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INTERLEUKIN-1β, TUMOR NECROSIS FACTOR AND FORSKOLIN STIMULATE THE SYNTHESIS AND SECRETION OF GROUP II PHOSPHOLIPASE A_2 IN RAT MESANGIAL CELLS

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Treatment of rat glomerular mesangial cells with interleukin-1β, tumor necrosis factor or forskolin resulted in the release of phospholipase A_2 activity in the culture medium. Essentially all of this phospholipase A_2 activity was bound to immobilized monoclonal antibodies raised against rat liver mitochondrial 14 kDa group II phospholipase A_2. Gelfiltration confirmed the absence of higher molecular weight phospholipases A_2 in the culture medium. Immunoblot experiments showed the virtual absence of this 14 kDa group II phospholipase A_2 in unstimulated mesangial cells. The time-dependent increase of phospholipase A_2 activity in both cells and culture medium upon stimulation with interleukin-1β plus forskolin is accompanied with elevated 14 kDa phospholipase A_2 protein levels. These results indicate that the increased phospholipase A_2 activity upon treatment of mesangial cells with these stimulators is due to increased synthesis of group II phospholipase A_2. Over 85 % of this newly synthesized phospholipase A_2 appears to be secreted from the cells. © 1991 Academic Press, Inc.

PLA_2 is believed to play an essential role in inflammatory reactions through the release of arachidonate from membrane phospholipids for production of important lipid mediators such as eicosanoids and platelet activating factor (1). The low molecular weight (14 kDa) PLA_2 described so far can be divided into two groups, based on their aminoacid sequence (2). Group I PLA_2's are characterized by the presence of Cys11 and within mammals occur mainly in the pancreas. Several non-pancreatic mammalian tissues contain group II PLA_2, characterized by the lack of Cys11. Group II enzymes have been described for rat (3,4), rabbit (5) and human platelets (6), rat liver mitochondria (7) and human spleen (8). This PLA_2 type is also found in soluble form at inflammatory sites such as peritoneal exudates (9,10) and human rheumatoid arthritis (6,11,12). The high levels of group II PLA_2 at several inflammatory sites suggested that this type of PLA_2 is involved in the process of inflammation. In line with these findings, the PLA_2 purified from human synovial fluid proved proinflammatory (13) and injection of

Abbreviations: PLA_2, phospholipase A_2; IL-1β, interleukin 1β; TNF, tumor necrosis factor α; PBS, phosphate-buffered saline; McAb, monoclonal antibody; PGE_2, prostaglandin E_2.

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purified rat platelet PLA_2 exacerbated the paw oedema in rats with adjuvant arthritis (14). Activation of PLA_2 activity and PGE_2 production by TNF and IL-1, two potent inflammatory mediators, is evident from several observations. It has been demonstrated that PLA_2 can be activated in rabbit chondrocytes (15), rat chondrocytes (16), human synovial cells (17), rat mesangial cells (18) and MDCK cells (19) upon stimulation with IL-1β and/or TNF. In addition, after treatment with these cytokines an increased mRNA for group II PLA_2 was observed in rabbit articular chondrocytes (20,21) and rat vascular smooth muscle cells (22).

Previous studies using cultured rat glomerular mesangial cells have shown that treatment with recombinant IL-1α, IL-1β and TNFα induced the release of PLA_2 activity into the medium in parallel to PGE_2 synthesis. Inhibition of these processes by actinomycin D and cycloheximide indicated the involvement of both transcription and translation (18). However, it remained to be established whether these effects where exerted at the level of PLA_2 itself or whether the synthesis of proteins affecting the activation or secretion of PLA_2 were involved. In addition, the type of PLA_2 enzyme responsible for the secreted PLA_2 activity had to be characterized, especially in view of the fact that a hormonally regulated 60 kDa form of PLA_2 has been described in rat mesangial cells (23,24). In this paper we demonstrate that the elevated rat mesangial cell PLA_2 activity upon treatment with IL-1β, TNF or forskolin is caused by induced synthesis and secretion of a 14 kDa group II PLA_2.

