Mechanism of Action and In Vivo Role of Platelet-Derived Growth Factor

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Platelet-derived growth factor (PDGF) is a major mitogen for connective tissue cells and certain other cell types. It is a dimeric molecule consisting of disulfide-bonded, structurally similar A- and B-polypeptide chains, which combine to homo- and heterodimers. The PDGF isoforms exert their cellular effects by binding to and activating two structurally related protein tyrosine kinase receptors, denoted the α-receptor and the β-receptor. Activation of PDGF receptors leads to stimulation of cell growth, but also to changes in cell shape and motility; PDGF induces reorganization of the actin filament system and stimulates chemotaxis, i.e., a directed cell movement toward a gradient of PDGF. In vivo, PDGF has important roles during the embryonic development as well as during wound healing. Moreover, overactivity of PDGF has been implicated in several pathological conditions. The sis oncogene of simian sarcoma virus (SSV) is related to the B-chain of PDGF, and SSV transformation involves autocrine stimulation by a PDGF-like molecule. Similarly, overproduction of PDGF may be involved in autocrine and paracrine growth stimulation of human tumors. Overactivity of PDGF has, in addition, been implicated in nonmalignant conditions characterized by an increased cell proliferation, such as atherosclerosis and fibrotic conditions. This review discusses structural and functional properties of PDGF and PDGF receptors, the mechanism whereby PDGF exerts its cellular effects, and the role of PDGF in normal and diseased tissues.

I. INTRODUCTION

Platelet-derived growth factor (PDGF) is a major mitogen for fibroblasts, smooth muscle cells, and other cells (for reviews, see Refs. 183, 377). Originally, PDGF was identified as a constituent of whole blood serum that was absent in cell-free plasma-derived serum (252, 396, 494); PDGF was subsequently purified from human platelets (19, 97, 184, 379). Although the α-granules of platelets are a major storage site for PDGF, recent studies have shown that PDGF can be synthesized by a number of different cell types.

Studies of PDGF and PDGF receptor gene targeting in mice have shown that PDGF has important functions as paracrine growth factor in development. Moreover, aberrant synthesis of PDGF and concomitant autocrine growth stimulation may be an important step in the neoplastic conversion of PDGF receptor-positive cells. Here, we describe the most important features of PDGF and PDGF receptors with regard to structure, signal transduction, and physiological and pathophysiological role in vivo.

II. PLATELET-DERIVED GROWTH FACTOR ISOFORMS

A. Structure of PDGF

Platelet-derived growth factor is a family of cationic homo- and heterodimers of disulfide-bonded A- and B-polypeptide chains. The mature parts of the A- and B-chains of PDGF are ~100 amino acid residues long and show ~60% amino acid sequence identity. Eight cysteine residues are perfectly conserved between the two chains; a similar spacing between cysteine residues is seen also in members of the vascular endothelial cell growth factor (VEGF) family (VEGF, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor) (reviewed in Ref. 219). Two of the cysteine residues (the second and the fourth) are involved in cysteine bonds between the two subunits in the PDGF dimer, and the other six are engaged in intrachain disulfide bonds (the first pairs with the sixth, the third with the seventh, and the fifth with the eighth) (166, 167, 340). Mutation of the interchain disulfide bonds is compatible with retained biological activity of PDGF (12, 238), presumably because the molecule still occurs as a dimer (371).

Platelet-derived growth factor-BB has been crystallized and its three-dimensional structure solved at 3.0 Å resolution (328). The two subunits in the dimer are arranged in an antiparallel manner. They consist of a tight knotlike structure in which one of the intrachain disulfides goes through the hole formed by the other two and the intervening sequences. From the cystine knot, two large loops stabilized by β-sheet interactions extend in one direction (loops 1 and 3) and a shorter loop 2 points in the opposite direction. Because of the antiparallel arrangement of the molecule, loops 1 and 3 of one subunit in the dimer will be close to loop 2 of the other.

The three-dimensional structure of PDGF-BB is not only similar to that of VEGF, which has a related amino acid sequence (313), but also shows some resemblance to those of nerve growth factor and transforming growth factor-β (TGF-β), despite the fact that the latter factors have no sequence similarity with PDGF (315). All these factors are dimers and show the characteristic cystine knot structure.

B. Biosynthesis and Processing of PDGF

Both the A-chain and the B-chain of PDGF are synthesized as precursor molecules that undergo proteolytic...
processing in the NH₂ termini and, in the case of the B-chain, in the COOH terminus (Fig. 1) (337). The mature native PDGF isoforms migrate in SDS-gel electrophoresis as components of a \(30\) kDa, whereas the reduced chains migrate as 15-kDa components. The A-chain has one potential site for attachment of \(N\)-linked carbohydrates, but PDGF is most likely not glycosylated. Recombinant PDGF has been produced in mammalian cells (339), baculovirus-infected cells (142), and yeast cells (236, 338) as well as in bacteria (111, 197).

Human platelets and cultures of cell lines naturally expressing both A- and B-chains contain all three PDGF isoforms, suggesting that the assembly of PDGF dimers is a random process (162, 164, 171). This notion is further supported by the observation that cells transfected with both A- and B-chains make all three isoforms (339) and that bacterially made PDGF chains can be assembled in vitro into homo- as well as heterodimers (198).

C. PDGF Genes

The genes for the A- and B-chains for PDGF are located on chromosomes 7 and 22, respectively (43, 89, 460). They are organized in a similar manner with seven exons. In each case exon 1 encodes the signal sequence, exons 2 and 3 encode precursor sequences that are removed during processing, exons 4 and 5 encode most of the mature protein, and exon 7 is mainly noncoding (61, 217, 394). Exon 6 encodes a COOH-terminal sequence that may be removed during the maturation of the B-chain; the A-chain occurs as two different splice forms, with and without the exon 6-encoded sequence.

D. Expression of PDGF

Platelet-derived growth factor is synthesized by many different cell types (Table 1). The synthesis is often increased in response to external stimuli, such as exposure to low oxygen tension (253), thrombin (90, 169), or stimulation with various growth factors and cytokines (reviewed in Ref. 42). Expression of PDGF-A also increases in human uterine smooth muscle cells during the physiological hypertrophy of pregnancy (299). Most cell types expressing PDGF make both A- and B-chains, but the expression of the two chains are independently regulated at the transcriptional as well as posttranscriptional levels (reviewed in Ref. 102).

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>PDGF-A</th>
<th>PDGF-B</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblasts</td>
<td>+</td>
<td>+</td>
<td>348, 377</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>+</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>Placental cytotrophoblasts</td>
<td>+</td>
<td>+</td>
<td>149, 377</td>
</tr>
<tr>
<td>Leydig cells</td>
<td>+</td>
<td>+</td>
<td>143</td>
</tr>
<tr>
<td>Kidney mesangial cells</td>
<td>+</td>
<td>+</td>
<td>2, 377</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>+</td>
<td>+</td>
<td>420</td>
</tr>
<tr>
<td>Vascular smooth muscle cells</td>
<td>+</td>
<td>+</td>
<td>324, 377, 419</td>
</tr>
<tr>
<td>Vascular endothelial cells</td>
<td>+</td>
<td>+</td>
<td>99, 377</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>+</td>
<td>+</td>
<td>325, 375, 386</td>
</tr>
<tr>
<td>Neurons</td>
<td>+</td>
<td>+</td>
<td>400, 507</td>
</tr>
<tr>
<td>Schwann cells</td>
<td>+</td>
<td>+</td>
<td>108</td>
</tr>
<tr>
<td>Oocyte, blastocyst</td>
<td>+</td>
<td>+</td>
<td>300</td>
</tr>
<tr>
<td>Uterus endometrium/myometrium</td>
<td>+</td>
<td>+</td>
<td>54, 299</td>
</tr>
<tr>
<td>Mammary epithelial cells</td>
<td>+</td>
<td>+</td>
<td>64</td>
</tr>
<tr>
<td>Retinal pigment epithelial cells</td>
<td>+</td>
<td>+</td>
<td>71</td>
</tr>
<tr>
<td>Macrophages</td>
<td>+</td>
<td>+</td>
<td>377, 424</td>
</tr>
<tr>
<td>Platelets/megakaryocytes</td>
<td>+</td>
<td>+</td>
<td>225, 377</td>
</tr>
</tbody>
</table>

PDGF, platelet-derived growth factor.
The 3.5-kb PDGF B-chain mRNA has an ~1-kb-long 5′-untranslated sequence that contains stable secondary structures and three small open reading frames, and it therefore acts as a potent translational inhibitor. The 5′-untranslated region contains an internal ribosomal entry site, which becomes more potent in the erythroleukemia cell line K562 upon their differentiation to megakaryocytic cells (38). Moreover, additional PDGF-B transcripts have been described; a 2.8-kb transcript that is present in endothelial cells lacks the long 5′-untranslated sequence and appears to be initiated from within the first exon genomic sequence (118), and a 2.6 kb transcript that is present in JEG-3 cells initiates at an alternative exon located in intron 1 (103). These alternative transcripts thus lack the translational inhibitory effect exerted by the exon 1 sequence of the 3.5-kb transcript. Interestingly, the expression of a 2.6-kb PDGF-B mRNA, lacking the inhibitory 5′-untranslated sequence, was found to correlate with increased PDGF B-chain immunoreactivity during rat brain development (408).

