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Platelet-derived growth factor and its role in health and disease

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Platelet-derived growth factor (PDGF) was first discovered in platelets because they are the principal source of mitogenic activity in whole blood serum for mesenchymal cells in culture. PDGF is ubiquitous in that it can be formed by a large number of normal cells as well as many varieties of transformed cells. However, its expression and biological activity appear to be controlled at a number of different levels. The molecule consists of two peptide chains (termed 'A' and 'B') and is found as one of at least three possible isoforms, (AB, AA or BB). Each of these isoforms binds to a high-affinity cell-surface receptor that is composed of two different subunits, each of which has specificity for one or the other of the peptide chains of PDGF. The two receptor subunits are present in differing amounts on different cell types, and therefore the capacity of the different isoforms of PDGF to induce mitogenesis depends on the specific PDGF isoform and the relative numbers of receptor subunits present on the responding cell. In addition to inducing cell replication, PDGF elicits a number of intracellular signals related to mitogenesis, is chemotactic, is a vasoconstrictor, activates leukocytes, and modulates extracellular matrix turnover. This growth factor is probably involved in a number of biologically important events including wound repair, embryogenesis and development, and inflammation, leading to fibrosis, atherosclerosis and neoplasia.

Introduction

Platelet-derived growth factor (PDGF), first discovered in 1974, is the principal source in whole blood serum of mitogenic activity for mesenchymal connective tissue-forming cells such as fibroblasts and smooth muscle cells (Ross *et al.* 1974; Kohler & Lipton 1974; Rutherford & Ross 1976). Although PDGF was initially discovered in platelets, it was soon observed that several diploid cells, including monocyte-macrophages, endothelium, smooth muscle cells and fibroblasts, can, when appropriately activated, form at least one of the three isoforms of PDGF. In addition, many neoplastically transformed cells express the normal cellular genes for PDGF and secrete this molecule as well. As a consequence, PDGF is found in many tissues and organs in both normal as well as several disease states, and has been postulated to play potentially important roles in diverse phenomena ranging from embryogenesis, growth and development, to wound healing, atherogenesis and neoplasia.

This review summarizes our current knowledge of the nature of the two peptide chains of PDGF, the recently discovered subunits of the PDGF receptor and their capacity to interact with the isoforms of PDGF, the cells that make PDGF, the biological responses it induces, and its potential roles in normal biology and in disease.

PDGF molecules

Since its discovery, PDGF has been purified from human platelets (Antoniades 1981: Heldin *et al.* 1981) and platelet-rich plasma (Deuel *et al.* 1981; Raines & Ross 1982), and exhibits

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multiple relative molecular mass forms ranging from 28000 to 35000. Reduction of PDGF destroys its biological activity and produces inactive polypeptides ranging in relative molecular mass from 12000 to 18000. Amino acid sequence analyses show that PDGF is comprised of two distinct but homologous chains, termed A and B, linked by disulphide bonds (Antoniades & Hunkapiller 1983; Waterfield *et al.* 1983; Johnsson *et al.* 1984). Until recently, it was unclear whether PDGF was comprised of heterodimers or approximately equivalent portions of homodimers. By using immunochemical, biochemical and chromatographic approaches, it has been shown that PDGF isolated from human platelets is 70–80 % PDGF-AB heterodimer with the remainder principally PDGF-BB homodimer with small but detectable amounts of PDGF-AA (Hammacher *et al.* 1988*a*; C. E. Hart *et al.* unpublished data). Homodimers of PDGF-AA have also been purified from the conditioned media of human tumour cell lines (Heldin *et al.* 1986; Westermark *et al.* 1986; Hammacher *et al.* 1988*b*). Thus all possible dimeric combinations of PDGF have been demonstrated.

