RAPID COMMUNICATION

Expression of Platelet-Derived Growth Factor and Its Receptors by Two Pre-B Acute Lymphocytic Leukemia Cell Lines

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Platelet-derived growth factors (PDGF) are potent regulators of cell proliferation. The three isoforms of PDGF AA, AB, and BB are encoded by two genes: PDGF A and PDGF B. The v-sis oncogene is homologous to the PDGF-B gene. v-sis can transform cells that express the appropriate PDGF receptors. Two different types of receptors, PDGF-α and PDGF-β, also encoded by two genes, have been identified. We show that two cell lines, SMS-SB and NALM-6, both derived from pre-B cell acute lymphocytic leukemias, express the PDGF-A chain gene, and one of them, SMS-SB, releases PDGF-A chains into the media. The SMS-SB cells also express the PDGF-β receptor, whereas NALM-6 cells express the PDGF-α receptor and bind PDGF. This extends the possible targets for PDGF to the B-cell lineage lymphocytes.

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PLATELET-DERIVED growth factor (PDGF) is a potent mitogen for cells of mesenchymal origin.1,2 PDGF was first recognized as a regulator of connective-tissue cell proliferation in the setting of vascular injury and wound healing.3 It has become clear recently that PDGF and its receptors play important roles in development and pathologic processes including cancer.4-10 Human PDGF is a disulphide-linked dimer consisting of two related polypeptide chains, designated A and B.1,11,12,13 That are the products of two distinct but closely related genes.14-15 In humans, the major isomorph in platelets is the heterodimer AB, whereas both AA and BB homodimers also exist.11,16 The different isoforms of PDGF differentially bind and activate the products of two genes that encode the PDGF-α and β receptors.15,17-19 The PDGF-α receptor interacts with all three isoforms of PDGF with about equal affinity, whereas the PDGF-β receptor preferentially binds BB homodimers and interacts with AB heterodimers with a reduced affinity.19-22 The expression of the A and B chains of PDGF has been studied in cells of hematopoietic origin. Megakaryocytes, myeloid and monocytic cells, and to a much lesser extent T cells, have been shown to produce PDGF.23-27 However, the state of PDGF expression in B-lineage cells remains largely unknown.

MATERIALS AND METHODS

Cell lines. SMS-SB25 and NALM-626,27 cells were described previously. Cells from both lines were maintained in serum-free Iscove’s medium16,31 (GIBCO, Paisley, Scotland) supplemented with a 1:100 dilution of insulin, transferrin, and selenium in bovine serum albumin linoleic acid (Collaborative Research). The cells were passaged when they reached a cell density of 2 to 4 × 10^5 cells/mL by dilution with fresh medium to a cell density of 5 × 10^3 cells/mL. Medium conditioned for 4 to 6 days, by which time the cell density had reached its maximum, was centrifuged for 10 minutes at 10,000 rpm to remove the cells. The supernatants were concentrated over a 10 K cut-off filter, and the growth factors precipitated in the cold by the addition of saturated ammonium sulphate to give a final concentration of 50%. The precipitate was isolated by centrifugation for 30 minutes at 10,000 rpm and washed with 55% cold ammonium sulphate. The precipitate was resuspended in 0.01 mol/L Tris pH 7.2, 0.001 mol/L EDTA, 0.1 mol/L NaCl (TES) and dialyzed against the same buffer. This ammonium sulphate fraction was assayed for PDGF in a radio receptor assay as described previously.12

Plasmids. The PDGF A and PDGF B and the α- and β-receptor plasmids were obtained from Dr Stuart Aaronson of the National Cancer Institute (Bethesda, MD). Inserts were isolated from agarose gels after excision of the cDNA fragments from the plasmids with the appropriate restriction endonucleases. The inserts were labeled with 32P in random priming with oligonucleotides using a kit from Pharmacia.

RNA. RNA was isolated from SMS-SB and NALM-6 cells using RNAZOL (Biogenesis, Bournemouth, UK). Poly-A plus RNA was isolated using oligo dT magnetic beads (Dynal, Wirral, UK). Either total or poly-A plus RNA was fractionated by electrophoresis through formamide-agarose gels.32 The RNA was vacuum blotted to nylon filters and hybridized with the probes mentioned in the figure legends. Except where stated, the RNA was isolated from cells grown in serum-free medium. For the serum-induction experiments, the RNA was extracted from 5 × 10^5 cells at various times after serum stimulation and cycloheximide addition.

PDGF binding assay. 125I-labeled PDGF BB was purchased from Amersham (Aylesbury, UK) as was unlabeled PDGF. The cells to be used in a binding assay were grown in serum-free medium as described above, and washed three times in Iscove’s medium supplemented with human serum albumin (HSA) at a final concentration of 100 μg/mL. A constant amount of radioabeled PDGF was incubated for 1 hour at 4°C in the presence or absence of unlabeled PDGF with 10^5 cells in a volume of 0.25 mL binding buffer: phosphate-buffered saline and 100 μg/mL HSA. After incubation the cells were washed five times in binding buffer and the radioactivity in the cell pellets determined in a gamma scintillation counter.

PDGF dose response. SMS-SB cells were washed in protein-free medium (GIBCO) and seeded in a 96-well plate at a density of 5 × 10^3 cells/well. Poly-A chain reaction (PCR). The PCRs were performed on cDNA made from RNA isolated from the cells. The cDNA synthesis and PCR reactions were performed according to the method of Tsai et al.12 The oligos for the PDGF-A chain spanned three in-

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51
equivalent of 169 ng/mL of PDGF (data not shown). Less than 2% of 125 PDGF-BB homodimers was competed by the conditioned medium, which indicated that SMS-SB cells were producing AB or AA isoforms of PDGF.

