

## History of Discovery Platelet-Derived Growth Factor

Daniel F. Bowen-Pope, Elaine W. Raines

**Abstract**—A growth-promoting activity released from activated platelets, the platelet-derived growth factor, was discovered and characterized while the cellular and molecular mechanisms underlying the formation of the lesions of atherosclerosis were being investigated. This review provides a personal account of the different challenges we faced 3 decades ago in this undertaking and describes how our path was influenced by our focus on a disease process and by the evolving general understanding of the molecular effectors of cell proliferation. (*Arterioscler Thromb Vasc Biol.* 2011;31:2397-2401.)

**Key Words:** growth factors ■ platelets ■ receptors ■ vascular biology

When the 2 of us arrived in the laboratory of Russell Ross in 1975 and 1979, platelet-derived growth factor (PDGF) had already been discovered but was still known only as a growth-promoting activity from platelets. We were tasked with characterizing the structure and function of PDGF and the cell surface receptor through which its actions were expected to be mediated. Several other groups, in the United States and in Sweden, were working on these same questions during this period. Rather than present our perspective as an account of a scientific competition between groups, or as a complete history with full citations and credits, we have chosen to describe the pathway followed by the group to which we belonged, headed by Ross, which focused on a disease process to identify a molecule and a mechanism. This allows us to reflect on how the approaches that we used were influenced by the assumptions, hypotheses, and tools of the era and how these changed with time.

### Prelude: Smooth Muscle Cell Proliferation as a Key Event in the Genesis of Lesions of Atherosclerosis

A new direction in cardiovascular disease research in the early 1970s was the exploration of the roles of vascular wall cells in the formation of atherosclerotic lesions. Although others in the vascular biology arena were focusing on the metabolism and pathophysiologic roles of circulating cholesterol and lipoproteins (for example, see Motulsky<sup>1</sup> for a description of the work for which Michael Brown and Joe Goldstein won the 1985 Nobel Prize in Medicine, and Brown and Goldstein<sup>2</sup> for their own account in *Arteriosclerosis, Thrombosis, and Vascular Biology* of their discovery of the low-density lipoprotein receptor), Ross and colleagues began investigating the properties and roles played by the smooth

muscle cells (SMCs) that constitute the bulk of the artery wall. Analysis of atherosclerotic lesions by electron microscopy demonstrated that the lesions are characterized by an accumulation of SMCs associated with abundant connective tissue matrix. SMCs had been thought of as contractile cells, but Ross and colleagues demonstrated that vascular SMC in vivo and in culture are also able to proliferate and to synthesize and secrete all 3 major constituents of connective tissue: collagen, elastic fiber microfibrils, and elastin.<sup>3,4</sup>

Ross and his collaborators then demonstrated that injuring an artery by removal of the endothelium with a balloon catheter resulted in an accumulation of SMCs in the intima that resembled early atherosclerotic lesions in humans.<sup>5</sup> These observations, together with previous studies suggesting the importance of the endothelial barrier, led Ross and John Glomset to hypothesize that endothelial injury increased penetration of certain plasma factors, which promoted SMC migration from the media and stimulated their proliferation to form an early atherosclerotic lesion.<sup>6</sup> Subsequent deposition of lipid from the plasma, and of connective tissue components secreted by SMCs, would then lead to maturation of the lesion into the more complex fibrous and lipid-laden lesions of atherosclerosis. Now they needed to identify the putative driving factor in plasma. It was within this context that a factor released from platelets, PDGF, was discovered and characterized.

### Discovery of a Platelet Factor Driving SMC Proliferation

Ross believed that the processes involved in atherosclerosis should be studied in an animal model as close to human as possible and focused on the nonhuman primate *Macaca nemestrina*. This was facilitated by the location of Regional

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Primate Center facilities in the adjacent wing of the University of Washington Health Sciences Building. To test their hypothesis that arterial SMC proliferation is stimulated by growth promoting substances that leak from the plasma through injured endothelium, Ross and colleagues tested various primate serum fractions for their ability to promote the growth of medial SMCs isolated from thoracic aortas of the macaques. Macaques are not large animals ( $\approx 10$  kg), and the local colony was unable to provide sufficient blood from which to prepare serum for these studies. To increase the frequency of collections, plasma was separated from cells, and the cells were reinjected into the donor (plasmapheresis), a well established method for increasing blood collection from human donors. Surprisingly, the serum prepared by clotting this cell-free plasma was able to maintain the viability of cultured primate SMCs but did not support their proliferation.

