Acta Medica et Biologica
Vol. 39, (Suppl.) 93-101, 1991

Mesangial Cell Proliferation and Membrane Phospholipid Metabolism

Yoshihiro FUJIWARA, Satoshi OCHI, Toshiro TAKAMA, SungHyo SHIN, Megumu FUKUNAGA, Kenji YOKOYAMA, Mari FUJIBAYASHI, Yoshifumi FUKUHARA, Naohiko UEDA, Takenobu KAMADA and ***Yoshimasa ORITA**

The First Department of Medicine, Osaka University Medical School 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan, *College of Biomedical Technology, Osaka University, Osaka, Japan

Summary. **The involvement of the phosphoinositide (PI) signaling pathway in the regulation of mesangial cell growth was investigated using cultured rat mesangial (M) cells. Angiotensin II (Ang II), which is known to contract M cells through stimulating the PI pathway, enhanced DNA synthesis in quiescent M cells in the presence but not in the absence of insulin. This suggests that Ang II is a competence-type growth factor. When Ang II-induced stimulation of the PI pathway was inhibited by the pretreatment with pertussis toxin, the stimulating effect on DNA synthesis was also abolished. This indicates that the stimulation of the PI pathway is an important signaling mechanism for Ang II by which it can exert its growth-promoting action on cells. Besides Ang II, a variety of M cell growth-promoting factors including endothelin, arginine vasopressin and platelet-derived growth factor are known to stimulate the PI pathway. Interleukin 6, a recently found potent growth factor for M cells, also stimulated the PI pathway. Thus, the PI pathway may be involved in the intracellular signal transduction of external growth factors for M cells.**

In addition, Ang II and thrombin stimulated the release of interleukin 6 and endothelin, respectively, from M cells probably through enhancing the PI pathway. Since both interleukin 6 and endothelin are known to be potent growth factors for M cells, these results suggest that the PI pathway in M cells is involved in the regulation of the release of autocrine growth factors.

Because the PI pathway is integrally related to mesangial cell proliferation and this, in turn, is one of the causes of the progression of glomerular diseases, control of the pathway should be useful.

INTRODUCTION

Proliferation of mesangial cells associated with matrix expansion is a common histologic abnormality in glomerular diseases.[1] Recent in vitro studies using cultured mesangial cells have revealed that mesangial cell growth is regulated by a number of positive and negative modulators[2-25] (Table 1), suggesting that ultimate morphologic and functional outcome in patients with mesangial proliferation depends on complex interactions among these factors. It is very difficult to know which factor is most responsible and thus, should be inhibited or stimulated in any given case involving mesangial proliferation.

Fortunately, a variety of growth-control factors utilize a limited repertoire of intracellular signal transduction systems. These include the phosphoinositide (PI) pathway, cyclic AMP system, cyclic GMP system and the tyrosine kinase system. Exploring the involvement of each of these signaling systems in the regulation of mesangial cell growth provides an efficient means by which the goal of the treatment of glomerular diseases can be realized. In this paper, we describe the close relationship that exists between mesangial cell growth and the PI pathway.

OUTLINE OF THE PI PATHWAY (Fig. 1)

The stimulation of the PI pathway by external stimuli such as Ca^{2+}-mobilizing hormones and several growth factors is a common mechanism in many different target cells.[26-35] The initial biochemical event in this pathway is receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), a minor phospholipid constituent of plasma membrane, by PI-specific phospholipase C. The phospholipase C is also termed phosphoinositidase C (PIC). PIP_2 hydrolysis by PIC generates two second messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG).

The primary function of IP_3 is to mobilize Ca^{2+}

Table 1. Growth control factors for mesangial cells

Stimulators	Inhibitors
Angiotensin II[2]	Atrial natriuretic peptide[21,22]
Arginine vasopressin[3]	Nitric oxide[23]
Bradykinin[4]	Prostaglandins E_2, I_2[48]
Endothelin[5,6]	Transforming growth factor-β[24,25]
Epidermal growth factor[7]	
Insulin[8,9]	
Insulin-like growth factor-I[8-11]	
Interleukin-1[12,13]	
Interleukin-6[14]	
Leukotriene D_4[15]	
Platelet-derived growth factor[16,17]	
Prostaglandin $F_2\alpha$[18]	
Serotonin[19]	
Thrombin[20]	
Transforming growth factor-α[7]	
Tumor necrosis factor[7]	