Using northern analysis we have observed the 1.9-, 2.3-, and 2.8-kb PDGF-A chain transcripts in RNA from SMS-SB cells and another pre-B-cell acute lymphocytic leukemia cell line, NALM-6 (Fig 1). We have also found transcripts for the A chain in a variety of hematopoietic cell lineages using PCR specific for the A chain of PDGF (Fig 2). No PCR PDGF-A chain products were detected in control reactions.

RESULTS AND DISCUSSION

While studying the growth factors produced by SMS-SB, derived from a human pre-B-cell acute lymphocytic leukemia, we found that serum-free conditioned media from the cells was mitogenic for fibroblasts. Subsequent analysis by radio receptor assays on human fibroblasts showed that the conditioned media competed effectively for binding of 125 PDGF-AB heterodimers to human fibroblasts, indicating that the 50% ammonium sulphate precipitate of serum-free conditioned medium from SMS-SB cells contained the

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**Fig 1.** Poly-A RNA was isolated from SMS-SB and NALM-6 cells grown in a serum-free medium. Five micrograms were separated according to size by formamide-agarose gel electrophoresis, blotted to filters and hybridized to p32 radiolabeled human PDGF-A chain-specific probe. The bands were visualized by autoradiography for 48 hours.

**Fig 2.** Detection of PDGF-A chain in cell lines by PCR. cDNA synthesized from total RNA was tested for the presence of PDGF-A chain sequences by PCR using PDGF-A chain-specific oligonucleotides, which would generate a 497-bp fragment (2A). The 497-bp fragment was detected by ethidium bromide staining of the PCR products after agarose gel electrophoresis (2B). The PCR reaction fragments were transferred to nitrocellulose and hybridized to PDGF-A chain-specific probes (2C). The cell lines were (1) SMS-SB poly A + RNA, (2) SMS-SB total RNA, (3) HL60, (4) U937, (5) RPMI8402, (6) NALL-1, (7) NALM-6, (8) REH, (9) HO, and (10) 321.
Serum induction of PDGF-A-chain mRNA. SMS-SB cells maintained in serum-free medium were stimulated by the addition of fetal bovine serum (FBS) to a final concentration of 10%. RNA was extracted before, 0, and at 30, 60, 120, and 240 minutes after the addition of FBS. Thirty minutes before serum addition cycloheximide (10 μg/mL) was added to one culture, RNA was extracted at 30, 60, 120, and 240 minutes after serum addition. Northern analysis was performed on 20 μg of total RNA using a PDGF-A-chain probe.

In SMS-SB cells the PDGF-A chain gene is induced by serum addition to cells maintained in serum free medium (Fig 3). Maximal induction is observed at 120 minutes after serum addition. The induction does not require protein synthesis, as it occurs in the presence of cycloheximide. However, unlike immediate early serum response genes, such as c-fos, in SMS-SB cells the induction of the PDGF-A-chain gene is not superinduced by the inhibition of protein synthesis. No mRNA transcripts for the B chain or PDGF were detected by Northern analysis, or by PCR using B-chain-specific oligonucleotide primers in either SMS-SB or NALM-6 cells (data not shown).

The RRA, Northern assay, and PCR assay all indicate that the vast majority of PDGF released by SMS-SB cells is the AA isoform.

The discovery that PDGF is encoded by the v-sis oncogene led to the study of PDGF and its receptors as a model for autocrine activation of cell proliferation. To determine if the PDGF-AA homodimers produced by SMS-SB cells could be one arm of an autocrine system, we examined the expression of PDGF receptor genes in SMS-SB and NALM-6 cells by Northern analysis of poly-A RNA from SMS-SB and NALM-6 cells with probes specific for either the α- or β-PDGF receptors. NALM-6 RNA had an easily detectable 6.4-kb α-receptor transcript, whereas this was barely visible in SMS-SB RNA; only SMS-SB RNA had the 5.3-kb β-receptor transcript (Fig 4B). On the face of it, SMS-SB cells might constitute a PDGF-based autocrine system, although PDGF-AA homodimers do not bind to the PDGF-β receptor, there appears to be a low level of PDGF-α receptor mRNA. On the other hand, NALM-6 cells are good candidates for an autocrine system based on PDGF, because they express the PDGF-α receptor that binds to the AA isoform of PDGF.

To determine if the PDGF receptors were expressed on SMS-SB or NALM-6 cells, 125I-labeled BB isofom of PDGF was used in PDGF binding assays. Specific binding was only detected on NALM-6 cells (Fig 5). To determine if we could detect a response to PDGF, SMS-SB cells, which are density dependent for growth, were seeded at low cell density in protein-free medium in the presence or absence of PDGF-AA. After 24 hours, the cells were pulse labeled with H3 thymi-
dine (Fig 6). SMS-SB cells were stimulated by PDGF AA in a dose-dependent fashion. This experiment was not possible as they are not density dependent for proliferation.

The data presented here widen the potential target range of PDGF to include at least leukemic cells of the B lineage. It is not known whether the expression of either the PDGF-A chain and/or PDGF receptors is unique to leukemic cells, or reflects normal pre-B-cell gene expression. If PDGF-AA homodimers are produced by pre-B cells, they might function as paracrine factors in the bone marrow stimulating the adherent cells to release needed mitogens for the developing pre-B cells. Alternatively, the expression on pre-B cells of the appropriate PDGF receptors might allow the cells to respond chemotactically to PDGF in the marrow or effect cell to cell interaction by altering the cell's cytokeratin.34 In any case, the finding that two pre-B ALL cell lines produce PDGF-A-chain homodimers and PDGF receptors highlights the need to investigate the possibility that PDGF may function as a lymphokine.

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LH Tsai, L White, E Raines, R Ross, RG Smith, W Cushley and B Ozanne