The simplest response to this failure of SMC to proliferate in primate plasma-derived serum would have been to resume the use of whole blood serum. Instead, these observations were considered data from an unintended experiment<sup>7</sup> and were interpreted to reflect the generation or release of growth-promoting factors by blood cells during the process of clot formation. Platelets were the obvious source because they were known to release their storage granule contents during clotting. Purified platelets were found to reconstitute the growth promoting activity to plasma when added before calcium addition to induce clotting, as did platelet releasate when added to plasma-derived serum.<sup>8</sup> At this point, the putative factor was given a name (PDGF), and the response-to-injury hypothesis for the etiology of atherosclerosis was refined to emphasize the ability of platelets to focally release a potent SMC growth factor, PDGF, in areas of endothelial injury or disruption, exactly where the SMC proliferation later occurred.<sup>9</sup>

### Activity to Protein: A Large-Scale Challenge

With the cellular source of PDGF identified, the next major hurdle was the identification of its protein structure(s) and determination of whether it represented a single growth factor or a combination of different factors. At this time, molecular biology still lagged far behind protein biochemistry, and there was only 1 feasible pathway: purify the protein responsible for the activity to near homogeneity, determine its physical properties, and obtain enough of its protein sequence to deduce a nucleotide sequence. Only then could the underlying gene be identified.

Ross was determined to investigate PDGF as the causative mitogen in atherosclerosis, and this dictated that we adhere as closely as possible to human or primate sources and use vascular SMCs as assay cells. To provide enough starting material from which to purify the putative PDGF, we arranged to obtain outdated human platelet-rich plasma preparations from blood banks. These preparations had no clinical use and allowed us to process the equivalent of 2000 L of human blood per week. Unbeknownst to us, they also represented a health risk, because we worked with human platelets for several years before the AIDS virus was recognized in 1981. As the platelet lysate was fractionated by

sequential chromatographic steps, we followed the biological activity by its ability to stimulate tritiated thymidine incorporation in vascular SMCs, and later in mouse 3T3 cells, which were much easier to maintain in large numbers. We made the switch to 3T3 cells with some trepidation because it was not completely clear at the time whether vascular SMCs and 3T3 cells, an undefined mouse embryonic connective tissue cell, would both respond to the same predominant mitogen in serum.

PDGF turned out to be difficult to purify completely, in part because there was so little of it in blood, with just nanograms of PDGF accounting for the mitogenic activity in 1 mL of whole human blood. Purification to near homogeneity required an 800 000-fold purification over its representation in outdated human platelet-rich plasma. Only during the final steps was it possible to see protein bands on SDS-PAGE and to know that we were looking at PDGF: it appeared to be an approximately 30 000-Da disulfide-bonded dimer of approximately 14 000 Da and 17 000-Da subunits with distinct characteristics by peptide mapping.<sup>10</sup>

### Cloning and Expression of PDGF

After PDGF was purified, a consortium of groups obtained enough protein sequence to recognize that 1 chain of PDGF (the B-chain) is virtually identical to the putative protein product of the transforming gene (*v-sis*) of the simian sarcoma virus.<sup>11</sup> This was the first time that a retroviral oncogene had been found to encode a growth factor, and it remains a unique example. Knowing the sequence of the *Pdgfb*, and later of *Pdgfa*, allowed PDGF to be identified via transcript expression and allowed for expression of recombinant protein. We soon began to benefit from the latter via the rise of the biotech industry. The first biotech company, Genentech, was founded in 1976 in the San Francisco Bay area. In 1981, Earl Davie and colleagues in Seattle, WA, founded a biotech company, Zymose (later renamed Zymo-Genetics), to use yeast fermentation technology to efficiently produce recombinant proteins. Davie was the chair of the Department of Biochemistry, University of Washington in which Ross held a joint appointment and in whose space our laboratory was situated. The focus of Zymose was protein factors in blood, especially clotting factors, so when the genes for human *Pdgfb* and *Pdgfa* were cloned, Zymose developed a procedure for producing PDGF in yeast.<sup>12</sup> As consultants and collaborators in this endeavor, we now had access to essentially unlimited quantities of purified recombinant PDGF-AA and PDGF-BB, enough to use at high concentrations as standards, binding competitors, etc, and the isoform purity provided insight into receptor binding specificity (see below).

### Finding a PDGF Receptor

During the 1970s, it had been clearly demonstrated that 2 polypeptide growth factors, insulin and epidermal growth factor (EGF), each had a specific transmembrane receptor. We expected that PDGF would have an analogous receptor. When we had purified human platelet PDGF to near purity, we radiolabeled it with <sup>125</sup>I and used approaches analogous to those used to identify insulin and EGF receptors to demon-

strate that PDGF-responsive cells, including vascular SMCs and fibroblasts, had specific binding sites for PDGF. The apparent affinity of the PDGF receptor (PDGFR) for PDGF [Kd=0.1 ng/mL] was greater than that of the EGF and insulin receptors for their ligands and was not competed by EGF or insulin.<sup>13</sup> PDGF receptors could be detected on many stromal cell types, eg, fibroblasts and SMCs, but not on epithelial cell types. By chemically cross-linking the <sup>125</sup>I-PDGF bound to cells, we could deduce that the <sup>125</sup>I-PDGF was bound to a detergent-soluble protein of ≈175 000 Da, which we called the PDGFR.

