Locally acting growth factors for vascular smooth muscle cells: endogenous synthesis and release from platelets

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ABSTRACT Release of platelet-derived growth factor (PDGF) from platelets has been postulated to stimulate at least some of the cell proliferation seen at sites of tissue damage, both beneficially (wound healing) and perniciously (during formation of atherosclerotic lesions). Two other growth factors have been localized to the platelet: epidermal growth factor and transforming growth factor. These factors may function synergistically with PDGF in promoting smooth muscle cell proliferation in the injured vessel wall. PDGF-like molecules (PDGF-c) that bind to the PDGF receptor and are at least partially recognized by antiserum against PDGF may also be synthesized by vessel wall cells themselves under certain circumstances. Arterial endothelial cells secrete several mitogens, one of which is a PDGF-c. Release is greatly stimulated by exposure of the cells to physiologic concentrations of thrombin. Also, aortic smooth muscle cells from 2-week-old rats secrete mitogenic levels of PDGF-c. In this case, PDGF-c accounts for all the mitogenic activity in conditioned medium (when assayed on 3T3 cells). Smooth muscle cells obtained from adult rat aortae secrete 150-fold less PDGF-c. In a third example, when adult rat carotid arteries are damaged with a balloon catheter, smooth muscle cells migrate into the intima of the artery and proliferate. By 2 weeks, the number of smooth muscle cells in the artery has doubled. When these intimal smooth muscle cells are cultured, they are found to secrete PDGF-c. These findings suggest that activation of endogenous synthesis of PDGF-c may contribute to the smooth muscle cell proliferation seen in response to vascular injury.

Circulation 72, No. 4, 735-740, 1985.

IT IS NOW recognized that smooth muscle cell proliferation plays an important role in the formation of the proliferative lesions of atherosclerosis. The involvement of platelet-derived growth factor (PDGF) in this process was suggested by an observation made by Russell Ross and his collaborators in the early 1970s that cultured arterial smooth muscle cells grew poorly in plasma-derived serum and that addition of platelet extract greatly increased the growth rate. Ross and his collaborators proposed that platelets contained a growth factor, now called platelet-derived growth factor or PDGF, which might contribute to the proliferation of vascular smooth muscle cells that occurs beneficially at sites of wound healing and perniciously during the process of formation of atherosclerotic lesions.

PDGF-like molecules (PDGF-c) from platelets. In normal blood, all PDGF seems to be sequestered within the platelets — none can be detected in the plasma. At sites of platelet adhesion and/or activation, platelets release the contents of their alpha granules. The PDGF that is released is predicted to bind to, and activate, nearby smooth muscle cells. This model provides a mechanism for the topical administration of growth factors to sites at which they are necessary for promoting tissue regeneration. Discoveries in the last few years, however, suggest both narrower and wider views of the role of PDGF in vascular biology: wider in that they suggest PDGF may be provided at sites of vascular trauma by mechanisms in addition to platelet-degranulation, and narrower in that they suggest PDGF is not the only important polypeptide growth factor present in platelets.

It was difficult to study PDGF critically when it could be identified only as a growth-promoting substance from platelets. However, PDGF has now been purified in several laboratories, it has been partially sequenced, and antiseras have been raised against it. It is known to be a protein of approximately 30,000 daltons with a basic isoelectric point and considerable hydrophobicity. With availability of pure PDGF, the molecule could be radioiodinated and the
properties of its cell-surface receptor could be determined. The binding of radiiodinated PDGF to PDGF-responsive cells has proven to be a useful method for determination of which cells are likely to be responsive to PDGF and has also allowed us to develop a sensitive radioreceptor assay for specifically detecting PDGF-c in test samples.23, 24 We have found that PDGF-responsive cells show very high affinity binding of [125I]-PDGF (figure 1).23, 25-27 This binding site does not cross react with any other growth factor or protein tested except at much higher concentrations.28 The number of binding sites varies within rather broad limits. In general, we find that connective tissue cells, including vascular smooth muscle cells, express PDGF receptors, while epithelial, skeletal muscle, and nerve cells do not.29 Cells from species as evolutionarily distant from humans as fish and rodents still bind [125I]-human PDGF with comparable affinity (figure 1). This high degree of conservation is consistent with the possibility that PDGF and its receptor serve some important role in vertebrate physiology. The techniques we have developed for determining expression of PDGF receptors and for measuring levels of PDGF-c have allowed us to extend the original hypothesis about the involvement of PDGF and PDGF-c molecules in vascular physiology.