MATERIALS AND METHODS

Recombinant IL-1β was prepared by the Biotechnology Department of Ciba Geigy Ltd., Basel, Switzerland; recombinant TNFα was from Boehringer Mannheim, F.R.G. and forskolin from Calbiochem, Luzern, Switzerland. [1-^{14}C] Linoleic acid was obtained from Amersham International. Ultrogel AcA54 was obtained from LKB (France). Nitrocellulose membranes were from Schleicher and Schull, Dassel, F.R.G. Alkaline-phosphatase conjugated goat anti-mouse immunoglobulines (GAMAP) and prestained markers were products of Bio-Rad Laboratories. The substrates for the color development, i.e. nitro blue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate were obtained from Sigma, St. Louis, MO. Cyanogenbromide activated Sepharose 4B was a product of Pharmacia, Uppsala, Sweden.

Cell culture and incubation. Cultivation and characterization of rat mesangial cells was performed as described previously (18,25). Briefly, the cells were grown in RPMI 1640 supplemented with 20% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and bovine insulin at 0.66 units/ml (Sigma). For the experiments passages 6-12 of mesangial cells were used. Confluent mesangial cells cultured in 16 mm diameter wells were washed twice with PBS and incubated with 1 ml of RPMI 1640 containing 0.1 mg/ml of fatty acid-free bovine serum albumin (Sigma) with or without agents for the indicated time periods. Thereafter, the medium was withdrawn and centrifuged for 10 min at 1000 rpm in an IEC Centra-7R lab centrifuge. The supernatant was removed and frozen in liquid nitrogen and stored until assayed for PLA_2 activity. When used, the cells were washed once and dissolved in PBS and frozen in liquid nitrogen until further analysis of protein content and PLA_2 activity. Protein was determined by the method of Bradford (26) with bovine serum albumin as standard.

Phospholipase A_2 activity. The PLA_2 activity was assayed using 200 μM sn-2-labeled [1-^{14}C] linoleoylphosphatidylethanolamine (specific activity 1000 dpm/nmol), in 0.1 M Tris/HCl pH 8.5 in the presence of 10 mM $CaCl_2$ and 0.05% Triton X-100. Reactions were stopped after 30 min and the liberated ^{14}C-labeled fatty acids were extracted by a modified Dole extraction procedure (27). The substrate 1-acyl-2-[1-^{14}C] linoleoylphosphatidylethanolamine was prepared as described earlier (28).

Immunoprecipitation of PLA_2 by monoclonal antibodies. Monoclonal antibodies against rat liver mitochondrial PLA_2 were prepared, purified and coupled to CNBr-activated Sepharose 4B as described before (7,29). In PLA_2 binding experiments mesangial cell supernatants were incubated with an excess of McAb-Sepharose suspension for 2 h at room temperature in the presence of 0.5 M salt. After centrifugation the supernatants were carefully removed and assayed for residual PLA_2 activity. The pellets were washed once with PBS and the bound PLA_2 was released from the McAb-Sepharose complex with 0.1 M glycine/HCl buffer (pH 2.5) containing 0.5 M KCl. After centrifugation the supernatants were assayed for PLA_2 activity.

Immunoblotting. Samples were resolved by SDS-PAGE using a 12.5% polyacrylamide gel according to Laemmli (30). The electrophoresis was carried out at a constant voltage of 200 V. For immunoblotting the proteins were transferred to nitrocellulose (2 mA/cm^2 for 45 min) (31). Non-specific binding sites on the blot were blocked with 0.1% (w/v) milk powder in PBS for 45 min at room temperature followed by the incubation with primary antibody; a solution of 0.01% milk powder and a 1:60 dilution of each of the hybridoma cell culture supernatants 2E7C3 and 2B9B7C3 (29). The McAb incubation was performed for 1 hour at R.T. The membrane was washed 3 times with 0.01% milk powder and incubated with alkaline phosphatase conjugated rabbit anti-mouse IgG for 1 hour at R.T. in 0.01% milk powder (w/v) in PBS. After washing the alkaline phosphatase activity was detected by developing the blots in p-nitro blue tetrazolium (NBT; 0.33 mg/ml) and 5-bromo-4-chloro-3-indoyl-phosphatase (BCIP; 0.17 mg/ml) in 0.1 M Tris/HCl (pH 9.5) containing 0.1 M NaCl and 5 mM $MgCl_2$. BCIP was dissolved in N,N-dimethylformamide and NBT was dissolved in 70% (v/v) N,N-dimethylformamide in water. The reaction was stopped by rinsing the filter with water.