The PDGF-B gene promoter has been found to contain a regulatory element, called Sis proximal element (SPE), at nucleotides −64 to −45 (104, 211, 212, 240). A CACCC motif within SPE that binds Sp1 and Sp3 transcription factors is important for transcription of the PDGF-B gene in U-20S osteosarcoma cells (273) and in K562 cells (274). Consensus binding sites for AP-1-like and Ets-like transcription factors were also observed at nucleotides −92 to −85 and −80 to −70, respectively (240). Elements involved in throbmin-induced PDGF-B transcription in endothelial cells (412) and in 12-O-tetradecanoylphorbol 13-acetate (TPA)-dependent promoter activation in K562 cells (352), have also been identified, as well as a shear-stress response element in endothelial cells (383) that is activated by NFκB (240, 241). An enhancer-like transcriptional activator region composed of at least two distinct elements that is active in the choriocarcinoma cell line JEG-3 has also been identified in the first intron of the PDGF-B gene (131). There is also evidence for negative regulation of the PDGF-B promoter since cultured dermal fibroblasts contain a transcriptional activator for the PDGF-B promoter but do not express the protein (101). No silencer could, however, be identified in a region from −12 to −25 kb relative to the transcription start site (101); the mechanism for inhibition of PDGF-B transcription, thus, remains to be elucidated. Analysis of somatic cell hybrids between PDGF-B expressing melanoma cells and hamster fibroblasts revealed that the negative regulation of the B-chain is probably mediated by a trans-acting factor (269).

Also, the transcription of the PDGF-A gene is regulated by positive and negative regulatory elements. A GC-rich region located at nucleotides −115 to −33 has been shown to contribute >80% promoter activity (220). The region contains three consensus Sp1 binding sites and two overlapping Egr-1 sites. In smooth muscle cells, Sp1 and the related Sp3 bind to the region and activate the A-chain promoter independently or additively (431). A variety of stimuli, including TPA, PDGF, and mechanical injury, induce Egr-1 expression in smooth muscle cells; the induced Egr-1 displaces members of the Sp family and activates the PDGF-A promoter further (431). Egr-1 is also involved in activation of a shear-stress-response element in the A-chain promoter in endothelial cells (239). In mesangial cells, TPA induces the synthesis of a nuclear protein, PDGF-A gene binding protein-1, which binds to the sequence −102 to −82 relative to the transcription start site (45). Elements located at nucleotides −223 to −135 and −71 to −55 have been shown to be important for serum-induced and TPA-induced expression of PDGF-A, respectively (242, 461). Methylation of the PDGF-A gene promoter in vitro was found to repress its activity (278).

The expression of PDGF-A is repressed by several stimuli, such as glucocorticoid treatment of smooth muscle cells (318) and aging of human fibroblasts (226). Several regions involved in negative regulation of the PDGF-A gene have been identified. The nucleotide sequences located between −1029 and −883 and between −1800 and −1029 exert negative control of the PDGF-A gene in the renal epithelial cell line BSC-1 (221, 282). Moreover, the Wilms' tumor suppressor gene product (WT1) binds to several sites in the region −643 to −8, relative to the transcription start site, and functions as a powerful repressor of the A-chain (136, 491). Platelet-derived growth factor-A is overexpressed in certain Wilms' tumors; it is therefore possible that loss of WT1 may lead to overexpression of PDGF-A and thereby contribute to the etiology of the tumor. There is also a negatively acting control element in the first intron that is active in HeLa cells but not in A172 glioblastoma cells (326, 492).

E. Retention Sequence in the COOH Terminus of PDGF

In both the A- and B-chains, exon 6 encodes a basic sequence that mediates interaction with components of the extracellular matrix and also may cause retention inside the producer cell (237, 264, 336, 367, 380). Platelet-derived growth factor binds to various types of collagens (440), thrombospondin (193), and BM-40/osteopontin/SPARC (378); however, the major component of the matrix involved in PDGF binding is likely to be heparan sulfate (286). The binding involves an electrostatic interaction between basic residues in the retention motif (14, 286) and negatively charged groups in heparan sulfate, including N-sulfated saccharide domains containing both 2-O- and 6-O-sulfate groups (121).

The precursor of the B-chain may thus be retained in...
the matrix; after maturation when the COOH-terminal retention sequence has been cleaved off, the molecule may become more diffusible. Moreover, the compartmentalization of the two splice forms of the A-chain of PDGF may be different. The more common short splice form of the A-chain, which lacks the COOH-terminal retention motif, may diffuse more easily through the tissue and affect cells at some distance from the producer cell, whereas the long form with the retention motif will be restricted to stimulate the producer cell and cells in the immediate environment. The availability of matrix-stored PDGF may also be regulated by proteolysis of matrix molecules (122, 251, 446).

F. PDGF Binding Proteins

Platelet-derived growth factor does not only interact with matrix molecules but also with soluble proteins. Like many other cytokines, PDGF binds to α- and β-receptors, and it also binds to 2-macroglobulin (reviewed in Ref. 260). This interaction, which involves PDGF-BB but not PDGF-AA (59), regulates the amount of PDGF available for interaction with receptors. Another PDGF binding protein was isolated from a rat neural retina cell line and called PDGF-associated protein (PAP) (124). PAP binds PDGF with low affinity and was found to enhance the activity of PDGF-AA but depress the activity of PDGF-BB. Moreover, the extracellular part of PDGF α-receptor (see sect. III) has been detected in normal human plasma; it is possible that such circulating soluble receptors can compete with cell-associated PDGF receptors for ligand binding (466).

G. Receptor Binding Epitopes in PDGF

With the use of site-directed mutagenesis, the receptor binding epitopes in PDGF have been localized. The amino acid residues most important for receptor binding are localized in loops 1 and 3, but loop 2 is also involved (13, 81, 119, 265, 335). Thus each PDGF molecule contains two symmetric receptor binding epitopes, each one built up by structures from both chains in PDGF. Platelet-derived growth factor-BB interacts with α- and β-receptors with similar affinity. The interaction appears to involve overlapping but not identical regions in the ligand, since residues in loop 2 are more important for binding to the β-receptor than to the α-receptor (13).

H. Conclusions

Platelet-derived growth factor is a family of heterodimeric or homodimeric isoforms of A- and B-polypeptide chains that are synthesized as precursor molecules undergoing proteolytic maturation. The synthesis of PDGF isoforms is carefully regulated, and their action on receptors is modulated by interaction with components in the matrix as well as with soluble binding proteins.

III. PLATELET-DERIVED GROWTH FACTOR RECEPTORS

A. Structure of PDGF Receptors

Platelet-derived growth factor isoforms exert their effects on target cells by activating two structurally related protein tyrosine kinase receptors. The α- and β-receptors have molecular sizes of ~170 and 180 kDa, respectively, after maturation of their carbohydrates. Extracellularly, each receptor contains five immunoglobulin-like domains, and intracellularly there is a tyrosine kinase domain that contains a characteristic inserted sequence without homology to kinases (78, 295, 505). The structures of PDGF receptors are similar to those of the colony stimulating factor-1 (CSF-1) receptor (85) and the stem cell factor (SCF) receptor (506).

The human α-receptor gene is localized on chromosome 4q12, close to the genes for the SCF receptor and VEGF receptor-2 (448), and the β-receptor gene is on chromosome 5 (505) close to the CSF-1 receptor gene (390).

B. Homo- and Heterodimeric Receptor Complexes

Because PDGF isoforms are dimeric molecules, they bind two receptors simultaneously and thus dimerize receptors upon binding (49, 180, 418). The α-receptor binds both the A- and B-chains of PDGF with high affinity, whereas the β-receptor binds only the B-chain with high affinity. Therefore, PDGF-AA induces αα-receptor homodimers, PDGF-AB αβ-receptor homodimers or ββ-receptor heterodimers, and PDGF-BB all three dimeric combinations of α- and β-receptors (Fig. 1) (163, 223, 418).

C. Cellular Effects Mediated by PDGF Receptors

The three dimeric PDGF receptor combinations transduce overlapping, but not identical, cellular signals. Whereas the heterodimeric receptor complex may have unique properties (see sect. IV), most information is available about the functional roles of PDGF receptor homodimers (Table 2). Both α- and β-receptor homodimers transduce potent mitogenic signals. There is, however, a difference between the receptors regarding their effects on the actin filament system. Whereas both receptors stimulate edge ruffling and loss of stress fibers, only the β-receptor mediates the formation of circular actin structures on the dorsal surface of the cell (113).
TABLE 2. Cellular effects mediated by PDGF α- and β-receptor homodimers

<table>
<thead>
<tr>
<th>Effect</th>
<th>α-Receptor</th>
<th>β-Receptor</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell growth</td>
<td>Stimulation</td>
<td>Stimulation</td>
<td>183, 377</td>
</tr>
<tr>
<td>Actin reorganization</td>
<td>Stimulation of edge ruffling and loss of stress fibers</td>
<td>Stimulation of edge ruffling, loss of stress fibers, and stimulation of circular ruffles</td>
<td>113</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>Stimulation or inhibition depending on cell type</td>
<td>Stimulation</td>
<td>201, 255, 334, 428, 429, 513, 515</td>
</tr>
<tr>
<td>Ca²⁺ mobilization</td>
<td>Weak stimulation</td>
<td>Stimulation</td>
<td>100</td>
</tr>
<tr>
<td>GAP junctional communication</td>
<td>?</td>
<td>Inhibition</td>
<td>202</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>?</td>
<td>Inhibition</td>
<td>504</td>
</tr>
</tbody>
</table>

GAP, GTPase activating protein.

Moreover, activation of the β-receptor stimulates chemotaxis; in contrast, activation of the α-receptors inhibits chemotaxis of certain cell types including fibroblasts and smooth muscle cells (255, 429, 513), whereas chemotaxis of certain other cell types is stimulated (201, 334, 428, 515). Both the α-receptor and the β-receptor mediate an increase in intracellular Ca²⁺ concentration, albeit the β-receptor more efficiently than the α-receptor; pretreatment with PDGF-AA depressed the Ca²⁺ mobilization after PDGF-BB stimulation (100). Platelet-derived growth factor also inhibits gap junctional communication between cells (202) and exerts an antiapoptotic effect (504).