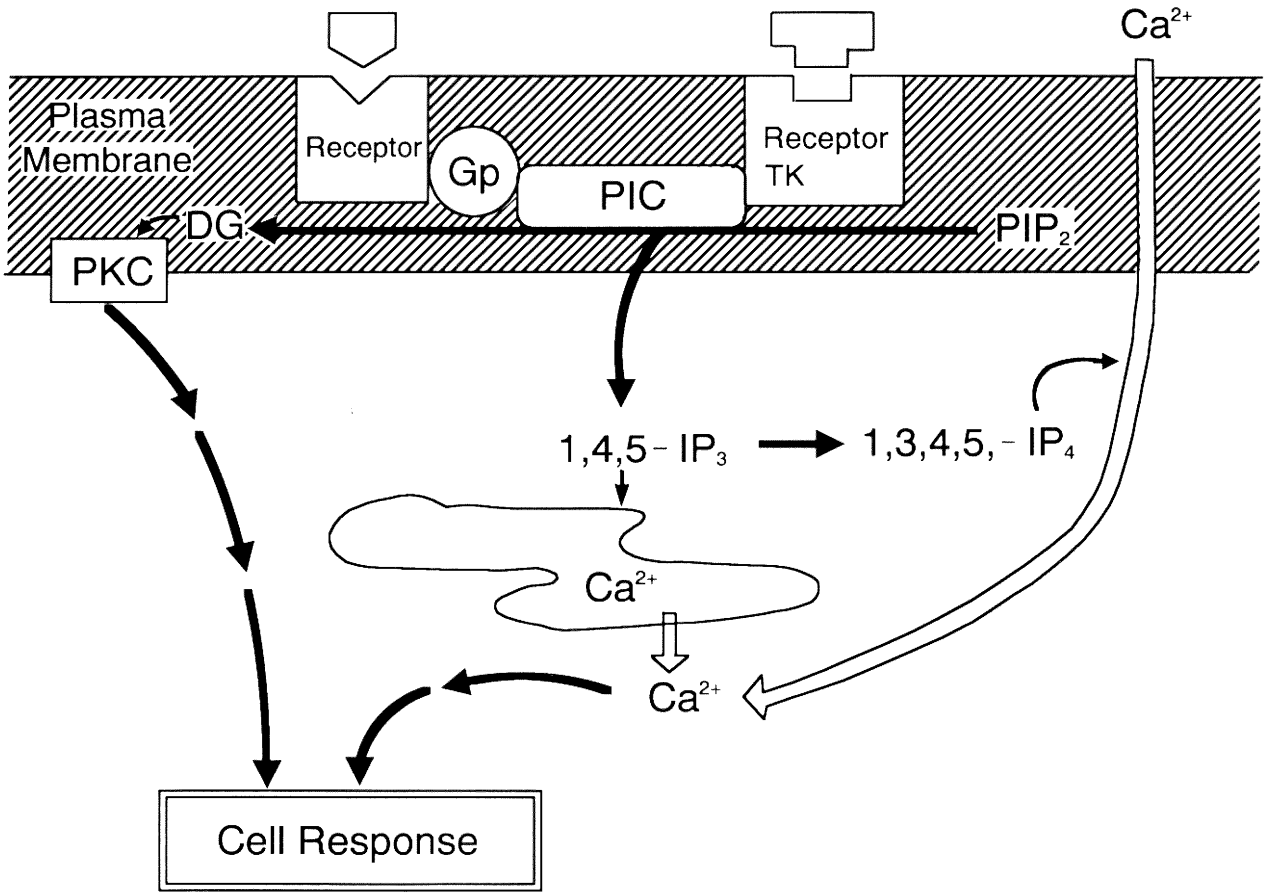

Fig. 1. Phosphoinositide signaling pathway. PIC; phosphoinositidase C, TK; tyrosine kinase, Gp; GTP-binding regulatory protein coupled to PIC, PIP_2; phosphatidylinositol 4,5-bisphosphate, DG; diacylglycerol, 1,4,5-IP_3; inositol 1,4,5-trisphosphate, 1,3,4,5-IP_4; inositol 1,3,4,5-tetrakisphosphate, PKC; protein kinase C.

from intracellular Ca^{2+} stores[36] such as calciosomes.[37] This leads to an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$). Recent evidence suggests that inositol 1,3,4,5-tetrakisphosphate (IP_4), a further phosphorylated product of IP_3, also plays an important role in the regulation of $[Ca^{2+}]i$ by stimulating Ca^{2+} influx through the plasma membrane.[38] DG, the other product of PIP_2 hydrolysis, activates protein kinase C (PKC) by influencing its affinity for Ca^{2+}.[39,40] Two limbs of this bifurcating pathway, the $IP_3/(IP_4)/Ca^{2+}$ limb and the DG/PKC limb, provide a control mechanism for a variety of cellular functions.

Upon receptor occupancy by certain agonists, PIC is activated by guanine nucleotide regulatory (G) protein in a manner similar to the stimulation of adenylate cyclase by a G protein.[34,41,42] The G protein (Gp) responsible for linking receptors to PIC has not yet been characterized. However, in certain cell types stimulated by certain agonists, the G protein is similar to adenylate cyclase-coupled inhibitory G protein (Gi) in that it is sensitive to pertussis toxin.[34,42,43] In contrast, receptor tyrosine phosphorylation is an intermediary mechanism between the binding of certain growth factors to receptors with tyrosine kinase activity and activation of PIC.[44]

ANGIOTENSIN II AND MESANGIAL CELL GROWTH

Of active substances which regulate mesangial cell functions, angiotensin II (Ang II) has been most extensively studied. The contraction in response to Ang II is a marker for cultured glomerular cells of mesangial origin.[45] From the time that it was report-