Both chains of PDGF are derived from genes that contain seven exons spanning approximately 24 kilobases (kb) of genomic DNA, and their similarity in intron–exon structures suggests that they may stem from a common ancestral gene (Chiu *et al.* 1984; Collins *et al.* 1985; Rao *et al.* 1986; Betsholtz *et al.* 1986; Collins *et al.* 1987*a*). The gene for the A-chain is located on the proximal arm of human chromosome 7 (Betsholtz *et al.* 1986), and three PDGF A-chain transcripts of 1.9, 2.3 and 2.8 kb have been detected in human tumour lines (Betsholtz *et al.* 1986; Betsholtz *et al.* 1987). In contrast, the PDGF B-chain gene is localized on human chromosome 22 (Dalla Favera *et al.* 1982; Swan *et al.* 1982), and its transcription is detected as a prominent band of 3.5–3.7 kb and minor bands of 2.6–2.7 kb and 1.3 kb (Barrett *et al.* 1984; Collins *et al.* 1985). Analysis of the cDNA clones of the PDGF A-chain predicts at least two forms of the A-chain due to alternative splicing (Betsholtz *et al.* 1986; Collins *et al.* 1987*b*; Tong *et al.* 1987; Rorsman *et al.* 1988). The cDNA clones isolated from endothelial cells all contain a 69 base pair (b.p.) deletion that codes for an A-chain precursor that contains 15 fewer amino acid residues and lacks the basic C terminus of the full-length cDNA isolated from a human clonal glioma cell line. Both forms of the PDGF A-chain are biologically active, but their biological significance remains to be determined (Beckmann *et al.* 1988). Analysis of regulatory elements of the PDGF B-chain genes have identified both transcriptional enhancer and silencer regions (Pech *et al.* 1989) and a region of the 5′ untranslated region of the PDGF B-chain transcript that inhibits translation (Rao *et al.* 1988). However, the existence of still additional negative control mechanisms beyond the regulation of promoter activity is suggested by these studies. Transcriptional and translational control represent just a portion of the regulation of the local concentration of this very potent biological molecule (see table 1).

Both the A- and B-chains of PDGF are synthesized as precursors and they share homology in part of their amino terminal propeptides. They also both contain classical hydrophobic signal sequences preceding the N-terminus of the propeptides. Only the B-chain has precursor sequences C-terminal to the sequence for the mature molecule. Analyses of active and inactive forms of PDGF have revealed multiple amino terminal sequences (Antoniades & Hunkapiller 1983; Waterfield *et al.* 1983; Johnsson *et al.* 1984). However, no peptides corresponding to 'pro' sequences from either the N-terminal region of the A- and B-chain or the C-terminal region of the B-chain have been detected in preparations from platelets, and therefore precursor sequences do not contribute to this heterogeneity. Rather, the heterogeneity is due to shortened forms of both mature chains, and these constitute 25–56 % of the total amino

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terminal sequences (Antoniades & Hunkapiller 1983; Johnsson *et al.* 1984; C. E. Hart *et al.* unpublished data). Cleavage of the N-terminus of the mature B-chain at threonine 33 is particularly prevalent, and this cleavage results in a loss of PDGF mitogenic activity (E. W. Raines *et al.*, unpublished data). Proteolytic cleavage thus represents another possible level of control of the biological activity of PDGF (see table 1).

TABLE 1. EVIDENCE FOR MULTIPLE LEVELS OF CONTROL OF LOCAL CONCENTRATIONS OF PDGF

control mechanism	example
transient induction of message transcription	activation monocytes; IL-1 stimulated fibroblasts
alternative splicing	at least two possibilities for A-chain of PDGF
dimer composition and processing	homodimers AA, BB and heterodimer AB
storage pools (inactive/active)	cultured endothelial cells release PDGF after treatment with thrombin or factor Xa
interaction with binding proteins and/or matrix	specific binding proteins inhibit cellular binding
proteolytic cleavage	cleavage products of PDGF have decreased activity
specificity of receptor distribution and expression	different receptor subunit specificities for different isoforms

PDGF RECEPTORS

PDGF binds with high affinity to specific cell-surface receptors. Upon binding to its receptor, PDGF evokes a large number of biological responses, ranging from stimulation of tyrosine kinase activity and phosphorylation of several intracellular molecules, to chemotaxis, vasoconstriction, and ultimately DNA synthesis and cell division. These will be discussed in greater detail below and are outlined in table 2.