RESULTS

Stimulation of mesangial cells by either IL-1β, TNF or forskolin caused a release of PLA_2 activity in the medium (Table I). Monoclonal antibody binding experiments, using McAb against rat liver mitochondrial PLA_2 (29), indicated that nearly all of the secreted PLA_2 activity was recognized and precipitated by the McAb-Sepharose, irrespective of the stimulator used. We checked with immunoblotting the presence of PLA_2 in the supernatant from the stimulated mesangial cells (Fig. 1). The blot clearly showed that the mediators caused the release of a 14 kDa enzyme. The intensity of the 14 kDa band correlated reasonably with the measured PLA_2 activities. To exclude the possibility of a proteolytic conversion of a high molecular weight form of PLA_2 into a 14 kDa enzyme after secretion, a stimulation with IL-1β plus forskolin was done in the presence of a mixture of peptidase inhibitors (compare legend Fig. 2). Western blot

Table I. Secretion of phospholipase A_2 activity upon stimulation of rat mesangial cells and binding of secreted enzyme to McAb-Sepharose

addition	Phospholipase A_2 activity ($nmol \cdot min^{-1} \cdot ml^{-1}$)	Phospholipase A_2 activity bound to McAb-Sepharose (%)
none	0.04	n.d.
IL-1β (1nM)	1.76	95
TNF (1nM)	0.54	93
IL-1β + TNF	1.30	96
Forskolin (10 μM)	0.28	94
IL-1β + Forskolin	2.25	95

The cells were stimulated for 48 hours by the indicated agents at concentrations shown in brackets. The preparation of cell supernatants, measurement of PLA_2 activity and assessment of binding to McAb-Sepharose were done as described under Materials and Methods. n.d., not done.

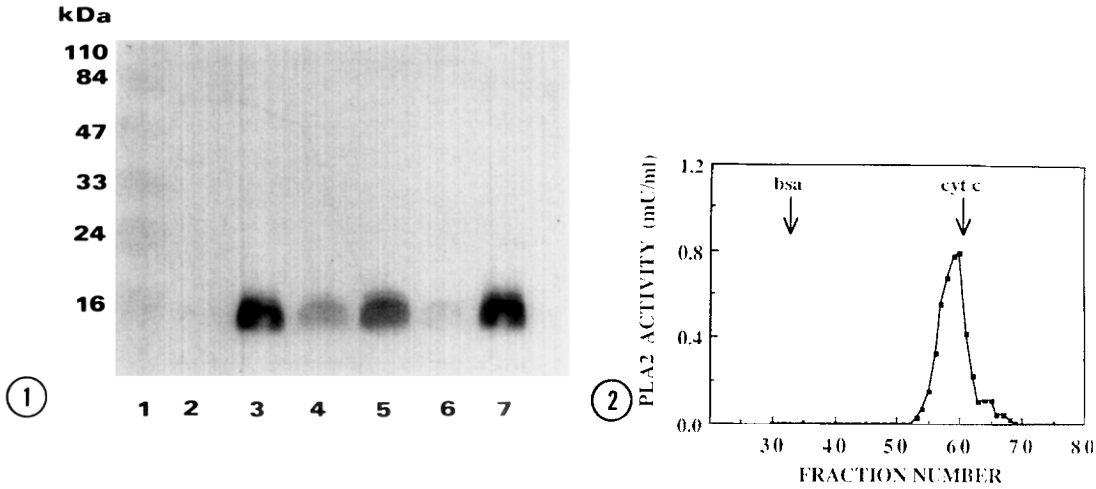

Figure 1. Immuno-detection of PLA_2 secreted from rat mesangial cells.
Rat mesangial cells were stimulated with IL-1β (1 nM, lane 3), TNFα (1 nM, lane 4) IL-1β and TNF (lane 5), forskolin (10 μM, lane 6) and IL-1β and forskolin (lane 7). Amounts corresponding to 500 μl of the supernatants were electrophoresed under non-reducing circumstances, transferred to nitrocellulose and detected as described in Materials and Methods. Lane 1 contained prestained markers and lane 2 the supernatant of control mesangial cells.