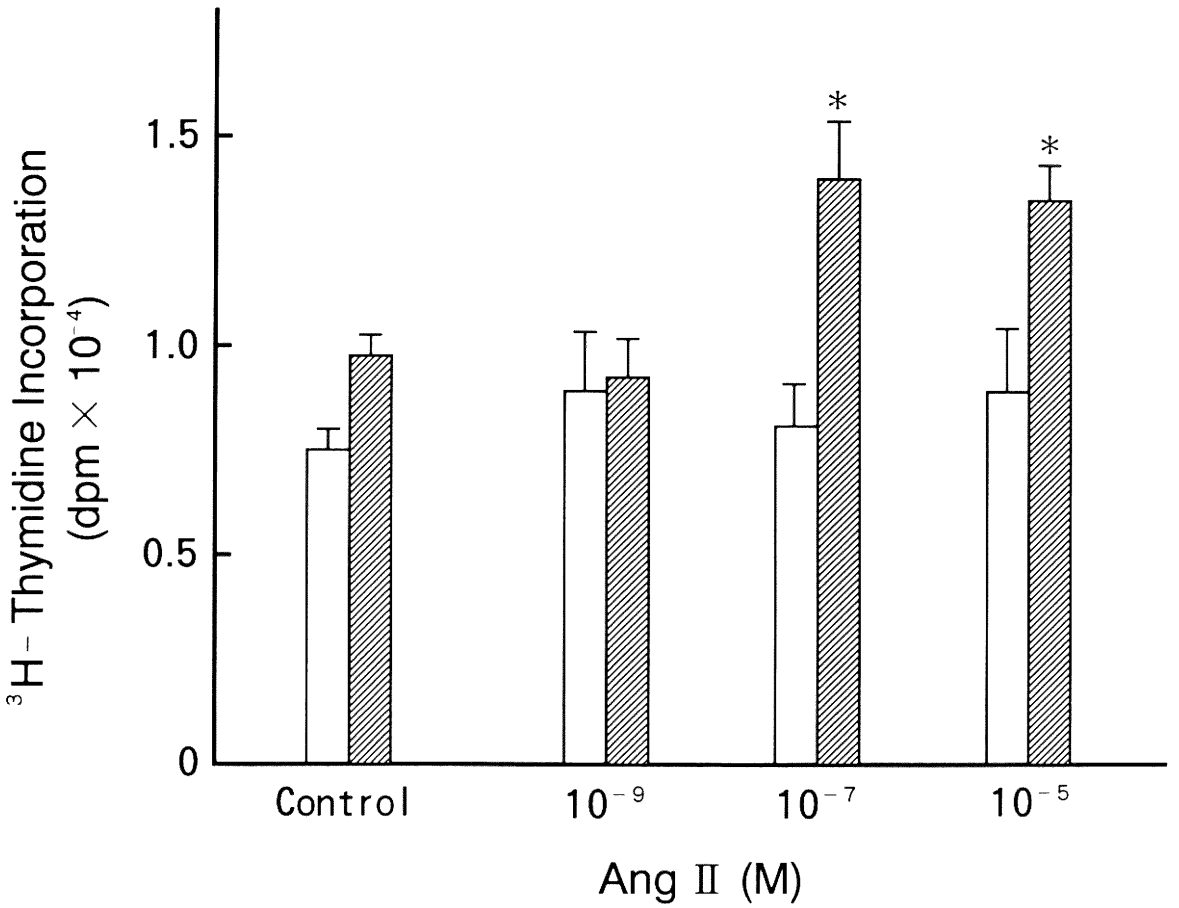

Fig. 2. The effect of Ang II on ^{3}H-thymidine incorporation into cultured rat mesangial cells. The primary cultured rat mesangial cells were seeded onto 35 mm plastic dishes. In the subconfluent state, the cells were incubated with fetal calf serum-free RPMI 1640 medium for 3 days in order for them to enter the quiescent phase. They were stimulated by Ang II for 24 h with (▨) or without (□) 5 μg/ml of insulin. Then, 1 μCi of ^{3}H-thymidine was added and the ^{3}H- radioactivity in TCA-insoluble fraction was measured after 24 h. Data are expressed as the means ± SE of triplicate experiments. *$p<0.05$ vs the controls with or without insulin.

ed that mas oncogene isolated from human epitheloid carcinoma encodes angiotensin receptor protein,[45] much attention has been given to Ang II as a growth factor in addition to its role as a potent vasoconstrictor. Therefore, we attempted to investigate possible growth-promoting action of Ang II on cultured rat mesangial cells.[2]

Cells were pretreated with fetal calf serum-depleted RPMI medium for 3 days prior to the experiments in order for them to enter the quiescent (G_0) phase. When stimulated by Ang II at a dose of 100 nM or more, 24 h-incorporation of ^{3}H-thymidine into the cells significantly increased in the presence but not in the absence of high concentration (5 μg/ml) of insulin (Fig. 2). In these experiments, insulin could be substituted for insulin-like growth factor I (IGF-I). In BALB/c 3T3 cells, it has been reported that there are two sets of growth factors: One is a competence factor which makes the cells competent to leave G_0 phase and enter the cell cycle, and the other is a progression factor which makes it possible for competence factor-treated cells (G_1 phase) to enter into DNA-synthetic (S) phase[47] (Fig. 3). Since insulin and IGF-I have been identified as progression factors, our results suggest that Ang II acts as a competence-type growth factor for mesangial cells.

Thus, Ang II not only regulates the glomerular ultrafiltration process by contracting mesangial cells,[48] but also may be involved in the pathogenesis and progression of glomerular diseases by stimulating mesangial cell growth.