D. Expression of PDGF Receptors

Because there are differences between α- and β-receptors in their binding specificity of PDGF isoforms and in the signals they transduce, the response of a cell to PDGF stimulation will be determined by which of the two receptor types the cell expresses. The classical target cells for PDGF, fibroblasts and smooth muscle cells, express both α- and β-receptors, but generally higher levels of β-receptors (Table 3). Other cell types express only α-receptors, such as the O-2A glial precursor cells (174), human platelets (478), and rat liver endothelial cells (186), whereas other cell types express only β-receptors, such as mouse capillary endothelial cells (435).

Importantly, the level of PDGF receptor expression on cells is not constant. For instance, the expression of β-receptors on connective tissue cells in vivo is low but increases during inflammation (400) or after explantation into tissue culture (405). Moreover, estrogen treatment increases both α- and β-receptors and PDGF in mouse uterus and vagina (151). On the other hand, stimulation with basic fibroblast growth factor (bFGF) selectively increases the expression of the α-receptor, but not the β-receptor, in vascular smooth muscular cells (416) and in bronchial smooth muscle cells (56), and lipopolysaccharide upregulates the α-receptor but not the β-receptor in rat lung myofibroblasts (83). Moreover, interleukin (IL)-1α and tumor necrosis factor (TNF)-α induce the α-receptor in osteoblast cultures from fetal rat bone (75, 468). On the other hand, IL-1 downregulates the α-receptor in human osteoblastic cells (500). Also, TGF-β stimulation of fibroblasts or mesothelial cells leads to a decrease in the expression of the α-receptor (57, 154, 262, 349). Protein kinase C (PKC) seems to be involved in negative regulation of the α-receptor, since overexpression of PKC-α suppressed the expression of the α-receptor but not the β-receptor (125).

The α-receptor promoter has been isolated (4, 232, 489) and shown to contain a binding site for the transcription factor GATA-4, which is responsible for parietal endoderm-specific α-receptor expression (488). A region between nucleotides −246 and −139, relative to the transcriptional initiation site, was found to enhance the promoter activity in spontaneously hypertensive rats, but not normal rats (248). Also, the mouse β-receptor promoter has been isolated (24); nuclear factor Y was found to control its basal transcription activity (209).

E. Activation of PDGF Receptors Through Dimerization

A common theme for activation of tyrosine kinase receptors is ligand-induced receptor dimerization, which juxtaposes the intracellular parts of the receptors and allows autophosphorylation of tyrosine residues in trans...
between the two receptors in the dimer (reviewed in Ref. 181).

Because PDGF is a dimeric molecule, it can bind two receptors simultaneously and thus form a bridge between the receptors. The ligand binding epitopes in PDGF α- and β-receptors are located in the three outermost Ig domains (177, 516); Ig domain 2 appears to be most important for ligand binding (285, 288, 302). The situation is analogous for VEGF, which binds with high affinity to Ig domain 2 of VEGF receptor-1, a tyrosine kinase receptor with seven Ig domains extracellularly; in this case, detailed information of the interaction is available following the crystallization of VEGF in complex with Ig domain 2 of the receptor (496).

In addition to the bridging effect of PDGF, the dimeric receptor complex is further stabilized by direct receptor-receptor interactions mediated by Ig domain 4 (285, 331, 426). The corresponding Ig domain in the SCF receptor (53), and in VEGF receptor-1 (27), is also involved in direct receptor-receptor interactions. Moreover, the complex between growth hormone and its receptor, for which the three-dimensional structure is known through crystallization, also involves direct receptor-receptor interactions. Thus, whereas receptor dimerization is driven by ligand binding, ligand-receptor complexes are often stabilized by direct interactions between receptors.

F. Conclusions

Isoforms of PDGF exert their cellular effects by inducing homo- or heterodimeric complexes of α- and β-tyrosine kinase receptors, resulting in cell growth, chemotaxis, actin reorganization, and prevention of apoptosis. The α- and β-receptors are expressed on overlapping but distinct cell types; the level of receptors at the cell surface can be modulated by external stimuli.

IV. INTRACELLULAR SIGNAL TRANSDUCTION

A. Autophosphorylation of PDGF Receptors

The autophosphorylation induced after dimerization of PDGF receptors serves two important functions. On one hand, phosphorylation of a conserved tyrosine residue inside the kinase domains (Tyr-849 in the α-receptor and Tyr-857 in the β-receptor) leads to an increase in the catalytic efficiencies of the kinases, as has been demonstrated for the PDGF β-receptor (116, 234) and the receptors for insulin (495), hepatocyte growth factor (319), fibroblast growth factor (303), and brain-derived neurotrophic factor (158). On the other hand, autophosphorylation of tyrosine residues located outside the kinase domain creates docking sites for signal transduction molecules containing SH2 domains.

B. Binding of SH2 Domain Proteins to PDGF Receptors

The SH2 domain is a conserved motif of ~100 amino acid residues that can bind a phosphorylated tyrosine in a specific environment (reviewed in Ref. 350). Recent observations have shown that signal transduction molecules contain several different types of motifs that mediate interactions between different components in signaling pathways. In addition to SH2 domains, also PTB domains recognize phosphorylated tyrosine residues. Moreover, SH3 domains recognize proline-rich sequences, PH domains membrane phospholipids, and PDZ domains COOH-terminal valine residues in specific sequence contexts (350).

A large number of SH2 domain proteins have been shown to bind to PDGF α- and β-receptors (Table 4). Some of these molecules are themselves enzymes, such as phosphatidylinositol 3′-kinase (PI 3-kinase), phospholipase C (PLC)-γ, the Src family of tyrosine kinases, the tyrosine phosphatase SHP-2, and a GTPase activating protein (GAP) for Ras. Other molecules such as Grb2, Grb7, Nck, Shc, and Crk are devoid of enzymatic activity and have adaptor functions, linking the receptor with downstream catalytic molecules. Also, members of the Stat family bind to the PDGF receptors. They are transcription factors that after phosphorylation on tyrosine dimerize and translocate into the nucleus where they affect the transcription of specific genes. Each SH2 domain molecule that binds to the PDGF receptors initiates a signal transduction pathway. The more important and well-characterized signaling pathways activated by PDGF receptors are described below. For a more comprehensive discussion, see Reference 182.

C. PI 3-Kinase

Members of the PI 3-kinase family that bind to and are activated by tyrosine kinase receptors consist of a regulatory subunit, p85, and a catalytic subunit, p110. Their preferred substrate is phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2], which is phosphorylated to phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3].

Phosphatidylinositol 3′-kinase has a central role in intracellular signal transduction; it can be activated by several different signals, it has a number of downstream effector molecules, and it mediates many different cellular responses, including actin reorganization, chemotaxis, cell growth, and antiapoptosis (reviewed in Ref. 476). The precise roles of the different effector molecules in the various responses remain to be elucidated. However, members of the Rho family of small GTPases, and in particular Rac, seem to be important for actin reorganization (175) and chemotaxis (195), and the serine/threonine kinase Akt/PKB for the antiapoptotic effect (106, 231).
D. PLC-γ

Phospholipase C-γ acts on the same substrate as PI 3-kinase, i.e., PI(4,5)P₂, the products, inositol 1,4,5-trisphosphate and diacylglycerol, mobilize intracellular Ca²⁺ from internal stores and activate certain members of the PKC family (39). The binding of PLC-γ to the PDGF receptor leads to its phosphorylation on specific tyrosine residues, whereby its catalytic activity increases (298, 485). Interestingly, full activation of PLC-γ is dependent on PI 3-kinase; the PI(3,4,5)P₃ formed by PI 3-kinase binds the PH domain of PLC-γ and may anchor the enzyme at the membrane (115). Phospholipase C-γ appears not to be of primary importance for the stimulation of cell growth and motility in most cell types; however, in certain cell types, PLC-γ affects these responses (reviewed in Ref. 222).

E. Src

Members of the Src family of tyrosine kinases are characterized by the presence of one SH3 domain and one SH2 domain in addition to the catalytic domain. In its inactive configuration, a phosphorylated COOH-terminal tyrosine residue in Src is involved in an intermolecular interaction (reviewed in Ref. 114). The binding of the SH2 domain to autophosphorylated PDGF receptors, in conjunction with the conformational change of the COOH-terminal domain, phosphorylation of other tyrosines in the molecule, activates Src. Src appears to be important for the mitogenic response of PDGF, since microinjection of blocking antibodies or a dominant negative Src molecule inhibits cell growth (reviewed in Ref. 114). However, direct binding of Src to the PDGF α-receptor is not necessary for mitogenic signaling via this receptor (196).

F. Grb2/Sos

Grb2 is an adaptor molecule with one SH2 domain and two SH3 domains; the latter domains mediate binding of Sos, a nucleotide exchange factor for Ras which converts inactive Ras-GDP to active Ras-GTP (reviewed in Ref. 414). The SH2 domain of Grb2 can bind directly to autophosphorylated PDGF receptors, or indirectly via other components, such as SHC or SHP-2; after binding to PDGF receptors, these molecules become phosphorylated on tyrosine residues that can be recognized by the SH2 domain of Grb2.

Activation of Ras is of major importance for several
cellular responses. Activated Ras binds to the serine/threonine kinase Raf-1 that initiates activation of the mitogen-activated protein (MAP) kinase cascade, a pathway which is implicated in stimulation of cell growth, migration, and differentiation. Interestingly, there is a cross-talk between Ras and PI 3-kinase, which interact physically and can activate each other (203, 392).

G. SHP-2

SHP-2 is a ubiquitously expressed tyrosine phosphatase with two SH2 domains, both of which need to bind to phosphorylated tyrosine residues for full activation of the catalytic activity (366).

Through its ability to dephosphorylate autophosphorylated PDGF receptors (250) and substrates for the PDGF receptors, SHP-2 is a potential negative modulator of signaling from the PDGF receptors. However, SHP-2 may also be involved in positive signaling through its ability to act as an adaptor that binds Grb2/Sos and thus to contribute to Ras activation (272), and through its ability to dephosphorylate the COOH-terminal tyrosine residue of Src and thus to contribute to Src activation (393a, 454).