### The PDGF Receptor Is a Tyrosine Kinase

The next obvious question to address was how PDGF binding to this receptor resulted in cell proliferation. By the 1960s, it had already been recognized that metabolic pathways could be regulated via phosphorylation of enzymes on serine and threonine, and in the 1970s, the EGF receptor was reported to have protein kinase activity, assumed to be against serine/threonine. It was not until 1979 that phosphorylation of tyrosine was first identified in a biological sample and viral transforming proteins were shown to be protein tyrosine kinases and to autophosphorylate on tyrosine. The EGF receptor was then reevaluated, and its kinase activity was determined to be against tyrosine.<sup>14</sup> It seemed possible that this exciting new enzymatic activity might be characteristic of other growth-stimulating receptors and oncogenes, including the PDGF receptor. In collaborations with the Hunter/Cooper and Krebs/Pike groups, we found that exposure to PDGF rapidly increased levels of phosphotyrosine<sup>15</sup> and that a receptor-sized transmembrane protein was itself immediately (auto)phosphorylated on tyrosine.<sup>16</sup> We had little further involvement in phosphorylation studies, but the PDGF receptor turned out to be a very fruitful system for working out mechanisms of signal transduction downstream of tyrosine kinase receptors. These studies were facilitated because fibroblast-like cells express relatively large numbers of PDGFR (so the phosphorylation signals are high) and because the biological responses of fibroblasts to PDGF (growth and chemotaxis) are large. Mutational analysis of autophosphorylation sites on the receptor led to an understanding of their function as docking/activation sites for downstream signaling effectors (reviewed in<sup>17,18</sup>).

### PDGF Is Synthesized and Secreted by Many Cell Types

As we considered the possible biological/pathological roles of PDGF, we were excited by the recently articulated hypothesis<sup>19</sup> that some protein growth factors, as contrasted with classical circulating endocrine hormones, could be secreted by a cell and act locally, either on neighboring cells (paracrine factors) or on the secreting cell (autocrine factors). Ross' original hypothesis was that PDGF functioned as both a hormone, in that it circulated to sites of action (stored in granules within platelets), and as a paracrine factor, in that it acted on target cells near the degranulating platelet. We now wondered whether platelets were the only source of PDGF. At that time, it was not easy to determine whether PDGF accounted for the growth factor activity in an experimental

sample. It was not practical to purify to homogeneity from a test sample and evaluate subunit composition by SDS-PAGE. No specific antibodies had been raised yet, and the underlying genes were not sequenced, so no transcript evaluation was possible. As an alternative, we developed a radioreceptor assay, in which the presence of functional PDGF-like molecules could be detected by ability to compete for binding of <sup>125</sup>I-PDGF to receptors on human fibroblasts.<sup>20</sup> The high affinity and specificity of the PDGF receptor made this assay very sensitive, and because PDGF from different species down to fishes bound to the human PDGFR, we could use the assay on samples from any species.<sup>21</sup>

We were surprised to find that many cells could secrete PDGF-like molecules, including vascular endothelial cells, macrophages, fetal/neonatal vascular SMCs, and oncogenically transformed fibroblasts. However, PDGF injected intravenously was rapidly cleared, with a  $t_{1/2}$  of less than 2 minutes, in part because of regulatory mechanisms such as its binding to plasma  $\alpha$ 2-macroglobulin that we identified. Detection of extracellular matrix retention sequences in specific forms of PDGF by our group and others suggested that these PDGF forms can be localized to generate concentration gradients. We did not detect PDGF receptors on endothelial cells or macrophages, so we proposed that these cells, like platelets, were involved in paracrine activation of fibroblasts and SMCs at sites of injury, both during normal response to injury and in chronic inflammatory conditions like atherosclerosis. Because normal adult SMCs and fibroblasts do express PDGFR and do not secrete PDGF, we suggested that growth of the artery wall during development may be driven by autocrine secretion of PDGF and that reactivation of such an autocrine loop in adults could play a role in driving proliferation of oncogenically transformed connective tissue cells and normal cells following injury. Altogether, this was an exciting time for generating autocrine/paracrine hypotheses (reviewed near the end of its glory days in<sup>22</sup>).