Figure 2. Ultrogel AcA-54 gelfiltration chromatography of the supernatant from IL-1β and forskolin stimulated mesangial cells.
The stimulation was done in the presence of peptidase inhibitors: antipain, phosphoramidon, leupeptin, pepstatin A, bestatin and amastatin (1 μg/ml each). The supernatant was dialyzed against AcA buffer (20 mM Tris/HCl pH 7.4, 1 M KCl, 2 mM EDTA and 20% glycerol). This solution (25 ml) was filtered over an AcA 54 column (144 x 3.7 cm) in AcA buffer at a flow rate of 40 ml/h. Fractions of 20 ml were collected. PLA_2 activity was measured in 50 μl aliquots of the fractions as described in Materials and Methods. Elution volume of cytochrome c, 12.5 kDa, and bovine serum albumin, 67 kDa is indicated by arrows.

analysis of this supernatant indicated a single 14 kDa enzyme (data not shown) thus precluding that this enzyme was formed by proteolytic conversion after secretion. Gelfiltration of the supernatant from cells stimulated with IL-1β plus forskolin in the presence of this cocktail of peptidase inhibitors also indicated the presence of a single peak of PLA_2 activity with an apparent molecular weight of 14 kDa (Fig. 2). This demonstrated that not only immuno-cross reactivity but also all enzymatic activity is confined to a 14 kDa enzyme. No indications for secretion of a 60 kDa PLA_2, reported to be present in mesangial cells (23), were obtained.

The increased PLA_2 protein levels in supernatants from stimulated cells (Fig. 1) suggest already that the enhanced PLA_2 activity in these supernatants (Table I) is not caused by activation of PLA_2. To investigate whether the enhanced PLA_2 protein levels in the culture supernatants are caused by enhanced secretion of preexisting cellular enzyme or represent induced synthesis and secretion a comparison of control and stimulated cells, rather than supernatants, was made. Western-blot experiments using McAb to detect the PLA_2 indicated much higher levels of the cross-reactive enzyme in stimulated cells than in control cells (Fig. 3). The detected PLA_2 which remained in stimulated cells showed a molecular weight of 14 kDa. In the control cells this enzyme is hardly detectable, indicating that IL-1β plus forskolin induce the synthesis of this 14 kDa PLA_2.

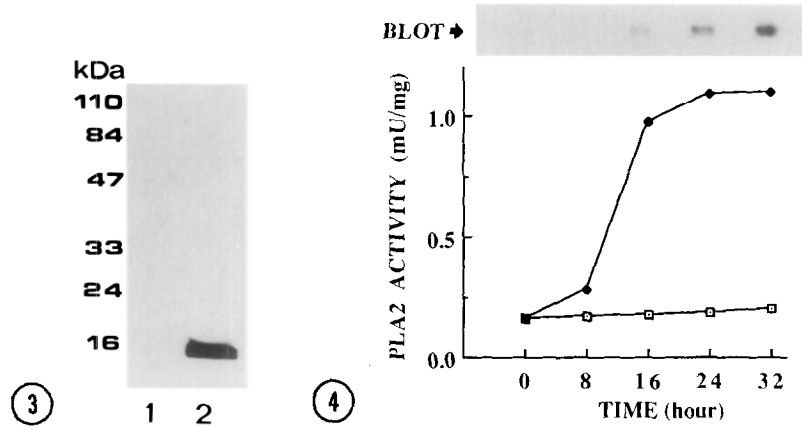

Figure 3. Immunoblot analysis of control and stimulated rat mesangial cells.
Lane 1: control cells; Lane 2: IL-1β plus forskolin stimulated cells. In each case 25 μg cellular protein was used.