THE PI PATHWAY AS A SIGNALING MECHANISM OF ANGIOTESIN II

We previously reported that Ang II stimulates the PI pathway both in cultured rat mesangial cells[49] and in isolated rat glomeruli.[50] Firstly, when the mesangial cells prelabeled with ^{3}H-arachidonic acid were stimulated by Ang II, PIP_2 labeling rapidly decreased while DG labeling increased. These changes occurred within 30 sec. The increase in DG was dose-dependent

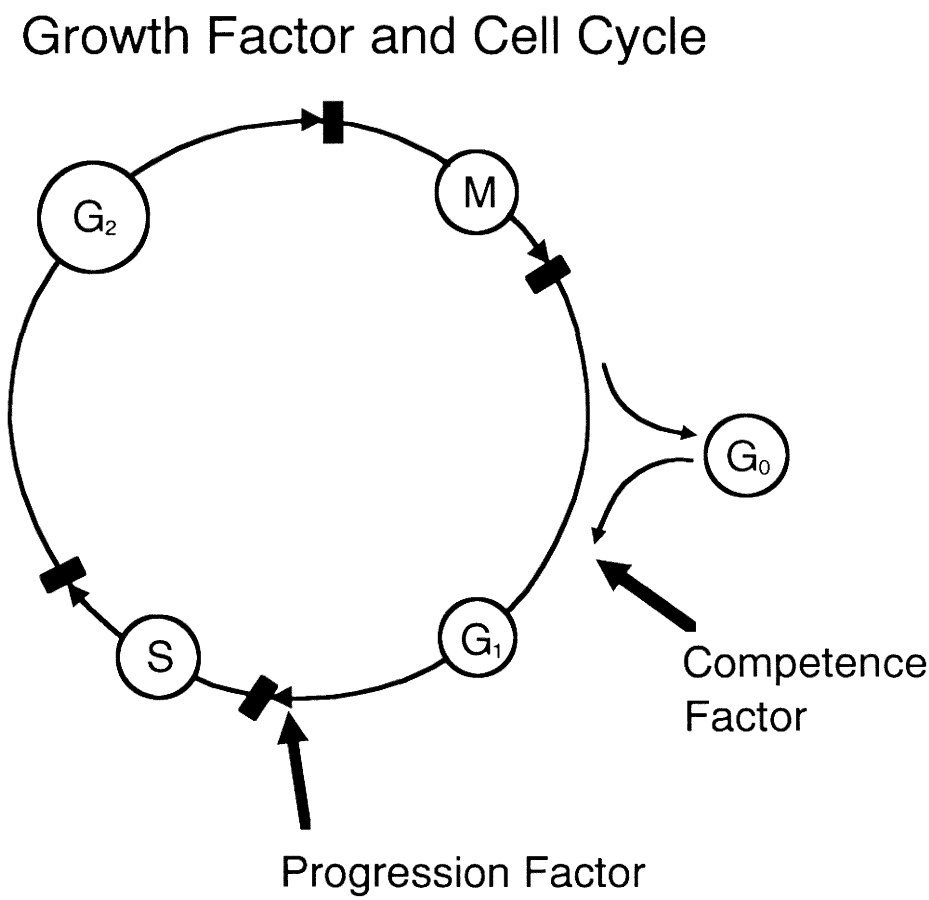

Fig. 3. Cell cycle and growth factors (47). G_0; quiescence, G_1; before DNA synthesis, S; DNA synthesis, G_2; after DNA synthesis, M; cell division.

within the range of 10 nM to 1 μM of Ang II. Furthermore, the 100,000 x g supernate of the cells contained Ca^{2+}-activated, phospholipid-dependent protein kinase (PKC). Therefore, the Ang II-induced increase in DG may activate PKC, leading to the regulation of various mesangial cell functions.

Next, to study the effect of Ang II on the metabolism of inositol phosphates, we developed a new method for analyzing individual inositol phosphates using an isocratic system of high-performance liquid chromatography (HPLC) equipped with a refractive index monitor.[51,52] This system enabled us to separate IP_3, its isomer inositol 1,3,4-trisphosphate (1,3, 4-IP_3) (which is a dephosphorylated product of IP_4), and more polar inositol phosphates including IP_4 from each other. This was achieved within 20 min with high resolution. When cultured rat mesangial cells prelabeled with ^{3}H-inositol were stimulated by Ang II, IP_3 sharply increased to reach a peak at 10 sec, and rather rapidly returned to around the control level. IP_4 also showed a rapid increase during the first 10 sec, though the subsequent decrease was more gradual than that of IP_3. By contrast, 1,3,4-IP_3 continued to increase gradually, and showed an upward trend even at 60 sec. The increase in IP_4 at 10 sec was dose-dependent within the range of 10 nM to 1 μM of Ang II.

All of the above mentioned results indicate that Ang II provokes PIP_2 hydrolysis by PIC, yielding DG and IP_3 in cultured rat mesangial cells. The generated IP_3 is further phosphorylated to IP_4, which is then dephosphorylated to 1,3,4-IP3. The activation of PKC by DG and the increase in $[Ca^{2+}]i$ by IP_3 and possibly by IP_4 play an important role in the regulation of mesangial cell functions by Ang II. The functional role of IP_4 in mesangial cells has not yet been established. Ang II induces the initial peak of $[Ca^{2+}]i$ probably via IP_3, followed by the sustained increase which is dependent on extracellular Ca.[2+53] If IP_4 stimulates Ca^{2+} influx through the plasma membrane in mesangial cells as reported in sea urchin eggs,[38] it might be involved in the sustained increase of $[Ca^{2+}]$ i. Consistent with this idea, the increase in IP_4 by Ang II was more persistently sustained than that of IP_3.[52]