**PDGF-c from endothelial cells.** Cultured endothelial cells have been reported to release growth-promoting activity into culture medium.29-31 We have used the PDGF radioreceptor assay to demonstrate that at least one of the mitogens made by cultured endothelial cells is PDGF-like, in that it binds to the PDGF receptor and is recognized by an antiserum prepared in goats immunized with pure PDGF.32 Comparisons between potency of endothelial cell conditioned medium in competing for PDGF binding to test cells and in stimulating 3H-thymidine incorporation to test cells suggest that the PDGF-c represents less than 25% of the total growth-promoting activity produced by endothelial cells.32, 33 This is supported by the observation that the antibody against PDGF, which completely neutralizes the PDGF binding competitor, only neutralizes about 25% of the growth-promoting activity of endothelial cell conditioned medium.

Both rapidly growing and quiescent cultured endothelial cells secrete PDGF-c constitutively, but the rate of release can be varied somewhat by specific conditions. Both tumor promoters and endotoxin can stimulate the rate of release about twofold.34 This increased rate of release seems to be associated with the injurious effects of these agents on the endothelial cells. We have also found34 that release of PDGF-c by cultured human and bovine endothelial cells can be stimulated at least threefold by physiologic concentrations of thrombin. This increased release is not associated with any change in the intracellular levels of PDGF-c, suggesting that thrombin is not stimulating release of preformed active PDGF. The effect of thrombin may be on the rate of synthesis of PDGF-c by endothelial cells or on the rate of processing of an inactive PDGF processor to a secreted active PDGF-c. The above findings with endothelial cells suggest that the original
hypothesis of the involvement of PDGF in response to injury might be extended to include the possibility that endothelial cells produce PDGF-c in the vicinity of an injury, possibly stimulated by the local formation of thrombin from prothrombin. This endothelial cell-derived PDGF-c could then serve to augment the PDGF that is provided by platelets.

**PDGF-c from smooth muscle cells.** A second vascular cell that can make a PDGF-c under certain conditions is the smooth muscle cell itself. We have recently reported\(^3\) that aortic smooth muscle cells cultured from the aortae of 2-week-old rats secrete substantial levels of PDGF-c, while smooth muscle cells from adult rats do not. This PDGF-c is recognized by goat anti-PDGF antiserum. We have used antibody neutralization to show that this PDGF-c is mitogenic for mouse 3T3 cells and is, in fact, the only such mitogen secreted by pup rat smooth muscle cells. This situation is quite different from that found with cultured endothelial cells and with blood platelets. In those cases the PDGF-c component accounts for only a portion of the total mitogenic activity. Since smooth muscle cells respond mitogenically to PDGF-c, the PDGF-c produced by pup rat smooth muscle cells may be an example of what Sporn and Todaro have called an “autocrine hormone,” a hormone that stimulates the cells that produce it.\(^3^6\) Autocrine production of PDGF-c by aortic smooth muscle cells may contribute toward stimulating the more rapid proliferation of these cells and the synthesis of connective tissue components that occurs during the period of growth of the young rat.\(^3^7,\)\(^3^8\)

We have recently reported\(^9\) that production of a PDGF-c by adult rat smooth muscle cells might also be activated under conditions associated with wounding. In these studies, the carotid artery was deendothelialized with a balloon catheter. Two weeks after this injury, extensive proliferation of smooth muscle cells in the intima of the artery had occurred.\(^4^0\) At this point the intima was dissected away from the media and cultured. When the PDGF-related phenotype of the intimal smooth muscle cells was compared with that of smooth muscle cells from uninjured media, it was found that the number of PDGF receptors, expressed by the intimal smooth muscle cells, was only 50% as high as the level of PDGF receptors expressed by cells from the uninjured media. In addition, cells from the intima produced 10-fold higher levels of a PDGF than did smooth muscle cells from the uninjured media. As is the case for the pup and adult rat smooth muscle cells (which maintain the differences in PDGF-related phenotype for at least 18 subcultures in vitro) the differences between intimal and medial smooth muscle cells seem to be at least partially stable in culture since the differences between medial and neointimal smooth cells were observed when cells that had been cultured in vitro for at least 3 weeks were used. These findings suggest that production of PDGF-c by smooth muscle cells might be activated in response to wounding and might supplement the PDGF derived from attached degranulated platelets and from activated endothelial cells in contributing toward the extensive and prolonged proliferation of smooth muscle cells in the intima of the wounded vessel. The relative importance of these three sources of PDGF in stimulating smooth muscle cell proliferation is not yet known and may depend on the type of injury and on the time after injury.