Early studies suggested that PDGF bound to a receptor of relative molecular mass 170000–180000 that appeared to be a monomeric cell-surface glycoprotein with an extracellular ligand-binding domain and an intracellular tyrosine kinase domain. This original view of the PDGF receptor is obviously too simple. The availability of recombinant PDGF-AA and PDGF-BB, and highly purified PDGF-AB lacking -AA and -BB, has made it possible to study the binding of each of the pure isoforms of PDGF (Hart *et al.* 1988; Seifert *et al.* 1989). In addition, monoclonal antibodies have been developed that recognize two different PDGF receptor proteins. These antibodies have been used to study binding competition of each of the different labelled pure isoforms of PDGF, and the data suggest that many cells contain more than a single class of PDGF receptor (Hart *et al.* 1988).

We have proposed (Seifert *et al.* 1989) that the mature high-affinity PDGF receptor consists of dimers of two PDGF receptor monomers, termed the α- and β-receptor subunits (see figure 1). The α-subunit can bind either the A- or the B-chain of PDGF, whereas the β-subunit can bind only the B-chain. PDGF-A is only able to bind to the α-subunit, whereas PDGF-B can bind to either the α- or the β-subunit. Exposure to the appropriate isoform of PDGF (AA, BB, or AB) recruits and apparently dimerizes the appropriate combinations of available α- or β-subunits on the cell surface (see figure 1). Direct evidence for ligand-induced receptor subunit

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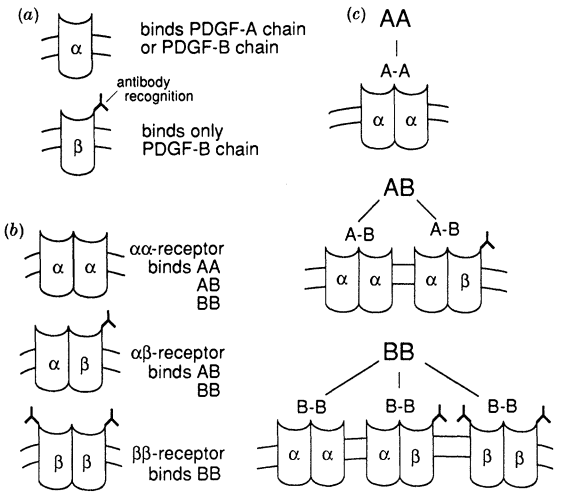

FIGURE 1. The PDGF receptor subunit model. (*a*) Receptor subunits. Two types of PDGF receptor subunits exist: α-subunits, which can bind PDGF A- and B-chains, and β-subunits, which can bind only PDGF B-chains. Monoclonal antibody PR7212 recognizes only the PDGF receptor β-subunit. Before ligand binding, PDGF receptor subunits are present on the cell surface as independent monomers or weakly associated dimers. (*b*) Receptor types. The two PDGF receptor subunits can form three types of high-affinity PDGF receptors: αα-receptors, αβ-receptors, and ββ-receptors. Antibody PR7212 recognizes the αβ-receptor and ββ-receptor via their β-subunits. (*c*) Receptor binding. PDGF-AA can only bind to PDGF αα-receptors, whereas PDGF-AB can bind to either PDGF αα-receptors or αβ-receptors. PDGF-BB can bind to all three PDGF receptor classes. (Reproduced from Seifert *et al.* (1989).)

dimerization and stabilization has been obtained by co-immunoprecipitation experiments using monoclonal antibodies specific for each of the receptor subunits (Seifert *et al.* 1989).

The sequences for both the β- and α-receptor subunits have been obtained from cDNA libraries from mouse placenta, cultured Swiss 3T3 cells, and human fibroblasts. The β-subunit of the mouse and human are highly homologous, and thus well conserved (Yarden *et al.* 1986; Gronwald *et al.* 1988; Claesson-Welsh 1988). The gene for the β-subunit has been localized on the long arm of human chromosome 5 (Yarden *et al.* 1986) close to the site of the gene for the cell stimulating factor (CSF)-1 receptor (Roberts *et al.* 1988). The α-subunit gene has been mapped to chromosome 4 (Matsui *et al.* 1989). The α- and β-subunits of the receptor are very similar in size, ranging in relative molecular mass from 170000–190000. The α-subunit has approximately 30% homology with the β-subunit; however the two subunits are highly homologous in several regions, particularly the transmembrane region and the split tyrosine kinase region (Matsui *et al.* 1989). There are marked differences in the number of α- versus β-subunits on different cells (Seifert *et al.* 1989).