H. GAP

GTPase activating protein binds to PDGF β-receptors but not to α-receptors (178). It converts Ras · GDP to Ras · GTP and thus has a modulatory role in Ras activation by PDGF receptors (475). The magnitude of Ras activation in PDGF-stimulated cells will thus be dependent on stimulatory as well as inhibitory signals.

I. Stat

The family of Stat molecules has seven members of which Stat1, Stat3, Stat5α and -β, and Stat6 have been shown to bind to the activated PDGF β-receptor and to be phosphorylated after PDGF stimulation; binding also occurs to the α-receptor, albeit only weakly (347, 474, 482). After phosphorylation on tyrosine, Stats dimerize and translocate to the nucleus, where they act as transcription factors (reviewed in Ref. 92). Stat molecules are important for the transduction of signals downstream of cytokine receptors; however, their importance in PDGF signaling remains to be elucidated.

J. Adaptors

Adaptors are molecules that are devoid of intrinsic catalytic activity; after binding to the PDGF receptors through their SH2 domains, they connect the receptor with downstream effector molecules. The regulatory subunits of PI 3-kinase and Grb2, which form complexes with the catalytic subunit of PI 3-kinase and Sos, respectively, are examples of adaptor molecules that have been discussed already. Other adaptor molecules that bind to PDGF receptors are Shc, Grb7, Nck, and Crk.

After binding of Shc to the PDGF receptors, it becomes phosphorylated on Tyr-317, which can be recognized by Grb2/Sos and thus contributes to Ras activation (398), as well as on Tyr-239 and Tyr-240, which initiates another signaling pathway leading to induction of c-myc (148). The SH2 domain of Grb7 has similar binding specificity to that of Grb2 (452, 512); its role in PDGF signaling remains to be determined. Nck is involved in activation of the JNK/SAPK serine/threonine kinase through interaction with the serine/threonine kinases PAK1 and NIK (55, 456). Crk, which is the only SH2 protein known that binds with significantly higher affinity to the α-receptor than to the β-receptor (511), forms a complex with the docking protein Cas and the nucleotide exchange protein C3G, which has been linked to the activation of JNK/SAPK (462).

K. Control of PDGF Signaling

Several mechanisms for modulation of signaling via PDGF receptors have been elucidated. For instance, MAP kinase, which is activated by Ras, phosphorylates and inactivates Sos, which thereby leads to a decreased Ras activation (369). Another negative-feedback mechanism involves cAMP-dependent protein kinase, which is activated by PDGF through induction of prostaglandin synthesis and activation of adenylyl cyclase (150); the cAMP-dependent protein kinase inhibits several of the pathways that are activated in PDGF-stimulated cells through phosphorylation of components in these pathways. Moreover, angiotensin II has been shown to delay PDGF-BB-induced DNA synthesis in vascular smooth muscle cells; the mechanism behind the effect remains to be elucidated (88).

A striking feature of PDGF signaling is that the strength of signals is modulated by the simultaneous activation of stimulatory and inhibitory signals. Thus the tyrosine phosphorylation induced by the PDGF receptors is balanced by activation of tyrosine phosphatases by PDGF. SHP-2 is one example that has been discussed above, but also other tyrosine phosphatases may be involved (40, 305). Another example is the binding of GAP to the receptor, which will counteract the Ras activation induced by Grb2/Sos binding to the receptor.

L. Different Signaling Via Homodimeric and Heterodimeric Receptor Complexes

Homodimeric αα- and ββ-receptor complexes give overlapping but distinctly different effects on target cells
but mRNA accumulation when cells were grown on collagen, a forms a complex with the findings that Tyr-754 in the receptor complexes. This possibility was substantiated by the observations that Tyr-754 in the homodimer (401) and that Tyr-771 in the β-receptor was phosphorylated to a higher degree in the heterodimer compared with the homodimer (401). The finding that PDGF-AB gives stronger mitogenic and chemotactic effects on cells expressing both α- and β-receptors (179, 401) suggests that the heterodimeric αβ-receptor complex may have unique properties. One mechanism for such a difference would be if receptors in a heterodimeric complex were autophosphorylated on different tyrosine residues compared with in the homodimeric receptor complexes. This possibility was substantiated by the findings that Tyr-754 in the α-receptor was phosphorylated to a higher degree in the heterodimer compared with the homodimer (401) and that Tyr-771 in the β-receptor was phosphorylated to a higher degree in the homodimer compared with the heterodimer (111a).

M. Cooperation With Integran Signaling

Most of the cell types that are responsive to PDGF are anchorage dependent, i.e., they are dependent for their growth on contacts with matrix molecules surrounding the cell. Such contacts are mediated by integrins, which are transmembrane receptors for matrix molecules. Binding of integrins to their specific extracellular matrix molecules leads to the formation of focal adhesions with the assembly of a large complex of signaling molecules around the cytoplasmic tails of integrins, including Src, PI 3-kinase, and Ras (reviewed in Ref. 79). Integrin signaling enhances growth factor-mediated cell proliferation and cell migration and is necessary to prevent apoptosis (20, 133). On the other hand, fibrillar collagen suppresses PDGF-induced DNA synthesis in arterial smooth muscle cells (256). This effect is likely to be mediated by an integrin-dependent suppression of cyclin E-Cdk2 activity.

A molecular basis for a cooperation between integrins and PDGF was unraveled by the demonstration that a highly phosphorylated subfraction of PDGF β-receptors forms a complex with αvβ3-integrins (415). Moreover, engagement of β1-integrins by plating of fibroblasts on collagen or fibronectin caused a transient tyrosine phosphorylation of PDGF receptors in the absence of PDGF (459). On the other hand, PDGF simulates the synthesis of the collagen binding integrin α2β1 (5, 246). A specificity in the cooperation between PDGF and integrins has been observed since PDGF was found to stimulate α2-integrin mRNA accumulation when cells were grown on collagen, but αv- and α5-integrin mRNA accumulation when cells were grown on fibronectin (501).

N. Conclusions

Ligand-induced PDGF receptor activation causes autophosphorylation of the receptors, which leads to increased catalytic activity of the kinases and to the formation of docking sites for downstream signal transduction molecules containing SH2 domains. Therefore, a number of signaling pathways are initiated, leading to different cellular responses. Signaling is modulated positively and negatively, extracellularly through interaction with matrix molecules and intracellularly through cross-talk with different signaling pathways.

V. INTERNALIZATION AND DEACTIVATION OF PLATELET-DERIVED GROWTH FACTOR RECEPTORS

Platelet-derived growth factor receptors are not uniformly distributed in the cell membrane, but rather concentrated in caveolae, distinct membrane invaginations which are involved in endocytosis (284). Ligand binding induces internalization of the ligand-receptor complex into endosomes (443). The PDGF-receptor complex then dissociates, and the receptor recycles to the cell membrane, or, alternatively, the ligand-receptor complex is degraded upon fusion of the endosomes with lysosomes. The latter pathway appears to dominate in the cell types studied. In addition to degradation in lysosomes, PDGF receptors also undergo cytoplasmic degradation in proteasomes after ubiquitination (306, 309). The PDGF β-receptor mutants with a decreased ability to undergo ubiquitination were found to have a longer half-life and to mediate a stronger mitogenic effect than wild-type receptors (307). This observation illustrates that the rate of deactivation of PDGF receptors is an important parameter in the regulation of the mitogenic response.

The internalization of PDGF receptors is a controlled process that is dependent on the kinase activity of the receptor (443) as well as on the interaction between the receptor and PI 3-kinase (218) and possibly other molecules (308).

VI. NORMAL IN VIVO FUNCTION OF PLATELET-DERIVED GROWTH FACTOR

A. Embryonic Development

The recent inactivation of the genes for PDGF A-chain (63) and B-chain (270) as well as PDGF α-receptor (442) and β-receptor (441) in mice has provided insight into the in vivo function of PDGF. The notion that PDGF and PDGF receptors have important roles during embryonic development is supported by the findings that in each case the mice died during embryogenesis or perinatally.

Targeting of the B-chain or the α-receptor gave similar phenotypes. In both types of mice, kidney development was severely affected with a total absence of mes-
anginal cell development (270, 441). This leads to a poor filtration in the glomeruli. There was also a defective development of blood vessels, with a dilated aorta and characteristic bleeding at about the time of birth. The bleeding was likely to be because of an inability of the newly formed blood vessels of the knock-out animals to attract pericytes (279). In contrast to the β-receptor knock-out mice, however, the B-chain knock-out mice also showed heart defects with an increased size and trabeculation of the myocardium. The fact that this defect is not seen in the β-receptor knock-out animals suggests that during normal development PDGF-BB may act via α-receptors in the heart.

Knock-out of the A-chain gene led to defect development of the alveoli of the lung, giving an emphysema-like phenotype and leading to death of the mice at ~3 wk of age (63). The failure of alveoli to form was coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development (280).

Inactivation of the α-receptor led to a phenotype that was more severe than the A-chain knock-out, including cranial malformations and deficiency of myotome formation (442). This is consistent with the possibility that PDGF-BB is the natural ligand for the α-receptor in some tissues. The spontaneous mouse mutant Patch involves a deletion of the α-receptor gene (453). The Patch mice have a phenotype similar to the α-receptor knock-out mice but have, in addition, a coat color defect that possibly is due to a perturbed expression of the neighboring gene for SCF receptor.

