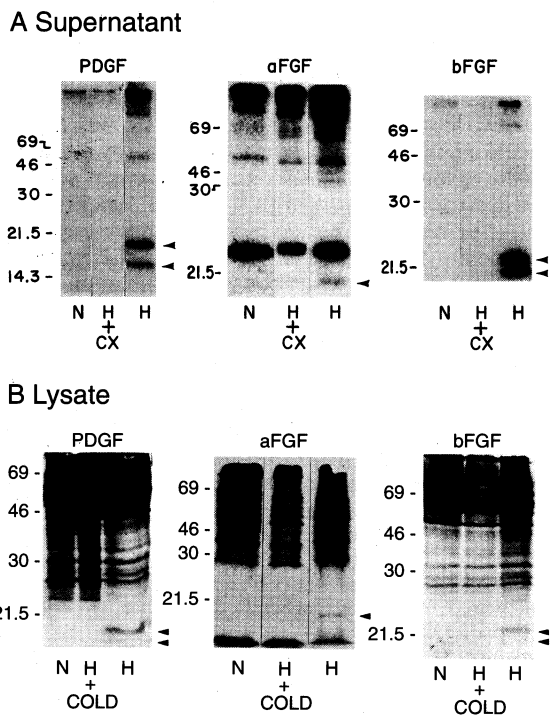


FIG. 3. Immunoprecipitation of culture supernatants and cell lysates from hypoxic or normoxic MPs to assess synthesis and release of PDGF and a/bFGF. MPs were exposed to hypoxia (H) or normoxia (N) for 24 h and metabolically labeled. Culture supernatants (A) or cell lysates (B) were immunoprecipitated with antibodies directed against PDGF, aFGF, or bFGF and then subjected to reduced SDS/PAGE and autoradiography. Where indicated, cycloheximide (CX; 10 μ g/ml) was added to MP cultures at the start of hypoxia or an excess of the respective unlabeled (+ cold) growth factor (1 μ g/ml) was added prior to the incubation with the antibody. The migration of hypoxia-induced bands representing PDGF, aFGF, and bFGF are indicated by the arrowheads. The positions of molecular mass standards (kDa) that were run simultaneously are indicated on the left of each autoradiogram.

aortic ECs in a dose-dependent manner (Fig. 1C), with a more striking induction of capillary EC proliferation than aortic EC proliferation. In contrast, conditioned medium from normoxic or hypoxic vascular smooth muscle cells or fibroblasts did not alter EC proliferation. MP elaboration of mitogenic activity for hypoxic ECs depended on the ambient oxygen concentration, as well as the duration of hypoxic exposure (Fig. 1D and E). To affirm that release of mitogens by hypoxic MPs was not due to nonselective release of intracellular contents as a result of cell death, MP viability was shown to be unaltered throughout the hypoxic exposure on the basis of their continued ability to exclude trypan blue dye. In addition, when hypoxic MPs were returned to normoxia, the secretion of growth factors as assayed by the ability of conditioned medium to stimulate EC proliferation returned to the normoxic baseline within 24 h (data not shown).

Characterization of Hypoxia-Induced, MP-Derived Growth Factors. Heparin chromatography was performed on conditioned medium from hypoxic MPs, and three pools of mitogenic activity for hypoxic ECs (Fig. 2A) were resolved as the column was eluted with an ascending salt gradient: pool I at 0.6–0.8 M NaCl, pool II at 1–1.2 M NaCl, and pool III at \approx 2 M NaCl. Low mitogenic activity was observed in the column flow through (Fig. 2B), suggesting that the three pools bound to the heparin column. Experiments with polyclonal antibodies directed against aFGF, bFGF, hst/K-FGF, VPF/VEGF, and PDGF were performed to identify the growth factor(s) responsible for the mitogenic activity in each of the three pools.