**Other growth factors from platelets.** Platelets also contain other growth factors that may also play roles in stimulating proliferation of vascular smooth muscle cells. With the use of antiserum against PDGF, it has been demonstrated that PDGF accounts for only about 50% of the growth-promoting activity present in human platelets, at least when assayed with attached cultured cells.\(^1^9\) * Two additional growth factors, or types of growth factor, are now known to be present in significant concentrations in human platelets: an epidermal growth factor (EGF)—like mitogen and a transforming growth factor (TGF). Despite its name, EGF is a potent mitogen for many connective tissue cell types, including smooth muscle cells.\(^4^1\) EGF was first discovered because of its presence in huge quantities within the mouse salivary glands.\(^4^2\) Although EGF is now one of the best-characterized growth factors, and has served as a model for many of the biochemical and cell biological studies performed with PDGF, its source and physiologic roles in humans are far from clear. It is not, for example, present in high concentrations in the human salivary gland. Even in the mouse, the relationship between the high concentrations in salivary glands and the concentrations seen in other tissues is far from clear since surgical removal of the salivary gland does not alter levels of EGF in other tissues and in blood.\(^4^3\) While investigating interactions between the receptors for PDGF and for EGF, we made measurements of EGF levels in blood, distinguishing what was in platelets from what was in plasma. We found that all EGF detectable in blood appeared to be present within the platelets.\(^4^4\) Oka and Orth\(^4^5\) made a similar observation and concluded that platelets are the major repository of EGF in man.

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Vol. 72, No. 4, October 1985
The third growth factor is a so-called transforming growth factor. TGFs are operationally defined as substances that promote the growth in suspension of cultured cells that otherwise require attachment to a sub-stratum. Ability to grow in soft agar is one of the characteristics of oncogenically transformed cells that most reliably distinguishes them from their nontransformed counterparts. Class alpha TGFs were first found to be secreted by cultured transformed cells. They resemble EGF in that they bind to the EGF receptor and are related to it in amino acid sequence. Class beta TGFs do not bind to the EGF receptor and depend on the presence of EGF in order to display optimal ability to promote growth in soft agar. Surprisingly, the normal tissue richest in class beta TGFs seems to be the blood platelets. Assoian et al. have provided evidence that the TGF-beta in platelets may be strongly supported in its activity by EGF and PDGF together, so that a platelet contains a complete set of the three growth factors needed to support optimal growth in soft agar: PDGF, EGF, and TGF-beta.

It is intriguing to speculate on why normal platelets contain factors that are associated with the production of the transformed phenotype. One possibility is that the environment of a smooth muscle cell in a damaged artery, and possibly in a normal artery as well, is not identical to that of an attached cell in monolayer culture and that it shares some characteristics with suspension in soft agar. Figure 2 is a transmission electron micrograph of the fibrous cap of a human atherosclerotic lesion. It illustrates the fact that smooth muscle cells in vivo are suspended within a complex extracellular matrix. It is possible that the normal function of the so-called TGFs is to stimulate the proliferation of just such cells in vivo and that certain oncogenic vi-
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ruses have acquired the ability to activate the gene for this growth factor. 54, 55

In summary, we would like to suggest that any model for describing the involvement of growth factors in the response of a vessel to injury should consider at least three factors: (1) that the cells adjacent to, or involved in, the damaged area may themselves synthesize mitogens to which they or their neighbors can respond, (2) that the blood platelets contain a variety of different growth factors, each of which may be able to stimulate growth under different conditions and/or may be able to act synergistically with other growth factors present in the platelet or in the wound environment, and (3) that the physiologic state, or local environment of a cell in vivo, may determine how cells will respond to different growth factors. Proliferation of cells in a wound may require factors that have been studied because of the association with oncogenic transformation, but that are encoded by cellular genes and presumably have functions in normal physiologic processes. Recent advances in our ability to specifically detect and measure growth factors that may be involved in these processes should allow us to test possibilities.

We would like to thank Mara Berkley for typing the manuscript.

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Locally acting growth factors for vascular smooth muscle cells: endogenous synthesis and release from platelets.
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Circulation. 1985;72:735-740
doi: 10.1161/01.CIR.72.4.735

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/72/4/735

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