Studies of the expression of PDGF A- and B-chains and PDGF receptors in different tissues during the development have revealed examples of expression of ligand and receptors in the same cells, suggesting autocrine stimulation, as well as in adjacent cell layers, suggesting paracrine stimulation (reviewed in Ref. 21). Maternal A-chain and α-receptor transcripts have been detected in embryos of several species (300, 343, 382), suggesting autocrine stimulation of growth of early blastomeres. The notion that PDGF A-chain has an important role in the early embryo is supported by the observations that about one-half of embryos lacking the A-chain gene die before embryonic day 10.5 (E10.5) (63) and that the Xenopus embryos injected with a dominant negative α-receptor mRNA stop cleaving and subsequently die (22).

At later stages of development, PDGF receptors are often expressed in mesenchymal structures and the corresponding ligands in adjacent epithelial layers. For example, at E7.5, the α-receptor is expressed in the mesoderm, with the exception of the primitive streak (333), and the A-chain is expressed in the nearby ectodermal and endodermal layers (332, 333, 344). The A-chain is expressed in the epidermal ectoderm, and the α-receptor is present in the dermis (333, 413). The A- and B-chains are expressed in lung epithelium and the α- and β-receptors in mesenchyme (63, 444). The A-chain is expressed in the mouse limb ectoderm and the α-receptor in the limb bud mesenchyme (332, 413). The B-chain is expressed in glomerular epithelium and the β-receptor in the metanephric blastoma (10). The PDGF A- and B-chains are expressed in Sertoli cells of the testis and PDGF α- and β-receptors in the adjacent peritubular myoid cells (143). These expression patterns suggest important roles for PDGF in paracrine stimulation of mesenchymal cells.

The PDGF receptors are also expressed in cells of nonmesodermal origin. The α-receptor is expressed in the ectodermally derived neural crest (192, 311, 332, 333, 343, 413), and the β-receptor is expressed on mammary epithelial cells (464); both receptors are expressed on retinal pigmented epithelial cells (70) and in the central nervous system (CNS) (see Table 3 and sect. viB).

With the consideration of the elaborate expression of PDGF A- and B-chains and α- and β-receptors in the embryo, it may be somewhat surprising that embryos with the genes for either of the ligands or receptors inactivated survive as long as they do. Part of the explanation is likely to be that α- and β-receptors, which mediate similar intracellular signals, can compensate for each other, since both receptors bind PDGF-BB with high affinity. There may also be a redundancy between the ligands; both PDGF-AA and PDGF-BB bind the α-receptor with high affinity.

B. CNS

Analyses of the temporal-spatial expression of PDGF ligands and receptors provide evidence for a role of PDGF in the development of the CNS through paracrine and autocrine stimulation. In mice, PDGF A-chain mRNA is found in neurons in spinal cord and dorsal root ganglia on E12, whereas it occurs in brain on E15 and continues to be expressed in most adult neurons (507). Analyses of the monkey Macaca nemestrina confirm these findings (409). Platelet-derived growth factor A-chain expression in neurons precedes that of astrocytes and may therefore constitute an earlier signal for oligodendrocyte development than astrocyte-derived PDGF (see also below).

Evidence for the expression of PDGF B-chain in the CNS was initially derived from in situ mRNA analysis and by the use of a transgenic mouse expressing a reporter gene under the transcriptional control of a putative B-chain promoter (409). The distribution of PDGF B-chain immunoreactivity confirms these findings; B-chain protein is found in neurons in several CNS regions of the embryo and in the adult (407). The earliest and strongest expression is recorded in the olfactory system, notably in olfactory nerve fibers and accessory olfactory nerve fibers. The PDGF B-chain content stays at a high level in the adult olfactory system. Because the primary sensory neurons of
the olfactory system retain their capacity to regenerate in the adult, this finding is in accordance with the suggested role of PDGF as a neurotrophic factor.

Expression of the PDGF α-receptor is found in glial precursors in various regions of the developing CNS. There is a transient expression of the α-receptor in the neuronal tube at E9 in the mouse (332, 413), whereas it occurs in the brain, brain stem, and spinal cord on E13.5. After E16, PDGF α-receptor expression is distinctly found in the O-2A oligodendrogial-astroglial precursors (372). This finding adds to the notion that the PDGF α-receptor is a critical determinant for the development of the oligodendrocyte compartment of the brain. Whereas the expression of PDGF α-receptor initially was thought to be restricted to the glial lineage (e.g., Refs. 508, 509), there is recent evidence that also this PDGF receptor type is expressed on postnatal neurons (342).

The distribution of PDGF receptors and the cognate ligands in the CNS suggests a functional role in the development and functional properties of the brain and spinal cord. With a few exceptions, however, information on the contribution of PDGF to the development and function of the CNS is as yet very scarce.

Studies of the growth and differentiation of the bipotential O-2A cells in vitro suggested an important role for PDGF in these processes. In their pioneering work, Raff and Noble (reviewed in 374, 376) defined the O-2A cell as a progenitor cell capable of differentiating into either type 2 astrocytes or oligodendrocytes. Although the O-2A cell was initially identified in the rat optic nerve, there is accumulating evidence that analogous cells are present in the entire CNS. In culture, the O-2A cells undergo premature differentiation to oligodendrocytes when deprived of growth factors; among these, PDGF is a critical factor. The mitogenic activity in astrocyte-conditioned medium was shown to be identical to PDGF-AA (386), and several studies have indicated that the presence of PDGF is obligatory for the proper timing of differentiation of the O-2A cells (173, 174, 375). Interestingly, the combined action of PDGF and bFGF leads to a complete block of differentiation and functional immortalization of the O-2A-cells (68). Moreover, PDGF added together with bFGF converts slowly proliferating adult O-2A progenitor cells to rapidly dividing cells with a phenotype similar to that of perinatal O-2A cells (498).

O-2A cells express PDGF α-receptors (173, 174) and will therefore respond to all isoforms of PDGF. Although astrocytes were initially supposed to be the single or main source of PDGF, several cell types in the CNS, including neurons, synthesize PDGF (see above).

Using the expression of the PDGF α-receptor as a marker in wild-type mice, Pringle and Richardson (373) identified a subset of neuroepithelial cells in the ventral half of the E14 rat spinal cord. These cells were seen to proliferate and migrate to populate the entire spinal cord. In vitro, all cells of the PDGF α-receptor phenotype derived from the ventral E14 spinal cord were found to differentiate into oligodendrocytes. No such cells were found in other regions (161). These data indicate that PDGF α-receptor-positive cells in the developing spinal cord are oligodendrocyte precursors (O-2A cells) and that most or all mature oligodendrocytes are derived from these cells. Although the PDGF α-receptor was used as a marker for the precursor cell, the actual role of PDGF in the development of oligodendrocytes in vivo has not yet been determined.

Probably because of the partial redundancy in the PDGF-PDGF-receptor pathway, analyses of the PDGF A- and B-chain knock-out mice have so far provided only limited information on the role of PDGF in the development of the O-2A lineage. Other in vivo data, however, suggest that PDGF is involved in the production of myelinating cells. Overproduction of PDGF in the rat CNS afforded by transplantation of PDGF-producing cells was found to lead to an expansion of the oligodendrocyte compartment, as a result of a reduced rate of apoptosis (29). Furthermore, PDGF has been found to reduce chemically induced rat oligodendrocyte death and enhance myelination in vitro (132). In conclusion, there is accumulating evidence that PDGF has a critical role in the development of the myelinating cells of the CNS. This finding has obvious therapeutical implications, particularly if PDGF can be used to induce remyelination.

Schwann cells, the myelinating cells in the peripheral nervous system, express PDGF β-receptors and respond to PDGF-BB with an enhanced proliferation in vitro (93, 108). In these cells, PDGF acts synergistically with agents that stimulate the formation of cAMP. The major effect of cAMP is to increase the expression of β-receptors (493). These observations suggest that PDGF might play a role in the development and maintenance of the peripheral neuronal system (109).

A neuroprotective and neurotrophic effect of PDGF has been recorded in cultures of rat dopaminergic neurons (341, 361). Thus addition of PDGF-BB to rat as well as human dopaminergic cells resulted in an increase in survival and neurite formation of tyrosine hydroxylase-positive cells (341). The presence of PDGF β-receptors on these cells was confirmed by immunohistochemistry. The effect of PDGF on cell survival was as marked as that of brain-derived neurotrophic factor, although PDGF was less efficient in promoting neurite outgrowth. The in vivo expression of PDGF was examined in 6-hydroxydopamine-induced lesions of dopaminergic neurons in the substantia nigra of rats (135). Expression of both the A- and B-chain was increased after injury, suggesting a role in compensatory neuroprotection and repair processes. The finding that PDGF exerts a neuroprotective effect on rat dopaminergic neurons exposed to 6-hydroxydopamine in culture (361) supports this notion. As suggested by Oth-
berg et al. (341), PDGF agonists may be potential therapeutic agents for patients with Parkinson’s disease. Platelet-derived growth factor and PDGF receptors are upregulated in infarcted human brain tissue, suggesting a role in neuroprotection and regeneration. An upregulation of PDGF and PDGF receptors was also found in facial nuclei in rats after axotomy (190). Neurons were found to have an increased expression of A- and B-chain mRNA as well as an increased expression of PDGF β-receptor. The density of PDGF α-receptor-positive astrocytes increased as well as their content of PDGF immunoreactivity.

C. Vascular System

The PDGF receptors are expressed on capillary endothelial cells (26, 35, 294, 425, 435), and PDGF has been shown to have an angiogenic effect (30, 321, 388, 410). The effect is, however, weaker than that of fibroblast growth factors or VEGF, and PDGF does not appear to be of importance for the initial formation of blood vessels, since no apparent vascular abnormality was observed during early embryogenesis in mice with genes for PDGF or PDGF receptors inactivated. However, in specific organs, the effect of PDGF on angiogenesis may be significant. Thus stimulation of PDGF-AB production in cardiac microvascular cells leads to induction of both von Willebrand factor and VEGF and VEGF receptor-2, suggesting an important role of PDGF in cardiac angiogenesis (110). Administration of PDGF-BB has been shown to induce functional anastomoses in vivo (65, 293). Moreover, PDGF B-chain produced by capillaries may have a generally important role to recruit pericytes that is likely to be required to promote the structural integrity of the vessels (86, 279, 458).