### Finding a Second PDGF Receptor

Not long after this, the PDGF receptor story got more complicated. A PDGF receptor was cloned<sup>23</sup> and could be expressed in naïve host cells, we had raised a monoclonal antibody that recognized a PDGF receptor, and we had adequate amounts of all 3 known isoforms of PDGF (recombinant PDGF-AA, and -BB from ZymoGenetics and -AB from human platelets) to do binding competition studies. We found that the cloned receptor, which was recognized by the monoclonal antibody, could not account for all of the binding of <sup>125</sup>I-PDGF, and we proposed the existence of 2 PDGFR proteins, PDGFR $\alpha$  and PDGFR $\beta$ , with PDGFR $\alpha$  able to bind PDGF A-chain and B-chain and PDGFR $\beta$  able to bind only B-chain.<sup>24</sup> Finally, we and others proposed that the functional receptor was a noncovalent dimer of 2 receptor proteins, with each subunit of dimeric PDGF binding to 1 PDGF receptor protein.<sup>25</sup> It is amusing to recollect that, at the time, the thought that there were 2 related PDGF receptors seemed extravagant. Now it is a commonplace that growth factors and their receptors exist in families, and the oddity is more that the PDGF receptor family has only 2 members.

## Two More PDGF Genes Are Found

Another decade passed before the final (as far as we know) players were identified in 2000 and 2001,<sup>26</sup> this time via database mining rather than via characterization of a biological activity. We were not involved in this, and we relate secondhand that the new PDGF members, PDGF-C and PDGF-D, were found via sequence similarity to vascular EGF family members and were initially thought to be new vascular EGF family members. When expressed as recombinant proteins, however, they were found to bind to PDGF receptors (expected to be the negative control) and not to vascular EGF receptors. They bind to PDGF receptors with slightly differing specificities than A-chain or B-chain, ie, C can bind only to PDGFR $\alpha$  and D can bind only to PDGFR $\beta$ . Unlike PDGF-A and -B, they are synthesized as inactive precursors that must be activated by proteolytic cleavage, eg, by thrombin/plasmin. This suggests a different level of regulation of function, ie, by proteolytic activation rather than regulated secretion. In general, because the 4 isoforms of PDGF act via only 2 receptor proteins and because the 2 receptors share most (but not all) signaling pathways,<sup>27</sup> much of the complexity of the PDGF system may serve to permit the maximal degree of specificity and flexibility in exactly where and when PDGF is available to drive a developmental program or to respond to a pathological threat.

## Central Role of PDGF in SMC Biology

Although the short format of this commentary does not allow a summary of experimental evidence demonstrating the PDGF function in vascular disorders that was the premise that led to its discovery, a major role for PDGF in mesenchymal biology, including vascular development and pathologies, is clearly established. Research over the past 2 decades has used multiple genetic and pharmacological approaches to probe PDGF functions in development and in a range of diseases.<sup>27,28</sup> The combined data point to a particularly important function for PDGF short-range paracrine release from adjacent cells to stimulate SMC/pericyte accumulation. This includes endothelial paracrine release of PDGF controlling pericyte and SMC proliferation, recruitment, and spreading during angiogenesis, arteriogenesis, and retinal diseases. SMC accumulation following vascular injury, such as atherosclerosis and pulmonary artery hypertension, is also dependent on PDGF paracrine release by endothelial cells, macrophages, and SMCs. Thus, PDGF, the growth factor activity for SMCs originally identified in platelet releasate, remains true to its origins, and analysis of PDGF functions continues to identify complex regulatory mechanisms to control its multiple functions.

## PDGF in the Clinic

Drugs that inhibit the tyrosine kinase activity of growth factor receptors, including the PDGF receptor, have been used with clinical successes against tumors. Clinical results for use of exogenous PDGF to enhance wound healing have also been successful. Recombinant human PDGF-BB delivered in a hydrogel (becaplermin, marketed as Regranex by Johnson & Johnson) is the only commercially available topical growth factor approved for use in cutaneous wound healing. In 1997,

the FDA approved Regranex for the treatment of chronic lower extremity diabetic neuropathic ulcers (reviewed in<sup>29</sup>). PDGF combined with bone matrix has also had clinical successes in enhancing bone repair, particularly in periodontal repair.<sup>30</sup> In contrast, PDGF receptor inhibitors have not yet proven successful against chronic vascular diseases such as atherosclerosis. For example, oral administration of imatinib, which inhibits the activity of PDGF receptors (as well as of c-kit and the BCR-ABL oncogene), did not reduce restenosis in patients treated for in-stent stenosis.<sup>31</sup> Success may require local delivery (eg, via drug-eluting stents) of higher doses of a more specific inhibitor. Thus, the short-range paracrine release of PDGF that appears to be biologically so important is also more difficult to approach therapeutically.

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## Disclosures

None.

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