Mitogenic activity in pool I was almost completely neutralized by polyclonal anti-PDGF antibody (Fig. 2CI). Immuno-

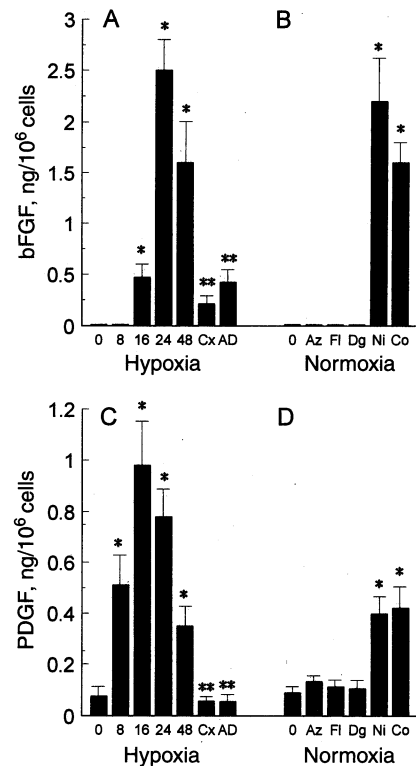


FIG. 4. Radioreceptor assays for PDGF and bFGF secreted by hypoxic MPs: time course study, and effects of metabolic inhibitors and heavy metals. MPs (3×10^4 cells/cm²) were incubated in serum-free medium under hypoxia ($pO_2 \approx 14$ torr) for the indicated times in hours (A and C) or in normoxia for 24 h in the presence of medium alone (0) or the indicated metabolic inhibitor or heavy metal (B and D). Az, sodium azide; Fl, sodium fluoride; or Dg, 2-deoxyglucose. Alternatively, either cobalt chloride (Co) or nickel chloride (Ni) was added to normoxic MP cultures for 24 h. The radioreceptor assay for PDGF and bFGF was performed on culture supernatants as described in the text. The mean \pm SEM of triplicate determinations is shown. Where indicated, cycloheximide (Cx; 10 μ g/ml) or actinomycin D (AD; 5 μ g/ml) was added to MP cultures at the start of hypoxia. The presence of nickel and cobalt salts did not alter binding of bFGF to the cells in the radioreceptor assay under our experimental conditions—i.e., the standard curve was unchanged. The experiment was repeated three times. * denotes $P < 0.01$ compared with normoxic controls, and ** denotes $P < 0.01$ compared with the 24-h hypoxic group.

precipitation of the supernatant of metabolically labeled, hypoxic MPs with this same antibody revealed two closely spaced bands (\approx 15 and 16 kDa; Fig. 3A). Similar bands were not observed in samples from hypoxic MPs incubated with cycloheximide or normoxic MPs (Fig. 3A). Immunoprecipitation of lysates from hypoxic MPs showed a major band, the appearance of which was blocked by excess unlabeled PDGF (and absent in lysates of normoxic MPs, Fig. 3B). These data suggest that the mitogenic activity of pool I was due to PDGF.

A similar analysis of mitogenic activity from pool II demonstrated that it could be almost completely neutralized by anti-aFGF antibody (Fig. 2CII). Immunoprecipitation of supernatants of metabolically labeled, hypoxic MPs with this same antibody revealed an \approx 14-kDa band, which was not observed in hypoxic cultures to which cycloheximide had been added or in normoxic cultures (Fig. 3A). Immunoprecipitation of cell lysates from hypoxic MPs demonstrated a major band (not observed in normoxic MP lysates), which disappeared on addition of excess unlabeled aFGF (Fig. 3B). These data suggest that the mitogenic activity of pool II was due to aFGF.