Platelet-derived growth factor has also been implicated in the regulation of the tonus of blood vessels. On one hand, PDGF induces constriction of different types of blood vessels (37, 405). On the other hand, PDGF-BB stimulation of endothelial cells induces a nitric oxide-mediated relaxation of rat aorta (87), and intravenous injection of PDGF-BB in rats was found to lower the systolic blood pressure through increased macrovascular compliance (206).

Another effect of PDGF that is of importance in the vascular system is its feedback control effect on platelet aggregation. Platelet-derived growth factor stimulation leads to decreased platelet aggregation (66). Human platelets, which are a rich source of PDGF, have PDGF α-receptors but not β-receptors (478), and PDGF receptors have also been demonstrated on megakaryocytes, the precursors of platelets (503). After thrombin-induced platelet aggregation, the content of the α-granulae, including PDGF, is released. The fact that thrombin-induced platelet aggregation is accompanied by activation of platelet PDGF α-receptors, and that this effect can be inhibited by PDGF antibodies, indicates that the PDGF released from platelets serves an autocrine feedback role in control of platelet aggregation (478).

D. Tissue Homeostasis

The interstitial tissue pressure, which is generally slightly negative, is carefully controlled to allow appropriate exchange of fluid and macromolecules between the extracellular compartment and the circulatory system. Platelet-derived growth factor has an important role to maintain the interstitial fluid pressure (393), probably through its ability to stimulate interactions between connective tissue cells and molecules the extracellular matrix.

E. Wound Healing

The healing of soft tissues involves reepithelialization, angiogenesis, and extracellular matrix deposition. Different types of growth factors regulate the different steps in the healing process. Three lines of studies support a role for PDGF in wound healing, i.e., investigations of the effects in vitro of PDGF on cell types of importance for wound healing, analyses of the expression of PDGF and PDGF receptors during the wound-healing process, and studies of the effect of topical application of PDGF to healing wounds.

Platelet-derived growth factor acts on several cell types involved in wound healing. It stimulates mitogenicity and chemotaxis of fibroblasts and smooth muscle cells and chemotaxis of neutrophils and macrophages (reviewed in Ref. 183). It also stimulates macrophages to produce and secrete other growth factors of importance for various phases in the healing process. Moreover, PDGF has been shown to stimulate production of several matrix molecules, like fibronectin (52), collagen (72), proteoglycans (417), and hyaluronic acid (185). Platelet-derived growth factor may also be of importance at later stages of wound healing, since it stimulates contraction of collagen matrices in vitro (80, 159), implicating a role in wound contraction in vivo. Moreover, PDGF stimulates the production and secretion of collagenase by fibroblasts (32), suggesting a role in the remodeling phase of wound healing.

For PDGF to affect wound healing in vivo it has to be present at the site of the wound. Early observations revealed that PDGF is released by platelets and secreted by activated macrophages (424), thrombin-stimulated endothelial cells (169), smooth muscle cells of damaged arteries (486), activated fibroblasts (348), as well as by epidermal keratinocytes (16), suggesting that PDGF is present in the wounded area. More recent studies have revealed that PDGF is present in wound fluid from soft tissue (230, 439).
and in tear fluid produced in conjunction with corneal wound healing (481). Interestingly, with the use of iso-
form-specific monoclonal antibodies, a markedly upregu-
lated level of the long form of PDGF-AA was observed in
capillaries and fibroblasts of acute wounds and in chronic
wounds treated with PDGF-BB, whereas in maturing
granulation tissue, the short form of PDGF-AA was prev-
alent; in contrast, normal skin and nonhealing dermal
ulcers did not contain PDGF (360). It remains to be elu-
cidated why the matrix binding long form of PDGF-AA is
more prevalent early during the healing process and the
more efficiently diffusible short form is more prevalent
during the later phase.

Another prerequisite for a role of PDGF in wound
healing is that cells in the wounded area express PDGF
receptors. Immunohistochemical staining for PDGF re-
ceptors has revealed that fibroblasts and smooth muscle
cells of resting tissues contain low levels of receptors.
However, the β-receptor is upregulated, e.g., during in-
flammation (384, 400, 465). Expression of β-receptors has
also been observed in epithelial cells after cutaneous
injury (18), although this remains controversial (360).
β-receptors have also been shown to be upregulated in
human gingival wounds (153) and in growth-activated
skin from chronic wounds and psoriatic lesions (258).
Impaired wound healing in diabetic mice was found to
correlate with reduced expression of PDGF A-chain and
α-receptor (34).

Early in vivo studies showed that direct application
of PDGF in chambers implanted into rats led to increased
formation of granulation tissue (155, 449). Subsequent
studies showed increased healing after local application
of PDGF-BB to incisional wounds in rat skin (358), exci-
sional wounds in rabbit ear (316) and guinea pig (267), full
and partial thickness burn injuries in pigs (91), periodont-
al wounds in dogs and monkeys (287, 404), and rat
medial collateral ligament injury (31). A single application
of PDGF-BB to incisional wounds increased the wound-
breaking strength to 150–170% of control wounds and
decreased the time of healing (357, 358). Wounds treated
with PDGF showed an increase of granulation tissue rich
in fibroblasts and glycosaminoglycans and an increased
rate of reepithelization and of neovascularization (316,
355, 356). Thus PDGF does not alter the normal sequence
of repair but increases its rate.

Clinical trials have revealed that PDGF-BB increases
the healing of decubitus ulcers (391). Analyses of sections
from healing human wounds showed that PDGF-BB in-
duces fibroblast proliferation and differentiation (359).
Importantly, PDGF-BB was found to increase healing also
in patients with decreased healing capacity, such as dia-
abetics (451).

To have a maximum effect on wound healing, PDGF-
BB has to be given at a high concentration. Alternative
modes of administration could therefore be advanta-
geous, such as retroviral-mediated gene transfer into ker-
atinocytes (112). Alternatively, nonreplicating and noncy-
topathic recombinant vaccinia virus encoding PDGF
could be used (327).

Overactivity of PDGF may be involved in the over-
healing response of keloids, since keloid fibroblasts were
found to have four to five times more PDGF α-receptors
compared with fibroblasts of normal skin (160).

 Whereas PDGF has a significant stimulatory effect on
healing of soft tissues, the effect on fracture healing is less
clear. Both PDGF and PDGF receptors are present in heal-
ing human fractures (15, 199), and stimulatory effects of
topically added PDGF-BB on fracture healing have been
noticed in different experimental models (301, 320, 484).
However, PDGF has also been seen to inhibit the bone
regeneration induced by osteogenin and instead induced a
soft tissue repair phenotype and response (290).

F. Conclusions

Platelet-derived growth factor has important func-
tions during the embryogenesis, in particular for the de-
velopment of the kidneys, blood vessels, lungs, and CNS.
In these organs, connective tissue-like cell types are de-
pendent on PDGF, including mesangial cells, pericytes,
alveolar fibroblasts, and glial cells. The significant role of
PDGF in the formation of connective tissue is also import-
ant during wound healing in the adult.

VII. PLATELET-DERIVED GROWTH FACTOR
IN DISEASE

A. PDGF in Autocrine Transformation

The PDGF gene has been transduced as the v-sis
oncogene by two acutely transforming retroviruses: SSV
(98) and Parodi-Irgens feline sarcoma virus (41). The v-sis
product is identical to the PDGF B-chain precursor and
is identically synthesized, assembled, processed, and exter-
nalized (337, 389). Both v-sis and its cellular counterpart,
c-sis, cause transformation by an autocrine mechanism
when expressed by PDGF-responsive cells, i.e., cells that
express either β- and/or α-receptors (33, 266). Obviously,
the PDGF A-chain gene transforms α-receptor-positive
 cells only (295, 296).

The ligand-receptor interaction in sis-transformed
cells has been studied in detail. The newly synthesized
PDGF binds to immature receptors already in the ER,
where it causes PDGF β-receptor autophosphorylation
(126, 235). Several investigators have argued that the
mitogenic and transforming signal is generated intracel-
ularly (36, 204, 235), as was originally suggested by Bet-
holtz et al. (44). Others have argued that the transforming
signal is generated only by receptors located at the cell surface (126, 168, 473). The latter hypothesis is strongly supported by the finding that SSV transformation can be blocked by PDGF antibodies (216). Moreover, VALS pose dotted with the PDGF b chain vector causes fibrosarcoma (351) in mice and glioma (472). Because it is unlikely that an autocrine growth stimulation is sufficient to cause a fully malignant phenotype, it is reasonable to believe that an aberrant activation of PDGF receptors is complemented by additional aberrations of as yet unknown nature in the virally induced tumors.

The sis gene not only transforms cells in culture but also causes tumors in experimental animals. Thus infection of SSV intramuscularly or intracerebrally causes fibrosarcoma and glioma, respectively, in marmosets (95). Similarly, a Moloney murine leukemia virus-based PDGF B-chain vector causes fibrosarcoma (351) in mice and glioma (472). Because it is unlikely that an autocrine growth stimulation is sufficient to cause a fully malignant phenotype, it is reasonable to believe that an aberrant activation of PDGF receptors is complemented by additional aberrations of as yet unknown nature in the virally induced tumors.