Of particular importance is the observation that the responsivity of a given cell type to the different dimeric forms of PDGF is highly dependent upon both the specific isoform of PDGF to which they are exposed, as well as the number of α- and β-subunits present on the cells. For example, human dermal fibroblasts respond poorly to PDGF-AA, but are highly responsive to PDGF-BB because they contain approximately 20:1 β- to α-subunits. In sharp contrast, NIH-3T3 cells and some strains of human arterial smooth muscle cells contain 1:1 ratio of β- to α-subunits. In these cells, PDGF-AA and PDGF-BB are equally good mitogens. Thus receptor subunit composition on the responding cells as well as amount and type of dimer composition of the PDGF ligand will co-determine the capacity of the different forms of PDGF to be mitogenic and to potentially exert the numerous biological responses that occur after binding.

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Biological actions of PDGF

PDGF is rapidly cleared from the blood when it is administered intravenously (Bowen-Pope *et al.* 1984*a*). This clearance may be mediated in part by PDGF binding proteins in plasma, such as α_2-macroglobulin (Raines *et al.* 1984; Huang *et al.* 1984), which inhibit PDGF binding to its receptor. Binding proteins therefore represent another possible level of control of local concentrations of PDGF (table 1).

As previously indicated, PDGF is a potent mitogen for mesenchymal connective tissue-forming cells. Before inducing such cells to traverse from G0/G1 into S, and thus to synthesize DNA, PDGF rapidly stimulates a number of cell processes (see table 2). It is important to note,

Table 2. Biological effects of PDGF immediate

effect	postulated role in biology and disease
1–5 min	
stimulation of phospholipase A2, phospholipase C, and prostaglandins	vasodilation; bone resorption; resultant hypercalcemia associated with neoplasia
stimulation of PI turnover	intracellular signalling
tyrosine phosphorylation, cytoplasmic and membrane	induction of growth response
vasoconstriction	altered vasoreactivity of atherosclerotic vessels
increased cytosolic free Ca^{2+}	intracellular signalling
Na^+/H^+ exchange	intracellular signalling
reorganization of vinculin and actin, membrane ruffling	increased translocation and cell motility
decreased EGF binding	modulation of the cellular response to EGF
30 min–4 h	
induction of 'early genes' c-*myc*, c-*fos* (nuclear) JE, KC, PDGF-A	regulation of gene expression and DNA replication
protein synthesis	structural and matrix proteins
increased LDL receptors, decreased HDL-mediated cholesterol efflux	increased cholesterol for membrane synthesis; conservation of mevalonate; accumulation of LDL in disease states
increased IGF-I binding	modulation of cellular response to IGF-I
chemotaxis of smooth muscle, fibroblasts, and retinal pigment epithelial cells	attraction of cells to sites of injury
chemotaxis and activation of neutrophils and monocytes	attraction of cells and stimulation of mediator release
6–48 h	
induction of interferon-β	inhibition of cell growth
collagen formation	fibrosis and connective tissue remodelling
increased secretion of IGF-I by smooth muscle and fibroblasts	enhanced growth
proliferation of connective tissue cells	wound repair, growth and development, fibrotic diseases, neoplasia, atherosclerosis; angiogenesis?
support of lens growth and transparency	growth and development
increased expression of MHC class II antigens on antigen-presenting cells	augmentation of the immune response
enhanced proliferation of antigen-driven T-cells and γ-IFN production	immune regulation
7–14 days	
enhanced proliferation of erythropoietic precursors	regulation of erythropoiesis

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however, that experiments defining the biological actions of PDGF preceded our understanding of the complexity of PDGF molecules and their receptors and the availability of pure reagents. Consequently, it is unclear in most of the cases discussed below which PDGF isoform and receptor subunits are responsible for the observed biological action.