The response of cultured mesangial cells to external stimuli might be altered by the culture procedure itself. To overcome this issue, we studied the effect of Ang II on the PI pathway using isolated glomeruli where the only known responding cell type to Ang II was mesangial cells.[50] When glomeruli isolated from Sprague-Dawley rat kidney were prelabeled with ^{3}H-glycerol and stimulated by Ang II, a decrease in PIP_2 labeling with an increase in DG labeling was observed. These changes occurred within 15 sec. Both the decrease in PIP_2 and the increase in DG induced by Ang II at 15 sec were dose-dependent. These results suggest that Ang II provokes PIP_2 hydrolysis by PIC in isolated glomeruli, a preparation more similar to in situ conditions than cultured glomerular cells. The stimulation of the PI pathway by Ang II in mesangial cells seems to play a pivotal role in their contractile response. We demonstrated that the initial phase of Ang II-induced mesangial cell contraction was dependent on intracellular Ca^{2+} release by IP_3, wihile the sustained phase on both extracellular Ca^{2+} influx possibly by IP_4 and PKC activation by DG.[54]

THE PI PATHWAY AND MESANGIAL CELL GROWTH

In addition to the pivotal role of the PI pathway in rapid responses such as the contraction of mesangial cells, the pathway seems to participate in Ang II-mediated regulation of intracellular events involved in mesangial cell growth.[2] When cultured rat mesangial cells were pretreated with pertussis toxin, an inhibitor of Gi,[43] Ang II-induced generation of IP_3 was completely inhibited. This indicates that mesangial Ang II receptors are coupled to PIC via pertussis toxin-sensitive G protein. The pretreatment with pertussis toxin also eliminated Ang II-induced increase in ^{3}H-thymidine incorporation into the cells. This suggests that the stimulation of the PI pathway is an essential prerequisite for Ang II by which it can exert its growth-promoting action on the cells.

A close relationship between the PI pathway and cell growth is generally suggested for the following reasons: 1) PKC, which is activated by DG, one of the messenger products of the PI pathway, is a target for tumor-promoting phorbol esters,[55] 2) the PI pathway is stimulated in cells transformed by tumor viruses and chemical carcinogens,[56] 3) several growth factors, including platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), stimulate the PI pathway,[56-58] and finally and most importantly, 4) antibody to PIP_2 abolishes cell growth induced by some types of growth factors.[59]

Contradicting these lines of evidence is a very recent report which revealed that overexpression of PIC in NIH 3T3 cells with the resultant enhancement of PIP_2 hydrolysis does not influence PDGF-induced DNA synthesis.[60] This finding suggests that PDGF-induced DNA synthesis uses biochemical pathways independent on the PI pathway, although the possibility cannot be excluded that a certain amount of PIP_2

hydrolysis is essential for the PDGF effect.

Of particular importance is the finding that, at least in mesangial cells, a considerable number of growth-stimulating factors thus far reported (Table 1) enhance the PI pathway (see Ref. #61, for review). These factors include Ang II,[49-52] arginine vasopressin, bradykinin, endothelin,[62] leukotriene D_4,[15] PDGF, prostaglandin (PG) $F_2\alpha$, serotonin and thrombin.[20] In addition, EGF stimulates the PI pathway in some cell types other than mesangial cells.[56,57] Insulin[63] and interleukin 1[64] cause the accumulation of DG via mechanisms other than PIP_2 hydrolysis. The accumulated DG leads to activation of PKC. Furthermore, we have recently found that interleukin 6 (IL-6), a newly discovered potent growth factor for mesangial cells,[14] stimulates the PI pathway in the cells: Human recombinant IL-6 caused a dose-dependent increase in IP_3 within 10 sec with a resultant increase in $[Ca^{2+}]i$ (unpublished data).

Given these lines of evidence, it can be concluded that the PI pathway is an important signal transduction mechanism of growth-stimulating factors for mesangial cells.

CROSSTALK AMONG INTRACELLULAR SIGNALING PATHWAYS

In contrast to the close relationship between the PI pathway and growth stimulation, other agents inhibit mesangial cell growth. These agents include cyclic AMP pathway stimulators PGI_2 and PGE_2,[18] and cyclic GMP pathway stimulators atrial natriuretic peptide[21,22] and nitric oxide.[23] As mentioned, substances such as Ang II, arginine vasopressin and endothelin stimulate the PI pathway and DNA synthesis in mesangial cells. They also enhance PGE_2 production in the cells (see Ref. #61, for review). Therefore, the PI and cyclic AMP pathways should constitute a negative feedback network by which mesangial cell growth is regulated.

Many potent growth factors, including EGF, IGF-I and PDGF, mediate growth-stimulating actions by binding to and activating cell surface receptors which have intrinsic protein tyrosine kinase activity (see Refs #65-67, for review). Receptor protein tyrosine kinases catalyze the phosphorylation of exogenous substrates as well as tyrosine residues within their own polypeptide chains. The tyrosine phosphorylated proteins may play a crucial role in transmitting the signal of growth factors into the cell nucleus. Also, in cultured rat mesangial cells we have found that PDGF induces tyrosine phosphorylation of several proteins including a 180 kDa protein.[68] The molecular weight of this protein was consistent with the reported value for PDGF receptor protein.[69] When tyrosine phosphatase was inhibited by vanadate[70] during the activation of the cells with PDGF, several additional proteins were phosphorylated including a 145 kDa protein. The molecular weight of this protein was similar to PIC which catalyzes PIP_2 hydrolysis.[71] Thus, the PI pathway and protein tyrosine phosphorylation may constitute a cross-talking network in the growth-regulating action of PDGF: tyrosine phosphorylation of the PDGF receptor by PDGF causes tyrosine phosphorylation of PIC which leads to stimulation of the PI pathway.