The transforming potential of the PDGF receptor pathway is further highlighted by studies of the mechanism of action of the E5 oncoprotein of bovine papilloma virus type 1. This virus causes fibropapillomas, i.e., benign skin tumors containing transformed epithelial as well as fibroblastoid cells. The E5 gene product is a 44-amino acid homodimeric, hydrophobic membrane protein (200). Several studies have shown that E5 forms a stable complex with the PDGF b-receptor and activates its intrinsic protein tyrosine kinase (146, 323, 353, 354); this event probably explains the transforming activity of E5 in fibroblasts (387). Mutational studies show that interaction between E5 and the b-receptor occurs via their hydrophobic membrane-spanning domains (82, 145, 447, 450). One possibility is that binding of E5 to the receptor causes dimerization and thereby receptor activation. Retention of the E5 protein in the endoplasmic reticulum/cis-Golgi has no effect on PDGF b-receptor autophosphorylation but abrogates transformation. This finding reinforces the notion that activated PDGF b-receptors are transformation incompetent when located intracellularly, although it cannot be formally excluded that the transforming signal of E5/PDGF b-receptor is generated, e.g., in trans-Golgi or in post-Golgi vesicles.

B. PDGF in Human Oncogenesis

The finding that PDGF causes transformation and malignant tumors in experimental systems raises the question of whether PDGF is involved as an autocrine growth factor in the development of spontaneous tumors in humans. This question has been addressed by several investigators, and a large number of tumor types have been studied with regard to their expression of PDGF and PDGF receptors. As is evident from Table 5, many tumors express PDGF and cognate receptors, and in these an autocrine stimulation of tumor cell growth may prevail. These studies have to be interpreted with some caution, however. First, in most cases, the expression of PDGF and PDGF receptors in the normal tissue from which the tumor is derived is not known. Second, in several tumor cases, only mRNA expression has been studied. Third, all studies have been restricted to the analysis of expression, and no attempts have been made to study receptor activation in terms of constitutive autophosphorylation or substrate binding. Finally, even if an autocrine receptor activation should occur, it is not known if this is a critical or even contributing event in the development of the tumor. With these caveats in mind, we and others argue that an autocrine PDGF receptor activation may be a rate-limiting event in the genesis of some tumor types, e.g., glioma and sarcoma (156, 157, 188, 189, 297, 434).

In human glioma, PDGF a-receptor-positive cells are found in all grades, although they occur at higher densities in high-grade tumors (189). Expression of the cognate ligand, PDGF A-chain, is dramatically increased from low or undetectable levels in low grade (I and II) to high levels in high grade (III and IV) tumors, suggesting a role in tumor progression. High levels of a-receptor expression are particularly found in tumors without epidermal growth factor (EGF) receptor gene amplification. In virtually all cases of glioblastoma multiforme (grade IV tumors), there is a high expression of either EGF receptors or PDGF a-receptors. Thus activation of either type of receptor may be of critical importance in tumor progression.

Compelling evidence for an involvement of PDGF and PDGF receptors in tumorigenesis is provided by findings of structural aberrations of the corresponding genes that lead to overexpression or expression of an abnormal protein. In a few cases of glioblastoma, the PDGF a-receptor gene is amplified (127, 189, 259), leading to receptor overexpression. In cases of dermatofibrosarcoma protuberans and giant-cell sarcoma, the PDGF B-chain gene is rearranged by a chromosomal rearrangement (432). Reciprocal translocations t(17;22; q22;q13) fuse the B-chain gene to the COLIA1 gene. This rearrangement leads to the synthesis of a chimeric protein with transforming activity in culture. In chronic myelomonocytic leukemia, the b-receptor gene is fused to an ets-like gene, TEL, by a t(5;12; q33;p13) translocation (74, 147). The resulting chimeric protein is likely to behave as a constitutively active PDGF b-receptor.

The growth of certain human glioma cells in vitro...
and in vivo (422) can be blocked by PDGF antagonists. Such studies have, however, been restricted to permanent cell lines that have been adapted and selected for growth in culture. Similar studies on xenotransplanted primary tissue are therefore warranted.

In addition to being an autocrine growth factor, PDGF is also involved in paracrine stimulation of stroma cells. In several tumor types, there is a complementary expression of PDGF in tumor cells and cognate receptors in stroma cells. Such tumors include, e.g., mammary carcinoma (11, 46), colorectal cancer (457), and small-cell lung carcinoma (233). Furthermore, the development of myelofibrosis in chronic myelogenous leukemia has been ascribed to PDGF. The potential role of PDGF in stroma development was demonstrated by Forsberg et al. (130). Xenotransplanted human melanoma cells expressing PDGF-BB formed stroma-rich and highly vascularized tumors that were devoid of necroses. In the absence of PDGF, these cells formed poorly vascularized and necrotizing tumors with no detectable stroma.

### C. Atherosclerosis

Whereas PDGF is expressed at low levels in arteries from healthy adults, its expression is increased in conjunction with the inflammatory-fibroproliferative response that characterizes atherosclerosis (reviewed in Ref. 395). Thus studies of balloon catheter-injured arterial tissue (224, 289, 469), naturally occurring atherosclerosis (276, 277, 400, 497), coronary arteries after percutaneous transluminal coronary angioplasty (471), and experimentally induced atherosclerosis (144, 397) revealed increased expression of PDGF and PDGF receptors in these lesions. These observations suggest that PDGF, produced by activated macrophages, smooth muscle cells, or endothelial cells, or released from platelets in thrombi, is important for the formation of the lesion. The reason for the increased production of PDGF and PDGF receptors in atherosclerotic lesions may be in response to external stimuli but may also be due to rheological changes; low

### TABLE 5. Expression of PDGF and PDGF receptors in tumor cells

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>A-Chain</th>
<th>B-Chain</th>
<th>α-Receptor</th>
<th>β-Receptor</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
<td>156, 157, 189</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>50</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>mRNA</td>
<td>mRNA</td>
<td>437</td>
</tr>
<tr>
<td>Meningioma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>50</td>
</tr>
<tr>
<td>Pituitary adenomas and normal pituitary</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>123</td>
</tr>
<tr>
<td>Soft tissue sarcoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>268</td>
</tr>
<tr>
<td>Fibrosarcoma protuberans</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>490</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>mRNA</td>
<td>455</td>
</tr>
<tr>
<td>Capillary</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>69</td>
</tr>
<tr>
<td>Mesothelioma and mesothelioma cell lines</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
<td>Protein</td>
<td>Protein</td>
<td>261, 262</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>28</td>
</tr>
<tr>
<td>Testicular germ cell tumors</td>
<td>mRNA</td>
<td>mRNA</td>
<td>1.5-kb Spliced transcript</td>
<td>Protein</td>
<td>312</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>194</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>480</td>
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<tr>
<td>Midgut carcinoid</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>187</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>76</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>Protein</td>
<td>Protein</td>
<td>Protein</td>
<td>Protein</td>
<td>11</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>421</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>84</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>46</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>mRNA</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>134</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia</td>
<td>mRNA</td>
<td>mRNA</td>
<td>Protein</td>
<td>Protein</td>
<td>107</td>
</tr>
<tr>
<td>Acute megakaryoblastic leukemia</td>
<td>PDGF-A and/or -B</td>
<td>Protein</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
<td>312</td>
</tr>
</tbody>
</table>

(479) and in vivo (422) can be blocked by PDGF antagonists. Such studies have, however, been restricted to permanent cell lines that have been adapted and selected for growth in culture. Similar studies on xenotransplanted primary tissue are therefore warranted.

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blood flow leads to an increased production of PDGF by endothelial cells (257, 304).

The involvement of PDGF in the atherosclerosis process has been confirmed experimentally using balloon catheterization of rat carotid arteries as a model. In the denuded artery, an increased amount of activated PDGF receptors is seen in the vessel wall (3, 345). The intimal thickening that follows this treatment was inhibited by administration of neutralizing PDGF antibodies (120). In addition, a low-molecular-weight PDGF receptor kinase inhibitor, AG-1295, was recently shown to inhibit neointima formation in a porcine restenosis model (25). Moreover, infusion of PDGF-BB into rats after carotid injury (210), or expression of recombinant PDGF-BB in porcine arteries (317), caused increased intimal thickening. The role of PDGF in the atherosclerotic lesions may be to stimulate smooth muscle cells to migrate from the media of the vessel to the intima layer and to proliferate and produce matrix molecules at this site (368).

Whereas PDGF-BB is important in the restenosis process, other mitogens, like FGF, are involved. Antibodies to PDGF inhibited intima thickening by ~50% and FGF antibodies also by ~50%; the combination of PDGF antibodies and FGF antibodies resulted in 84% inhibition (403). However, PDGF-AA appears to be less important, since no appreciable inhibition of intimal hyperplasia was observed after administration of PDGF-AA antiserum (172).

Platelet-derived growth factor is also involved in neointima formation in other model systems including human saphenous vein cultured in vitro (138). It is well known that hypercholesterolemia predisposes for atherosclerosis. Interestingly, PDGF A- and B-chain mRNA levels are increased in mononuclear cells from hypercholesterolemia patients (48). Moreover, the development of aortic lesions in cholesterol-fed rabbits was inhibited by prior immunization of the rabbits with PDGF-BB (402). These observations suggest that PDGF is involved in cholesterol-induced atherosclerosis.

Given the role for PDGF in restenosis, the PDGF β-receptor has been targeted in attempts to inhibit intimal hyperplasia. Neutralizing mouse or mouse/human chimera monoclonal antibodies against the receptor have been found to inhibit intimal thickening in injured arteries of the baboon, and a murine monoclonal antibody to inhibit intimal thickening of rat carotid arteries (172). Moreover, treatment of balloon-injured vessels in the rat with antisense oligonucleotides to PDGF β-receptor significantly decreased PDGF β-receptor expression and intimal thickening (433). These observations suggest that inhibitors of PDGF-BB or PDGF β-receptor may be useful clinically to prevent the restenosis that occurs at high frequency after coronary angioplasty. Clinical trails using the rather unspecific PDGF inhibitor Trapidil have already given encouraging results (291, 330).

D. Lung Fibrosis

Studies of the effects of antisense oligonucleotides on early lung explants revealed that both α- and β-receptors were of importance in lung growth, whereas the α-receptor was of specific importance to induce lung branching (445). There are several observations that support the notion that overactivity of PDGF is involved in the development of various fibrotic conditions in the lung.