One of the earliest results of PDGF binding to its receptor is activation of phospholipase type A (Shier 1980; Shier & Durkin 1982) and phospholipase C (Habenicht *et al.* 1981, 1985, 1986). Such phospholipase activation leads to release of free arachidonic acid, formation of diacylglycerol (Coughlin *et al.* 1980; Habenicht *et al.* 1981; Sawyer & Cohen 1981; Rozengurt *et al.* 1983), breakdown of phosphatidylinositol, and turnover of phosphatidylinositol 4-5-bisphosphate (PIP_2) (Chu *et al.* 1985; Hasegawa-Sasaki 1985; Matuoka *et al.* 1988). Such PIP_2 turnover may be important in the entry of cells into DNA synthesis.

Early changes induced by PDGF also include modulation of ion flux in cells. PDGF increases intracellular Ca^{2+} (Rozengurt *et al.* 1984; Frantz 1985) and the activity of the Na^+/H^+ antiport in cells (Paris & Pouyssegur 1984). These changes may be important in permitting cell division to occur.

When PDGF binds to its receptor, it stimulates phosphorylation of tyrosine moieties on the receptor by activating the tyrosine kinase activity of the receptor (Ek *et al.* 1982; Nishimura *et al.* 1982; Pike *et al.* 1983; Kazlauskas & Cooper 1988). Such receptor autophosphorylation on tyrosine may be critical to mitogenesis since receptor mutants in which the tyrosine kinase region has been altered are no longer able to respond mitogenically (Escobedo & Williams 1988). PDGF binding also leads to phosphorylation of several other cellular proteins, not only on tyrosine moieties, but on threonine and serine as well (Cooper *et al.* 1982; Kazlauskas *et al.* 1988).

PDGF induces expression of several 'early' genes (Stiles 1983; Linzer & Nathans 1983; Cochran *et al.* 1983; Rollins & Stiles 1988). The PDGF-induced genes appear to account for only approximately 0.1–0.3% of the total genes transcribed, and their induction is not dependent on protein synthesis. Two of the genes that are stimulated are the proto-oncogenes c-*myc* and c-*fos*, two nuclear proteins (Kelly *et al.* 1983; Cochran *et al.* 1984; Greenberg & Ziff 1984). However, expression of c-*myc* and c-*fos* in PDGF-stimulated cells does not appear to be sufficient or necessary for cell proliferation to occur (Coughlin *et al.* 1985). Other PDGF-induced 'early' genes, such as JE, KC, and PDGF-A, encode growth factor or cytokine-like molecules and thus may be further involved in supporting cell proliferation (Paulsson *et al.* 1987; Richmond *et al.* 1988; Rollins & Stiles 1988).

PDGF has been shown to indirectly stimulate the proliferation of cells by inducing the expression of other genes which amplify the proliferative response. Such an example is PDGF stimulation of somatomedin-C secretion by cultured fibroblasts and smooth muscle cells (Clemmons *et al.* 1981; Clemmons & Van Wyk 1985) as well as somatomedin-C receptors (Clemmons *et al.* 1980). Inhibition of endogenously produced somatomedin-C with specific antibodies decreases the proliferative response of these cells to PDGF (Clemmons & Van Wyk 1985). PDGF also enhances antigen-driven T-cell proliferation (Acres *et al.* 1985), perhaps via induction of γ-interferon (Johnson & Torres 1985). Finally, PDGF enhances *in vitro* erythropoiesis (Delwiche *et al.* 1985), which appears to occur indirectly because of stimulation of marrow stromal cells, which then release substances that induce the erythropoietic cells to proliferate.

PDGF is highly chemotactic for mesenchymal cells such as fibroblasts and smooth muscle

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(Grotendorst *et al.* 1981, 1982; Seppa *et al.* 1982). It has also been reported to be chemotactic for mononuclear cells and neutrophils (Deuel *et al.* 1982; Williams *et al.* 1983), although induction of monocyte chemotaxis is controversial (Graves *et al.* 1989). Granule release from neutrophils and monocytes (Williams *et al.* 1983; Tzeng *et al.* 1984, 1985) as well as release of superoxide anion by neutrophils is stimulated by PDGF (Tzeng *et al.* 1984).