Data accumulated thus far on growth-control factors for mesangial cells may lead to the conclusion that inhibition of the PI pathway and protein tyrosine phosphorylation, and stimulation of the cyclic AMP and cyclic GMP pathways are useful in preventing mesangial cell proliferation.

NEW ROLE OF THE PI PATHWAY IN THE REGULATION OF MESANGIAL CELL GROWTH

As described above, the PI pathway is an important primary signal which stimulates mesangial cell growth. Our recent findings provide a new distinct role for the pathway in the regulation of meangial cell growth.

Several growth factors for mesangial cells such as PDGF,[72] IL-6[14] and endothelin (our unpublished observation) are synthesized in and released from mesangial cells themselves. In other words, they act as autocrine growth factors. However, the regulatory mechanisms for their synthesis and release in the cells are largely unknown. We recently obtained two interesting results (unpublished data). One is that Ang II, which stimulates the PI pathway,[49-52] enhanced the release of IL-6 from cultured mouse mesangial cells. The other is that thrombin, which also stimulates the PI pathway,[20] enhanced the release of endothelin from cultured rat mesangial cells. Since both IL-6 and endothelin are growth factors for mesangial cells,[5,6,14] these findings suggest that the PI pathway plays a secondary signaling role in the regulation of mesangial cell growth through releasing autocrine growth factors. However, the involvement of these autocrine growth factors in the growth-stimulating actions of Ang II and thrombin should be further elucidated.

CONCLUSION

The PI pathway in mesangial cells, consisting of the $IP_3/IP_4/Ca^{2+}$ limb and the DG/PKC limb, is an important signaling mechanism by which external stimuli can induce responses. These include the rapid response of contraction as well as the long-term response of cell growth. The pathway is involved in the regulation of mesangial cell growth in two ways: One is as a primary signal for external growth factors to mediate their growth-stimulating actions, and the other is as a secondary signal by which the release of autocrine growth factors is regulated.

The inhibition of the PI pathway, thus, may provide a promising therapeutic approach for the control of mesangial cell proliferation, one of the causes of the progression of glomerular diseases.

REFERENCES

1) Wilson CB, Dixon DJ: Renal response to immunologic injury in the kidney. In: Brenner BM, Rector FC Jr (eds) The kidney, 3rd ed. WB Saunders, Philadelphia 1986, p800-890.
2) Fujiwara Y, Takama T, Shin SH, Ochi S, Fukunaga M, Orita Y, Kamada T: Angiotensin II stimulates mesangial cell growth through phosphoinositide cascade. *Kidney Int* **35**: 172, 1989.
3) Ganz MB, Pekar SK, Perfetto MC, Sterzel RB: Arginine vasopressin promotes growth of rat glomerular mesangial cells in culture. *Amer J Physiol* **255**: F898-F906, 1988.
4) Sterzel RB, Ganz MB, Perfetto M, Unwin R: Vasoactive peptides promote growth of cultured mesangial cells. *Kidney Int* **35**: 307, 1989.
5) Badr KF, Murray JJ, Breyer MD, Takahashi K, Inagami T, Harris RC: Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. *J Clin Invest* **83**: 336-342, 1989.
6) Simonson MS, Wann S, Mene P, Dubyak GR, Kester M, Nakazato Y, Sedor JR, Dunn MJ: Endothelin stimulates phospholipase C, Na^+/H^+ exchange, c-fos expression, and mitogenesis in rat mesangial cells. *J Clin Invest* **83**: 708-712, 1989.
7) Silver BJ, Jaffer FE, Abboud HE: Platelet-derived growth factor (PDGF) synthesis in mesangial cells: Induction by multiple peptide mitogens. *Proc Nat Acad Sci USA* **86**: 1056-1060, 1989.
8) Arnqvist H, Ballerman BJ, King GL: Receptor for and effects of insulin and IGF-1 in rat glomerular mesangial cells. *Amer J Physiol* **254**: C411-416, 1988.
9) Conti FG, Striker LJ, Lesniak MA, MacKay K, Roth J, Striker GE: Studies on binding and mitogenic effect of insulin and insulin-like growth factor I (IGF-I) in glomerular mesangial cells. *Endocrinology* **122**: 2788-2795, 1988.
10) Doi T, Striker LJ, Elliot SJ, Conti FG, Striker GE: Insulin-like growth factor-I is a progressive factor for human mesangial cells. *Amer J Pathol* **134**: 395-404, 1989.
11) Doi T, Striker LJ, Quaife C, Conti FG, Palmiter R, Behringer R, Brinster R, Striker GE: Progressive glomerulosclerosis develops in transgenic mice chronically expressing growth hormone and growth hormone releasing factor but not in those expressing insulin-like growth factor-I. *Amer J Pathol* **131**: 398-403, 1988.
12) Lovett DH, Ryan JL, Sterzel RB: Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. *J Immunol* **131**: 2830-2836, 1983.
13) Ooi BS, MacCarthy EP, Hsu A: Beta-endorphin amplifies the effect of interleukin-1 on mouse mesangial cell proliferation. *J Lab Clin Med* **110**: 159-163, 1987.
14) Horii M, Muraguchi A, Iwano M, Matsuda T, Hirayama T, Yamada H, Fujii Y, Dohi K, Ishikawa H, Ohmoto Y, Yoshizaki K, Hirano T, Kishimoto T: Involvement of IL-6 in mesangial proliferative glomerulonephritis. *J Immunol* **143**: 3949-3955, 1989.
15) Badr KF, Mong S, Hoover RL, Schwartzberg M, Ebert J, Jacobson HR, Harris RC: Leukotriene D_4 binding and signal transduction in rat glomerular mesangial cells. *Amer J Physiol* **257**: F280-F287, 1989.
16) Abboud HE, Poptic E, Dicorleto P: Production of platelet-derived growth factor like protein by rat mesangial cells in culture. *J Clin Invest* **80**: 675-683, 1987.
17) Shultz P, Dicorleto PE, Silver BJ, Abboud HE: Mesangial cells release a PDGF-like protein, express PDGF mRNAs and proliferate in response to PDGF. *Amer J Physiol* **255**: F674-F684, 1988.
18) Mene P, McDermott RD, Dunn MJ: Modulation of mesangial cell proliferation by prostaglandins. *Kidney Int* **33**: 276, 1988.
19) Takuwa N, Ganz M, Takuwa Y, Sterzel RB, Rasmussen H: Studies of the mitogenic effect of serotonin in rat renal mesangial cells. *Amer J Physiol* **257**: F431-F439, 1989.
20) Shultz PJ, Knauss TC, Mene P, Abboud HE: Mitogenic signals for thrombin in mesangial cells: regulation of phospholipase C and PDGF genes. *Amer J Ppysiol* **257**: F366-F374, 1989.
21) Johnson A, Lermioglu F, Garg UC, Morgan-Boyd R, Hassid A: A novel biologic effect of atrial natriuretic hormone: inhibition of mesangial cell mitogenesis. *Biochem Biophys Res Commun* **152**: 893-897, 1988.
22) Appel RG: Growth inhibitory activity of atrial natriuretic factor in rat glomerular mesangial cells.