Pool III, which had the greatest mitogenic activity in conditioned medium from hypoxic MPs, was neutralized in large part by anti-bFGF antibody (Fig. 2CIII). Immunoprecipitation

of supernatants from metabolically labeled, hypoxic MPs with this antibody demonstrated two closely spaced bands migrating at ≈ 18 and 20 kDa (Fig. 3A). Similar bands were not observed in hypoxic cultures incubated with cycloheximide, or normoxic cultures (Fig. 3A). Immunoprecipitation of cell lysates from hypoxic MPs showed a major band (≈ 20 kDa; not observed in normoxic MP lysates), whose appearance was prevented by addition of excess unlabeled bFGF (Fig. 3B). These data suggest that the mitogenic activity of pool III was due to bFGF.

Mitogenic activity from pools I and II was also tested with antibody to hst/K-FGF, a form of FGF (7), and VPF/VEGF (16), because these mitogens elute from immobilized heparin columns in the range of salt concentrations used for pools I and II. No neutralization or adsorption of MP-derived mitogenic activity for hypoxic ECs was observed with antibodies directed against either of these mitogens (data not shown). Consistent with these data is the observation that when unfractionated conditioned medium from hypoxic MPs was incubated with hypoxic capillary ECs, simultaneous addition of antibodies against a/bFGF and PDGF blocked EC proliferation by $\approx 80\%$ (Fig. 2D).

Kinetics of the Production of bFGF and PDGF by Hypoxic MPs. Conditioned medium from hypoxic MPs was evaluated for the presence of these growth factors by using radioreceptor assays. Secretion of bFGF into supernatants of hypoxic MPs occurred in a time-dependent manner (Fig. 4A). Because both cycloheximide and actinomycin D blocked the increased expression of bFGF, bFGF must be synthesized *de novo* rather than be released from intracellular stores.

To determine whether blockade of aerobic metabolism in the hypoxic environment caused the increased bFGF production, we investigated whether metabolic inhibitors could induce bFGF. Incubation of normoxic MPs cultures with 2-deoxyglucose, fluoride, or azide failed to induce bFGF production. [These are inhibitors of glycolysis, glycolysis and fatty acid oxidation, and aerobic metabolism, respectively (17); Fig. 4B.] In contrast, cobalt and nickel, which can mimic hypoxia-induced production of erythropoietin, possibly by interacting with a heme-containing protein (18), induced secretion of mitogenic activity by normoxic MPs.

Radioreceptor assays for PDGF performed on samples of hypoxic MPs also demonstrated a time-dependent release of this growth factor (Fig. 4C). As with bFGF, treatment with either cycloheximide or actinomycin D prevented secretion of PDGF, consistent with *de novo* biosynthesis. Studies with metabolic inhibitors and heavy metals also demonstrated a pattern similar to that observed with bFGF: metabolic inhibitors were without effect, but cobalt and nickel stimulated production of PDGF by normoxic MPs (Fig. 4D).

In summary, we have shown the following: (i) hypoxia, at levels similar to those observed in wounds (5), induces MPs to synthesize and secrete PDGF and a/bFGF; (ii) these mitogens stimulate the proliferation of hypoxic ECs; and (iii) heme-binding metals, such as cobalt or nickel, can simulate the effect of hypoxia on MP production of a/bFGF and PDGF. By analogy with studies on erythropoietin expression, the effect of hypoxia on growth factor gene expression in MPs may involve activation of DNA-binding activity to a site such as HIF-1 (19–21). Alternatively, more indirect mechanisms may be

involved, with production of cytokines, such as interleukin 1 (4, 22), involved as an intermediate step in hypoxia-induced expression of endothelial growth factors. On the basis of our results, we propose that angiogenesis under hypoxic conditions involves two key cellular components: MPs, which produce angiogenic stimuli under hypoxic conditions (by a mechanism potentially involving a heme-containing oxygen sensor; ref. 18), and hypoxic microvascular ECs, which maintain functional growth factor receptors but do not spontaneously proliferate under hypoxia. Taken together, these data indicate that hypoxic MPs can direct the migration and proliferation of hypoxic ECs in a paracrine fashion, potentially leading to new vessel formation in hypoxic foci infiltrated by mononuclear phagocytes.

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