PDGF also modulates the turnover of extracellular matrix components by stimulating formation of collagen (Canalis *et al.* 1981; Owen *et al.* 1982; Narayanan & Page 1983) as well as a marked increase in collagenase activity (Bauer *et al.* 1985; Chua *et al.* 1985). This capacity of PDGF to stimulate both synthesis and breakdown of connective tissue matrix suggests an important role in matrix turnover in a number of situations ranging from wound healing to various fibrotic diseases such as rheumatoid arthritis and atherosclerosis.

CELLS THAT SECRETE PDGF

Diploid cells

PDGF is expressed and secreted by both normal and transformed cells from epithelial tissue, connective tissue, muscle, nervous tissue and cells of hematopoietic origin (Ross *et al.* 1986; Raines *et al.* 1989). However, in normal diploid cells, the expression of PDGF appears to be tightly regulated. The first example of this was the demonstration that endothelial cells scraped from fresh bovine aorta or human umbilical vein contained only 10% and 1.3%, respectively, as much B-chain transcript as did the same cells grown in monolayer culture (Barrett *et al.* 1984). Further, with the formation of tube-like structures in culture, which more closely approximates their state *in vivo*, expression of both the A- and B-chain of PDGF decreases (Jaye *et al.* 1985; Tong *et al.* 1987). Analysis of other adult tissues from human, rhesus and rat have demonstrated mRNA levels 1–5% of that seen with activated cultured cells from the same tissues (Barrett & Benditt 1988; Majesky *et al.* 1988; Sjolund *et al.* 1988). All of these observations suggest that PDGF expression and secretion is a response to perturbations associated with culturing. The resulting expression and secretion of PDGF in the cultured normal cells is constitutive. Other normal cells, such as human monocytes and alveolar macrophages, can be induced to transiently express and secrete PDGF in response to activation (Shimokado *et al.* 1985; Martinet *et al.* 1986; Mornex *et al.* 1986). Another example of transient induction of PDGF is in cultured human fibroblasts, which normally do not express either the A- or B-chain of PDGF. However, in response to interleukin-1 and PDGF, fibroblasts transiently express and secrete PDGF-AA (Paulsson *et al.* 1987; Raines *et al.* 1989).

A number of factors have been demonstrated to modulate the constitutive expression and secretion of PDGF by normal cultured cells. Alteration of the substrate upon which rat smooth muscle cells are grown (Sjolund *et al.* 1988) and the addition of low-density lipoproteins or fish oils to endothelial cells (Fox & DiCorleto 1986; Fox *et al.* 1987; Fox & DiCorleto 1988) alter the secretion of PDGF. Specific mediators, such as coagulation factors, transforming growth factor-beta (TGFβ), and tumour necrosis factor-alpha (TNFα) can increase the capacity of cells to secrete PDGF. Thrombin and factor Xa, two products of the coagulation cascade, induce a rapid release of PDGF from cultured endothelial cells that precedes transcriptional activation and is not dependent on protein synthesis (Gajdusek *et al.* 1986; Harlan *et al.* 1986). This rapid release appears to be from a storage pool associated with a dense granule fraction after subcellular fractionation of endothelial cells (E. W. Raines, B. Ewenstein, J. S. Pober and

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R. Ross, unpublished data). Thrombin also increases PDGF A- and B-chain transcription levels in microvascular endothelial cells (Daniel *et al.* 1986; Starksen *et al.* 1987) and B-chain transcriptional rate (Kavanaugh *et al.* 1988), which is followed by the secretion of PDGF. TGFβ increases the transcriptional rate of both the A- and B-chain of PDGF, and the transcript induction is prolonged as compared with thrombin (Starksen *et al.* 1987). TNFα also increases PDGF A- and B-chain transcript expression and the release of PDGF (Hajjar *et al.* 1987; our unpublished observations).

Transformed cells

A large number of neoplastic transformed cells (either responsive or non-responsive to PDGF) secrete PDGF into their culture medium (reviewed in Bowen-Pope *et al.* 1984*b* and Ross *et al.* 1986). Cells that contain receptors can stimulate themselves in an autocrine fashion by expressing genes for one or another of the chains of PDGF. The importance of PDGF in the process of tumour formation and progression remains to be determined (see section on neoplasia).