FEBS Lett **238**: 135-138, 1988.
23) Garg UC, Hassid A: Inhibition of rat mesangial cell mitogenesis by nitric oxide-generating vasodilators. *Amer J Physiol* **257**: F60-F66, 1989.
24) Jaffer F, Saunders C, Shultz P, Throckmorton D, Weinshell E, Abboud HE: Regulation of mesangial cell growth by polypeptide mitogens. Inhibitory role of transforming growth factor beta. *Amer J Pathol* **135**: 261-269, 1989.
25) Yamashita W, MacCarthy EP, Hsu A, Gartside PS, Ooi BS: The effect of transforming growth factor-β non mouse mesangial cell proliferation. *Clin Exp Immunol* **77**: 285-288, 1989.
26) Hokin MR, Hokin LE: Enzyme secretion and the incorporation of ^{32}P into phospholipids of pancreas slices. *J Biol Chem* **203**: 967-977, 1953.
27) Michell RH: Inositol phospholipids and cell surface receptor function. *Biochim Biophyys Acta* **415**: 81-147, 1975.
28) Nishizuka Y: The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature* **308**: 693-698, 1984.
29) Nishizuka Y: Turnover of inositol phospholipids and signal transduction. *Science* **225**: 1365-1370, 1984.
30) Berridge MJ, Irvine RF: Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312**: 315-321, 1984.
31) Majerus PW, Connolly TM, Deckmyn H, Ross TS, Bross TE, Ishii H, Bansal VS, Wilson DM: The metabolism of phosphoinositide-derived messenger molecules. *Science* **234**: 1519-1526, 1986,
32) Berridge MJ: Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Ann Rev Biochem* **56**: 159-193, 1987.
33) Nishizuka Y: The molecular heterogeneity of protein kinase C and its implication for cellular regulation. *Nature* **334**: 661-665, 1988.
34) Catt KJ, Balla T: Phosphoinositide metabolism and hormone action. *Ann Rev Med* **40**: 487-509, 1989.
35) Berridge MJ, Irvine RF: Inositol phosphates and cell signalling. *Nature* **341**: 197-205, 1989.
36) Streb H, Irvine RF, Berridge MJ, Schulz I: Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **306**: 67-68, 1983.
37) Volpe P, Knause K-H, Hashimoto S, Zorzato F, Pozzan T, Meldolesi J, Lew DP: "Calciosome", a cytoplasmic organelle: The inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store of nonmuscle cells? *Proc Nat Acad Sci USA* **85**: 1091-1095, 1988.
38) Irvine RF, Moor RM: Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{2+}. *Biochem J* **240**: 917-920, 1986.
39) Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, Nishizuka Y: Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J Biol Chem* **254**: 3692-3695, 1979.
40) Takai Y, Kishimoto A, Kikkawa U, Mori T, Nishizuka Y: Unsaturated diacylglycerol as a possible messenger for the activation of calcium- activated, phospholipid-dependent protein kinase system. *Biochem Biophys Res Commun* **91**: 1218-1224, 1979.
41) Cockcroft S, Gomperts BD: Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature* **314**: 534-536, 1985.
42) Pfeilschifter JM: Cellular signalling in the kidney: The role of inositol lipids. *Renal Physiol Biochem* **12**: 1-31, 1989.
43) Nakamura T, Ui M: Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. A possible involvement of the toxin-specific substrate in the Ca^{2+}-mobilizing receptor-mediated biosignaling system. *J Biol Chem* **260**: 3584-3593, 1985.
44) Boyer JL, Hepler JR, Harden TK: Hormone and growth factor receptor-mediated regulation of phospholipase C activity. *Trends Pharmacol Sci* **10**: 360-364, 1989.
45) Tanaka T, Fujiwara Y, Orita Y, Sasaki E, Kitamura H, Abe H: The functional characteristics of cultured rat mesangial cell. *Jap Circ J* **48**: 1017-1029, 1984.
46) Jackson TR, Blair LAC, Marshall J, Goedert M, Hanley MR: The mas oncogene encodes an angiotensin receptor. *Nature* **335**: 437-440, 1988.
47) Stiles CD, Capone GT, Scher CD, Antoniades HN, VanWyk JJ, Pledger WJ: Dual control of cell growth by somatomedins and platelet-derived growth factor. *Proc Nat Acad Sci USA* **76**: 1279-1283, 1979.
48) Dworkin LD, Ichikawa I, Brenner BM: Hormonal modulation of glomerular function. *Amer J Physiol* **244**: F95-F104, 1983.
49) Orita Y, Fujiwara Y, Ochi S, Tanaka Y, Kamada T: Calcium-activated, phospholipid-dependent protein kinase in cultured rat mesangial cells. *FEBS Lett* **192**: 155-158, 1985.
50) Ochi S, Fujiwara Y, Orita Y, Tanaka Y, Shin SH, Takama T, Wada A, Ueda N, Kamada T: Phosphoinositide turnover enhanced by angiotensin II in isolated rat glomeruli. *Biochim Biophys Acta* **927**: 100-105, 1987.
51) Shin SH, Fujiwara Y, Wada A, Takama T, Orita Y, Kamada T, Tagawa K: Angiotensin II-induced increase in inositol 1,4,5-trisphosphate evidenced by refined high performance liquid chromatography. *Biochem Biophys Res Commun* **142**: 70-77, 1987.
52) Shin SH, Fujiwara Y, Takama T, Ochi S, Wada A, Fukunaga M, Orita Y, Kamada T, Tagawa K: Analysis of inositol tris- and tetrakisphosphate in mesangial cells by HPLC. *Kidney Int* **35**: 1320-1323, 1989.