Figure 4. Induction of PLA_2 activity and PLA_2 protein in rat mesangial cells as a function of time.
Mesangial cells were incubated with 1 nM IL-1β plus 10 μM forskolin (♦) or without mediators (□) for the indicated time periods. Thereafter, PLA_2 activity was determined in cell homogenates as described under Materials and Methods and 30 μg protein was used for Western blotting and PLA_2 immunostaining. Blots of control cells contained no detectable PLA_2 and only blots of stimulated cells are given at the top.

A comparison of the amount of secreted PLA_2 activity with that remaining in the cell after stimulation with IL-1β plus forskolin indicated that the activity remaining in the stimulated cells was only 14% of that recovered in the supernatant. The specific PLA_2 activity in stimulated cells amounted to 1.54 and 1.10 $nmol \cdot min^{-1} \cdot mg^{-1}$ in two experiments versus 0.17 and 0.16 $nmol \cdot min^{-1} \cdot mg^{-1}$ in control cells. This 6 to 9-fold increase in specific cellular PLA_2 activity is less than that suggested by the Western blot experiment of Fig. 3, most likely because of the

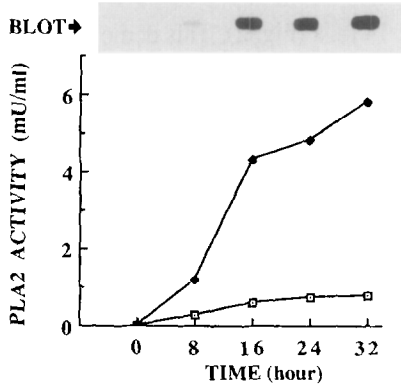

Figure 5. Secretion of PLA_2 activity and PLA_2 protein into rat mesangial cell culture supernatants as a function of time.
Conditions as in figure 4, except that 200 μl of the culture supernatants were used for Western blotting. Blots at the top represent those from the supernatants of stimulated cells. Supernatants from control cells gave no signal.

presence of other PLA_2's, such as a 60 kDa enzyme, that are not detected by the McAb in the blot experiment.

The time-dependency of PLA_2 synthesis and secretion induced by IL-1β plus forskolin was next investigated by activity measurements and Western blot analysis of cells and supernatants. As can be seen in figure 4 a time-dependent accumulation of PLA_2 activity in the cells, after a lag period of approximately 8 hrs, is accompanied by increased 14 kDa PLA_2 protein levels. The time-dependent secretion of this enzyme, both with respect to activity and protein levels, is shown in figure 5. Control cells showed no increase in PLA_2 activity (Fig. 4) and little secretion (Fig. 5). We calculated that at each time point of the stimulation (8 to 32 hrs) at least 88 % of the total PLA_2 activity was present in the supernatant indicating that the newly synthesized PLA_2 is mainly secreted.

DISCUSSION

IL-1α, IL-1β and TNF have been shown to induce PGE_2 synthesis in parallel to PLA_2 release into the medium of cultured rat glomerular mesangial cells (18). The relatively long lag time of approximately 8 hrs and the inhibition by actinomycin D and cycloheximide indicated the involvement of transcription and translation in these processes. Subsequent experiments showed that IL-1β, but not TNF, treatment augmented the formation of PGE_2 from exogenous arachidonic acid suggesting that only the former cytokine increased cyclo-oxygenase activity (32). In addition, both Il-1β and TNF pretreatment enhanced angiotensin II and A23187-induced arachidonic acid release in line with the augmented PLA_2 activity (32). IL-1β increases 14 kDa PLA_2 mRNA levels in rabbit chondrocytes (20,21) and rat vascular smooth muscle cells (22), but the affected enzyme in rat mesangial cells has not been identified.