PDGF *IN VIVO*

Wound repair

During the process of wound repair, the blood coagulation response that immediately results from injury is followed by a massive influx of polymorphonuclear neutrophilic leukocytes, monocytes and lymphocytes. Both the platelets involved in blood coagulation and the monocytes that become actively phagocytic are capable of secretion of cytokines and growth factors, including PDGF, into the wound fluid. PDGF may be involved in activation of these infiltrating cells, and recent data suggests that there may be receptors for PDGF on microvascular endothelial cells (Hermansson *et al.* 1988). Thus PDGF may also play a role in inducing angiogenesis at the wound site.

Grotendorst *et al.* (1985) demonstrated that the addition of PDGF to Hunt–Schilling wound chambers containing collagen gel induced an early influx of connective tissue cells, increased DNA synthesis, and increased collagen deposition. Sprugel *et al.* (1987) examined the effect of implantation of PDGF, TGFβ, basic fibroblast growth factor (FGF), or EGF in wound chambers made of polytetrafluoroethylene containing collagen or collagen–heparin mixtures. They observed that PDGF, basic FGF, and TGFβ each induced granulation tissue formation within ten days after subcutaneous implantation of the wound chamber, but that PDGF was particularly effective in inducing such wound repair. PDGF may be important in wound repair because it can be derived from most of the cells involved in the different phases of the process, including platelets, monocyte-macrophages, endothelium, fibroblasts and smooth muscle. The possible use of recombinant forms of PDGF in enhancing wound repair in deficient wound-healing circumstances such as diabetes may be important in the development of agents to improve this process.

Embryogenesis and development

PDGF may play a role in embryogenesis and development. Goustin *et al.* (1985) demonstrated B-chain expression in first-trimester human placentas. Taylor & Williams (1988) further observed placental expression of transcripts for the A- and B-chains of PDGF and for the β-subunit of the PDGF receptor. Early expression of PDGF has been found in *Xenopus* embryos (Mercola *et al.* 1988) and in pre-implantation mouse embryos (Rappolee *et al.* 1988). In *Xenopus* embryos, both forms of the PDGF A-chain are encoded by maternal mRNAs. Thus PDGF

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may be involved during a number of stages of normal development. Much remains to be learned about this process.

Atherosclerosis

Advanced lesions of atherosclerosis are proliferative smooth muscle lesions in the intima (the innermost layer) of medium and large arteries that can lead to myocardial infarction, cerebral infarction and peripheral vascular disease. The initiating event in the development of lesions is thought to be endothelial injury. The earliest lesion of atherosclerosis, the fatty streak, begins as an essentially pure monocyte-derived macrophage lesion that contains some lymphocytes. With time, this lesion may progress to one that contains not only monocyte-macrophages and T-lymphocytes, but large numbers of smooth muscle cells. The principal cellular component that leads to lesion enlargement and occlusion of vessels such as the coronary, carotid, cerebral, or peripheral arteries results from the migration of smooth muscle cells from the media into the intima and to their further proliferation and deposition of connective tissue within the intima. It has been suggested that activated platelets or macrophages (Gerrity 1981*a*, *b*; Faggiotto *et al.* 1984*a*, *b*; Rosenfeld *et al.* 1987*a*, *b*) may be critical cells involved in the early deposition of growth factors and cytokines, including both chains of PDGF, that can play roles in inducing migration and proliferation of smooth muscle cells in the intima. Smooth muscle cells from the lesions of atherosclerosis express the A-chain of PDGF (Libby *et al.* 1988; Wilcox *et al.* 1988), and thus may participate in lesion expansion as a result of autocrine stimulation.