Idiopathic pulmonary fibrosis is an inflammatory and fibrotic condition in which alveolar macrophages are an important source of PDGF and other factors (292, 483). Normal alveolar macrophages express PDGF in vitro, and this expression can be upregulated by a variety of stimuli (176, 310). However, also the alveolar epithelium of patients with idiopathic pulmonary fibrosis produce PDGF and other cytokines (17). Mesenchymal cells have more PDGF β-receptors than α-receptors and thus respond better to PDGF-BB or PDGF-AB than to PDGF-AA (60, 73). Upregulation of the α-receptor on rat pulmonary fibroblasts was observed in response to stimulation with IL-1 (281) or exposure to asbestos (58).

Overexpression of PDGF has also been seen in other forms of lung fibrosis, including bronchiolitis obliterans-organizing pneumonia (23), obliterative bronchiolitis after transplantation (191), histiocytosis X (470) and coal workers’ pneumoconiosis (477), as well as fibrosis following hypoxic pulmonary hypertension (227), breathing of high concentrations of oxygen (165, 370), and exposure to asbestos (283). An increased amount of PDGF is also seen in bronchial lavage of lungs after acute injury (438, 487).

Studies of a genetic form of lung fibrosis, Hermansky-Pudlak syndrome, provided evidence that PDGF has a causal role in lung fibrosis; in this disease, an increased concentration of PDGF was observed in bronchial lavage fluid even before the patients had any symptoms (170).

Experimental evidence for a role of PDGF-BB in lung fibrosis was obtained by the demonstration that intratracheal injection of PDGF-BB causes transient proliferation of pulmonary mesenchymal and epithelial cells accompanied by collagen deposition (510). Moreover, administration of neutralizing PDGF antibodies or anti-sense PDGF oligonucleotides inhibited silica particle-induced pulmonary fibrosis in a mouse model (329).

E. Kidney Fibrosis

As mentioned in section vA, PDGF is important for the development of mesangial cells of the kidney during embryonic development (10, 270). Recent studies have shown that overactivity of PDGF is involved in the pathogenesis of several glomerular diseases that are characterized by mesangial cell proliferation, including IgA nephropathy, membranoproliferative glomerulonephritis, and lupus nephritis (reviewed in Ref. 1).
Mesangial cells cultured in vitro both produce and respond to PDGF (129, 427, 430). In normal kidney tissue, the expression of PDGF and PDGF receptor is low; the expression is increased in conjunction with glomerulonephritis in patients (140, 322) as well as in animal models (141, 205, 514). Whereas PDGF B-chain is clearly most important, the A-chain can also contribute (275). The anti-Thy-1 model in rats is a much-studied model for glomerulonephritis. It resembles IgA nephropathy, the most common form of glomerulonephritis in humans. Injection of complement-fixing antibodies against the mesangial cell antigen Thy-1 results in mesangiolysis. After infiltration of platelets and monocytes/macrophages, a massive proliferation of mesangial cells is seen after 2–6 days (214). Thereafter, a phase characterized by extracellular matrix deposition ensues, whereafter the lesion is healed. In this model, the expression of PDGF and PDGF receptors correlates with the mesangial cell proliferation (205). The notion that PDGF has a causative role in this process is furthermore supported by the finding that administration of neutralizing PDGF antibodies slows down the proliferation of mesangial cells 4 days after injury (215). The antibodies had no effect on day 2, however, suggesting that the early phase of mesangial proliferation is driven by other factors. Moreover, infusion of PDGF-BB to normal rats induced mild mesangial proliferation and a massive proliferation in rats given subnephritic doses of anti-Thy-1 sera (128).

Platelet-derived growth factor is not the only growth factor or cytokine involved in the development of glomerulonephritis. Isaka et al. (208) used an in vivo transfection technique to compare the effects of PDGF and TGF-β on glomerulonephritis; whereas introduction of PDGF B-chain caused increased mesangial cell proliferation, TGF-β caused primarily extracellular matrix accumulation. The importance of TGF-β in the development of glomerulonephritis was underscored by the observation that infusion of the TGF-β binding proteoglycan decorin inhibits the development of glomerulonephritis in the anti-Thy-1 model (62).

Platelet-derived growth factor overactivity may be involved also in another fibrotic condition in the kidney, i.e., tubulointerstitial fibrosis, since administration of PDGF-BB induced tubulointerstitial proliferation and increased production of collagen (463). This observation is of importance, since impaired renal function correlates more closely with histological changes in the tubular and interstitial compartments in the kidney than with changes in the glomerular compartment (6).

A role for PDGF in chronic vascular rejection of kidneys has been inferred from the observations that PDGF receptors (117) and PDGF (9) increase in conjunction with renal rejection.

F. Other Fibrotic Conditions

Platelet-derived growth factor has also been implicated in liver cirrhosis. In this condition fat-storing cells (Ito cells) dedifferentiate into myofibroblast-like cells that respond to PDGF (186, 362, 363). After liver injury, e.g., after administration of carbon tetrachloride, the amounts of PDGF and PDGF receptors in the liver increase (364, 499). An immunohistochemical analysis revealed that PDGF A- and B-chain are present in infiltrating inflammatory cells and along vascular structures in fibrous septa and PDGF receptors in mesenchymal cells, fibrous septa and around sinuses; the expression of receptors correlated with the severity of the lesion (365).

Another fibrotic condition where PDGF may be involved is palmar fibrosis (Dupuytren’s contracture). Both PDGF A- and B-chain were expressed by primary cell cultures from palmar fibromatosis, and the expression increased when the cells were subjected to mechanical strain; neutralizing PDGF antibodies decreased DNA syntheses in fibrotic cultures (7, 8).

There are also other conditions where PDGF may be involved. In chronic synovial inflammation, PDGF (406) and PDGF receptors (385, 399) are upregulated, suggesting a role for PDGF in mesenchymal cell proliferation in rheumatoid arthritis. In scleroderma, an autoimmune disease characterized by progressive fibrosis in the skin and in visceral organs, PDGF β-receptor (249) and PDGF (397) are upregulated. In patients with myelofibrosis of the bone marrow, PDGF levels in serum and urine have been found to be elevated (139) and the content of PDGF in platelets correspondingly decreased (229).

G. Conclusions

Overactivity of PDGF has been linked to several different disorders. In progression of glioblastoma and sarcomas, PDGF often causes autocrine stimulation of tumor cell growth. In addition, paracrine stimulation of stroma cells by PDGF made by tumor cells is also important for the balanced growth of different cell types in tumors. Overactivity of PDGF has also been implicated in atherosclerosis and several fibrotic conditions, including lung fibrosis, kidney fibrosis, liver cirrhosis, and myelofibrosis.

VIII. PLATELET-DERIVED GROWTH FACTOR ANTAGONISTS

As discussed in sections VI and VII, PDGF is implicated in autocrine and paracrine stimulation of cell growth in several different pathological conditions. Potent and specific PDGF antagonists could therefore be clinically useful.

Antagonists that interfere with the binding of PDGF
to its receptors have already been shown to have effects in animal models; a PDGF antisera inhibits neointimal smooth muscle cell accumulation in a restenosis model in the rat (120). Other possibilities to bind and inhibit PDGF include use of soluble extracellular domains of the PDGF receptors (105), PDGF binding DNA aptamers (152), or low-molecular-weight compounds binding PDGF (314). Monoclonal PDGF receptor antibodies that block ligand binding and receptor activation have been described and are also potentially useful as PDGF antagonists (263, 285, 381).

An interesting and potentially useful approach to inhibit PDGF action is by inhibiting the PDGF receptor kinase. Low-molecular-weight compounds, tyrphostins, have been described that efficiently inhibit tyrosine kinases (reviewed in Ref. 271). Some of these compounds show selectivity for inhibition of PDGF receptor kinases (47, 67, 254, 411, 423); however, their precise specificities remain to be determined. A PDGF receptor kinase inhibitor was recently shown to inhibit neointimal thickening in vivo (25).

IX. FUTURE PERSPECTIVES

During the 25 years since its discovery, PDGF has been one of the most studied growth factors. With the recent knock-out of the genes for PDGF and PDGF receptors, important roles for PDGF during embryonic development have been elucidated. However, because mice with the genes for either of the two ligand chains or the two receptors inactivated die around birth, much work remains to investigate the roles for PDGF after birth. Because PDGF and PDGF receptors are expressed at high amounts in the CNS, it will be of particular interest to explore the possibility that PDGF is of importance for the function of the CNS.

Studies during the recent years have provided an initial insight into the mechanism of signal transduction via tyrosine kinase receptors. Several different signal transduction pathways have been identified and linked to the cellular effects of growth factors. The exact mechanism whereby PDGF and other growth factors stimulate cell growth is, however, still unknown. Different research groups have reported slightly different results, partly because different cell types have been studied. The precise role of individual pathways thus remains to be determined. It is clear, however, that there is an elaborate cross-talk between different signaling pathways and that the ultimate strength of a response is determined by the balance between positive and negative signals.

The observations that PDGF and PDGF receptors are expressed on various cell types in a healing wound and that addition of PDGF increases the rate of wound healing in animal models and patients suggest that PDGF might find clinical utility to enhance wound healing. Platelet-derived growth factor is one of several growth factors that speeds up wound healing, and it remains to be determined which of the factors, or combination of factors, gives the best results in patients with a decreased ability to heal wounds, such as diabetic patients.

There is good evidence that PDGF overactivity is involved in the development of several serious disorders, including certain malignancies, atherosclerosis, and various fibrotic conditions. The development of clinically useful PDGF antagonists is therefore highly warranted. One promising type of antagonist is inhibitors of the PDGF receptor kinases. Several such inhibitors have been described; future studies will aim to identify potent inhibitors that are specific for PDGF receptors and that do not inhibit other kinases.

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