Exocrine 14 kDa PLA_2 can be distinguished in pancreatic type I and non-pancreatic type II enzymes based on their amino acid sequence (2). Some tissues, e.g. rat spleen, contain both group I and group II enzymes (33) that could be distinguished by immunological criteria. It is currently not known whether these different enzymes occur in single cell types. Rat mesangial cells contain an additional PLA_2 that showed an apparent molecular weight of 60 kDa upon gelfiltration (23,24) and of 110 kDa upon SDS-PAGE (34). In this paper we have investigated which mesangial cell PLA_2 is affected by cytokines using gelfiltration and McAb to differentiate the various forms. Previous experiments have shown that these McAb recognize rat group II 14 kDa PLA_2 but not the analogous rat group I enzyme (29). IL-1β, TNF and forskolin, either alone or in combination, stimulated the release of PLA_2 activity into the culture medium (Table I). The stimulation of PLA_2 secretion by forskolin was previously observed for rat vascular smooth muscle cells (22,35) and rat mesangial cells (36). Evidence has been provided that different mechanisms underly forskolin- and TNF-induced PLA_2 release from smooth muscle cells but that in both cases the secreted enzyme was of the group II type (37). Similarly, immunobinding (Table I) and immunoblotting (Fig. 1) experiments showed that treatment of rat mesangial cells with either the proinflammatory cytokines IL-1β and TNF or the cAMP-elevating agent forskolin induced the secretion of group II 14 kDa PLA_2. Gelfiltration of supernatants from cells stimulated in the presence of protease inhibitors (Fig. 2) indicated that

no 60 kDa PLA_2 was secreted and at the same time excluded the possibility that the 14 kDa PLA_2 originated from proteolytic cleavage of larger molecular weight forms of PLA_2 after secretion.

Immunoblot experiments of control and stimulated cells (Figs. 3 and 4) indicated that the group II PLA_2 was hardly present in unstimulated cells and became detectable after approximately 8 hrs of stimulation in both cells and supernatants (Fig. 5). Hence, the enhanced cellular PLA_2 activity upon stimulation (Fig. 4) is due to new synthesis of PLA_2 protein and cannot solely be explained by activation of preexisting PLA_2 through e.g. translocation of enzyme from cytosol to membranes, synthesis of activators or phosphorylation processes. The fact that the enzyme was hardly present in unstimulated cells also excludes the possibility that IL-1β, TNF and forskolin induced release of PLA_2 from the cells is caused by increased secretion of preexisting cellular enzyme. Rather, these agents appear to affect group II PLA_2 gene expression resulting in higher levels of PLA_2 protein, about 90% of which appears to be secreted from the cells. In this respect it is worth mentioning that analysis of group II PLA_2 genes has revealed the presence of sequences coding for secretory signals (6,12,38,39). Whether IL-1β and forskolin influence gene expression in mesangial cells by different mechanisms as recently reported for rat vascular smooth muscle cells (37) remains to be investigated. The previously observed (18) close correlation between secretion of PLA_2 activity, herein identified as 14 kDa group II PLA_2, and PGE_2 formation is in line with the concept that group II PLA_2's play an important role in inflammatory processes. Whether the PLA_2 released in the extracellular environment contributes to arachidonate release for PGE_2 formation after stimulation of mesangial cells remains to be elucidated.

REFERENCES

1. Van den Bosch, H. (1980) Biochim. Biophys. Acta 604, 191-246.
2. Heinrikson, R.L., Krueger, E.T. and Keim, P.S. (1977) J. Biol. Chem. 252, 4913-4921.
3. Hayakawa, M., Horigome, K., Kudo, I., Tomita, M., Nojima, S. and Inoue, K. (1987) J. Biochem. 101, 1311-1314.
4. Hayakawa, M., Kudo, I., Tomita, M., Nojima, S. and Inoue, K. (1988) J. Biochem. 104, 767-772.
5. Mizushima, H., Kudo, I., Horigome, K., Murakami, M., Hayakawa, M., Kim, D.K., Kondo, E., Tomita, M. and Inoue, K. (1989) J. Biochem. 105, 520-525.
6. Kramer, R.M., Hession, C., Johansen, B., Hayes, G., McGray, P., Pinchang Chow, E., Tizard, R. and Pepinsky, R.B. (1989) J. Biol. Chem. 264, 5768-5775.
7. Aarsman, A.J., De Jong, J.G.N., Arnoldussen, E., Neys, F.W., Van Wassenaar, P.D. and Van den Bosch, H. (1989) J. Biol. Chem. 264, 10008-10014.
8. Kanda, A., Ono, T., Yosida, N., Tojo, H. and Okamoto, M. (1989) Biochem. Biophys. Res. Commun. 163, 42-48.
9. Forst, S., Weiss, J., Elsbach, P., Maraganore, J.M., Reardon, L. and Heinrikson, R.L. (1986) Biochemistry 25, 8381-8385.
10. Chang, H.W., Kudo, I., Tomita, M. and Inoue, K. (1987) J. Biochem. 102, 147-154.
11. Hara, S., Kudo, I., Chang, H.W., Matsuta, K., Miyamoto, Y. and Inoue, K. (1989) J. Biochem. 105, 395-399.
12. Scilhamer, J.J., Pruzanski, W., Vadas, P., Plant, S., Miller, J.A., Kloss, J. and Johnson, L.K. (1989) J. Biol. Chem. 264, 5335-5338.
13. Vadas, P. and Pruzanski, W. (1986) Lab. Invest. 4, 391-404.
14. Murakami, M., Kudo, I., Nakamura, H., Yokoyama, Y., Mori, H. and Inoue, K. (1990) FEBS Lett. 268, 113-116.
15. Chang, J., Gilman, S.C. and Lewis, A.J. (1986) J. Immunol. 136, 1283-1287.
16. Suffys, P., Van Roy, F. and Fiers, W. (1988) FEBS Lett. 232, 24-28.