53) Hassid A, Pidikiti N, Gamero D: Effects of vasoactive peptides on cytosolic calcium in cultured mesangial cells. *Amer J Physiol* **251**: F1018-F1028, 1986.
54) Fujiwara Y, Kitamura E, Ueda N, Fukunaga M, Orita Y, Kamada T: Mechanism of action of angiotensin II on isolated glomeruli. *Kidney Int* **36**: 985-991, 1989.
55) Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y: Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257**: 7847-7851, 1982.
56) Whitman M, Cantley L: Phosphoinositide metabolism and the control of cell proliferation. *Biochim Biophys Acta* **948**: 327-344, 1988.
57) Rozengurt E: Early signals in the mitogenic response. *Science* **234**: 161-166, 1986.
58) Williams LT: Signal transduction by the platelet-derived growth factor receptor. *Science* **243**: 1564-1570, 1989.
59) Matsuoka K, Fukami K, Nakanishi O, Kawai S, Takenawa T: Mitogenesis in response to PDGF and bombesin abolished by microinjection of antibody to PIP_2. *Science* **239**: 640-643, 1988.
60) Margolis B, Zilberstein A, Franks C, Felder S, Kremer S, Ullrich A, Rhee SG, Skorecki K, Schlessinger J: Effect of phospholipase C-γ overexpression on PDGF-induced second messengers and mitogenesis. *Science* **248**: 607-610, 1990.
61) Mene P, Simonson MS, Dunn MJ: Physiology of the mesangial cell. *Physiol Rev* **69**: 1347-1424, 1989.
62) Fukunaga M, Ochi S, Takama T, Yokoyama K, Fujiwara Y, Orita Y, Kamada T: Endothelin-1 stimulates prostaglandin E_2 production in an extracellular calcium-independent manner in cultured rat mesangial cells. *Amer J Hypertens.* (in press)
63) Low MG, Saltiel AR: Structural and functional roles of glycosyl-phosphatidylinositol in membranes. *Science* **239**: 268-275, 1988.
64) Kester M, Simonson MS, Mene P, Sedor JR: Interleukin-1 generates transmembrane signals from phospholipids through novel pathways in cultured rat mesangial cells. *J Clin Invest* **83**: 718-723, 1989.
65) Hunter T, Cooper JA: Protein-tyrosine kinases. *Ann Rev Biochem* **54**: 897-930, 1985.
66) Yarden Y, Ullrich A: Growth factor receptor tyrosine kinases. *Ann Rev Biochem* **57**: 443-478, 1988.
67) Ullrich A, Schlessinger J: Signal transduction by receptors with tyrosine kinase activity. *Cell* **61**: 203-212, 1990.
68) Ochi S, Fujiwara Y, Yokoyama K, Fukunaga M, Shin SH, Orita Y, Fukuhara Y, Ueda N, Kamada T: Signaling mechanism of platelet-derived growth factor in cultured rat mesangial cells. *Contr Nephrol* (in press)
69) Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U, Fried VA, Ullrich A, Williams LT: Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature* **323**: 226-232, 1986.
70) Swarup G, Cohen S, Garbers DL: Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. *Biochem Biophys Res Commun* **107**: 1104-1109, 1982.
71) Wahl MI, Olashaw NE, Nishibe S, Rhee SG, Pledger WJ, Carpenter G: Platelet-derived growth factor induces rapid and sustained tyrosine phosphorylation of phospholipase C-γ in quiescent BALB/c 3T3 cells. *Mol Cell Biol* **9**: 2934-2943, 1989.