Analysis of human carotid artery lesions of atherosclerosis circumstantially supports a role for PDGF expression in lesions. The levels of PDGF B-chain mRNA were fivefold higher in atherosclerotic plaques than the low level constitutively expressed in the normal artery (Barrett & Benditt 1987). Barrett & Benditt (1988) analysed fractions of very advanced atherosclerotic plaques for PDGF genes and found a correlation of the PDGF A-chain with c-*fms* (the CSF-1 receptor in macrophages) and the B-chain of PDGF with von Willebrand factor (an endothelial-specific protein). Wilcox *et al.* (1988) used *in situ* hybridization to examine lesions of atherosclerosis obtained by carotid endarterectomy, and observed that the B-chain hybridized with endothelial cells of interplaque arteries and the A-chain with intimal smooth muscle-like cells. In both of these studies of human tissues, the lesions were very advanced and contained extensive necrosis. Further data is needed at the cellular level of early and intermediate lesions to determine the role of each of the cells in lesion genesis and progression, since the important proliferation-inducing events occur during development of the lesions.

Neoplasia

Interest in PDGF in relation to neoplasia began with the observation that simian sarcoma virus (SSV) transformed cells secrete a protein $p28^{sis}$, coded for by the oncogenes v-*sis* derived from the retrovirus. This protein is highly homologous (93%) with the B-chain of PDGF (Doolittle *et al.* 1983; Waterfield *et al.* 1983; Johnsson *et al.* 1984). The virus was originally derived from a woolly monkey that had multiple subcutaneous fibrosarcomas (Thielen *et al.* 1971; Deinhardt *et al.* 1972). Transformation by SSV *in vitro* appears to be limited to connective tissue cells that express the PDGF receptor (Leal *et al.* 1985).

A large number of cells obtained from naturally occurring human tumours as well as cells transformed *in vivo* by retroviruses and a series of adenoviruses produce PDGF-like molecules. It is unclear whether expression of PDGF is associated with the initial transformation event or subsequent to initiation. However, in cells that express PDGF receptors, PDGF may also

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contribute to tumour growth and progression. Many tumour cells that express and secrete PDGF do not have receptors and thus are not able to respond mitogenically to PDGF. These tumours, such as colon carcinomas, may secrete PDGF that may bind to cells in the adjacent connective tissue stroma. In such a scenario, PDGF could stimulate proliferation of connective tissue formation in a desmoplastic response. Thus PDGF may affect some tumours in an autocrine fashion, and may affect adjacent tissues in a paracrine fashion.

Inflammation and fibrosis

Several fibrotic phenomena are associated with inflammatory disease. Perhaps one of the most prevalent of these is chronic synovitis, in which an increase in the β-subunit of the PDGF receptor has been demonstrated in the inflamed tissue (Rubin *et al.* 1988*a*, *b*). Significant levels of PDGF have also been observed in the synovial fluid of patients with both osteoarthritis (Hamerman *et al.* 1987) and rheumatoid arthritis (E. W. Raines, F. M. Brennan, D. Chantry, M. Turner, R. Maini, M. Feldmann & R. Ross, unpublished results). In the latter case, PDGF may induce the synovial proliferation that results in pannus formation, fibrosis and the resultant clinical sequelae.

Similarly, PDGF may be secreted in numerous other responses such as interstitial pulmonary fibrosis. PDGF is secreted by activated alveolar macrophages (Shimokado *et al.* 1985). Mornex *et al.* (1986) demonstrated that alveolar macrophages obtained from individuals with idiopathic pulmonary fibrosis, sarcoidosis and histiocytosis X transcribe the gene for the B-chain of PDGF. Martinet *et al.* (1986) showed that macrophages from patients with pulmonary fibrosis secrete increased amounts of PDGF.

Conclusions

Platelet-derived growth factor is a ubiquitous molecule that can play important roles in normal wound repair, in embryogenesis and development, in neoplasia, and in the intimal proliferative smooth muscle response in atherosclerosis. Although PDGF can be expressed and secreted by a large number of cells, its expression and biological activity appear to be controlled at many different levels. Understanding these levels of control should provide opportunities to intervene in the activities of this molecule at sites where its activities are undesirable, and perhaps to augment PDGF action where it could be beneficial, as in deficient wound repair. PDGF, like numerous other growth factors and cytokines, exerts its actions in a complex environment in which other growth factors and cytokines also induce cellular responses. The integration of these responses and the presence of inhibitors that can modify these responses will determine the net effects induced by PDGF.

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