17. Godfrey, R., Johnson, W. and Hoffstein, S.T. (1987) Biochem. Biophys. Res. Commun. 142, 235-241.
18. Pfeilschifter, J., Pignat, W., Vosbeck, K. and Märki, F. (1989) Biochem. Biophys. Res. Commun. 159, 385-394.
19. Leighton, J. and Pfeilschifter, J. (1990) FEBS Lett. 259, 289-292.
20. Lyons-Giordano, B., Davis, G.L., Galbraith, W., Pratta, M. and Arner, E. (1989) Biochem. Biophys. Res. Commun. 164, 488-495.
21. Kerr, J., Stevens, T., Davis, G., McLaughlin, J. and Harris, R. (1989) Biochem. Biophys. Res. Commun. 165, 1079-1084.
22. Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) FEBS Lett. 261, 171-174.
23. Gronich, J.H., Bonventre, J.V. and Nemenoff, R.A. (1988) J. Biol. Chem. 263, 16645-16651.
24. Bonventre, J.V., Gronich, J.H. and Nemenoff, R.A. (1990) J. Biol. Chem. 265, 4934-4938.
25. Pfeilschifter, J., Kurtz, A. and Bauer, C. (1984) Biochem. J. 223, 855-859.
26. Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
27. Van den Bosch, H. and Aarsman, A.J. (1979) Agents Action 9, 382-389.
28. Van den Bosch, H., Aarsman, A.J. and van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 348, 197-207.
29. De Jong, J.G.N., Amesz, H., Aarsman, A.J., Lenting, H.B.M. and van den Bosch, H. (1987) Eur. J. Biochem. 164, 129-135.
30. Laemmli, U.K. (1970) Nature 227, 680-685.
31. Towin, H., Stachelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350-4354.
32. Pfeilschifter, J. and Mühl, H. (1990) Biochem. Biophys. Res. Commun. 169, 585-595.
33. Ono, T., Tojo, H., Kuramitsu, S., Kagamiyama, H. and Okamoto, M. (1988) J. Biol. Chem. 263, 5732-5738.
34. Gronich, J.H., Bonventre, J.V. and Nemenoff, R.A. (1990) Biochem. J. 271, 37-43.
35. Pfeilschifter, J., Pignat, W., Märki, F. and Wiesenberg, I. (1989) Eur. J. Biochem. 181, 237-242.
36. Pfeilschifter, J., Leighton, J., Pignat, W., Märki, F. and Vosbeck, K., (1991) Biochem. J., in press.
37. Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) J. Biol. Chem. 265, 12745-12748.
38. Komada, M., Kudo, I., Mizushima, H., Kitamura, N. and Inoue, K. (1989) J. Biochem. 106, 545-547.
39. Komada, M., Kudo, I. and Inoue, K. (1990) Biochem. Biophys. Res. Commun. 168, 1059-1065.