Phospholipid metabolism and concanavalin A stimulation of thymocytes from magnesium-deficient rats and magnesium-deficiency-induced T-cell lymphoma

By *R. Averdunk* and *T. Günther*

Institute for Clinical Chemistry and Institute for Molecular Biology and Biochemistry, Freie Universität Berlin

Zusammenfassung

In Thymozyten von Mg-arm ernährten Ratten waren die Aktivität von Phospholipase A_2 (gemessen an Hand der Freisetzung von inkorporierter ^{14}C-Arachidonsäure), die Aktivität von Lysophosphatid-CoA-Acyltransferase (gemessen mit Hilfe des Einbaus von ^{14}C-Ölsäure) und die Aktivität der Phosphatidylmethyltransferase (gemessen an Hand des Einbaus von ^{3}H-Methylgruppen aus Methionin) um 50 – 100 % höher als bei normalen Thymozyten. Diese Ergebnisse lassen sich mit der Ca^{2+}-Abhängigkeit dieser Enzyme und dem verstärkten Turnover und der geänderten Verteilung des Ca^{2+} in Mg-Mangel-Thymozyten erklären. Die Phospholipase A_2 und Acyltransferase wurden in Mg-Mangelthymozyten durch ConA weniger stimuliert als in normalen Thymozyten. Als Ursache kommen die erhöhte Grundaktivität und demzufolge ein verringertes Substratangebot sowie die verminderte Zahl von Con A-Rezeptoren in Frage. In den durch Mg-Mangel erzeugten Lymphomzellen des Thymus waren die Aktivitäten dieser Enzyme etwa so hoch wie in normalen Thymozyten. Die Stimulierbarkeit des Phospholipid-Stoffwechsels durch ConA und die Anzahl der ConA-Rezeptoren war in den Lymphomzellen niedriger als in normalen Thymozyten.

Summary

In thymocytes from Mg-deficient rats, the activity of phospholipase A_2, measured by the release of incorporated ^{14}C-arachidonic acid, the activity of lysophosphatide CoA acyltransferase, measured by incorporation of ^{14}C-oleic acid, and the activity of phosphatidylmethyltransferase, measured by incorporation of ^{3}H-labelled methyl groups from methionine, were 50 – 100 % higher than in normal thymocytes. These results can be explained by the Ca^{2+}-dependency of these enzymes and by the increased turnover and distribution of Ca^{2+} in Mg-deficient thymocytes. Phospholipase A_2 and acyltransferase in Mg-deficient thymocytes were less stimulated by Con A than in normal thymocytes. The reason may be the higher basal activity and thus limited availability of substrate and a reduced number of Con A receptors. In Mg-deficiency-induced lymphoma of the thymus, unstimulated activities of these enzymes were similar to those in normal thymocytes. There was less Con A stimulation of PL metabolism and fewer Con A receptors in lymphoma cells than in normal thymocytes.

Resumé

Dans les thymocytes de rats carencés en Mg l'activité de la phospholipase A_2, mesurée par la libération de l'acide arachidonique ^{14}C incorporé, l'activité de la lysophosphatide CoA acyltransferase, mesurée par l'incorporation de l'acide oleique ^{14}C et l'activité de le phophatidylmethyltransferase, mesurée par l'incorporation de groupes methyles marqués par le ^{3}H de la méthionine s'avère 50 à 100 % plus élevez que celle de thymocytes normaux. Ces résultats peuvent s'expliquer par la Ca^{2+}-dépendance de ces enzymes et le turnover accru et la distribution du Ca^{2+} dans les thymocytes carencés en Mg. Phospholipase A_2 et acyltransferase des thymocytes carencés en Mg sont moins stimulés par la Con A que les thymocytes normaux. La raison peut être une activité basale plus haute avec disponibilité limitée de substrat et nombre réduit de récepteurs pour la Con A. Dans le lymphoma induit par carence magnésique du rat, les activités non stimulées de ces enzymes sont identiques à celles de thymocytes normaux. Il y a une moindre stimulation par la Con A du métabolisme des PL et un nombre réduit de récepteurs à la Con A dans les cellules lymphomateuses que dans les thymocytes normaux.

Abbreviations

PL	phospholipid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
LysoPC	lysophosphatidylcholine
ConA	concanavalin A
TCA	trichloroacetic acid

Introduction

Young rats chronically fed with a Mg-deficient diet evidenced a reduction in the immunoglobulin concentration in serum as well as the stimulation of lymphocytes by mitogens [3]. For review see [2]. About 15 % of these rats developed a malignant T-cell lymphoma of the thymus [4]. Mg-deficient thymocytes and lymphoma cells showed a change in the cell membrane permeability of Na^+, K^+ and Ca^{2+} [1, 4] and the intracellular Ca^{2+}-distribution [4]. The cellular concentration of Na^+ and Ca^{2+} increases [4]. An alteration of Ca^{2+} metabolism with an increase of intracellular Ca^{2+} concentration is one of the early events in lymphocyte activation [18]. The activation of lymphocytes — as, for example, by ConA — after receptor binding and the activation of phospholipase C and diacylgycerol kinase lead to the formation of phosphatidic acid (particularly from PC or PI). Phosphatidic acid (or lysophosphatidic acid formed by phospholipase A_2) may induce gating and influx of Ca^{2+} [8]. Inositoltrisphosphate, produced by phospholipase C from PI, may also liberate stored intracellular Ca^{2+} [10]. The simultaneously formed diacylgycerol may activate protein kinase C [15]. The increased intracellular Ca^{2+} concentration may stimulate phospholipase A_2 [5, 8], a Ca^{2+}-dependent enzyme [19]. Phospholipase A_2 releases arachidonic acid from various phospholipids (PC, PE and PI) according to the cell type [7, 19]

and thus regulates production of prostanoids. It is possible that PC, formed by methylation of PE after ConA stimulation, may be preponderantly hydrolyzed by phospholipase A_2 [5]. However, the latter result was doubted [7, 14].

Methylation of PE may be a consequence of the increased Ca^{2+} influx [6, 11]. There is evidence that Ca^{2+} regulates phospholipid methyltransferase [11]. However, methylation may also precede Ca^{2+} influx [5]. The exact sequential relationship between PL turnover and Ca^{2+} metabolism remains unclear.

To explain the reduced functions of the immune system in Mg deficiency, we tested whether signal transmission and the reactions of PL metabolism are changed in Mg-deficient thymocytes and Mg-deficiency-induced lymphoma cells. For this purpose, we measured the effect of ConA on oleate incorporation in PL with intact cells and a membrane preparation as well as the effect of ConA on PL methylation (phospholipidmethyltransferase) and on arachidonic acid release (phospholipase A_2) in these cells.

Methods

Female Wistar Rats, weighing 60 g, were fed an Mg-deficient diet (Mg content: 3 mmol/kg; Ca content: 250 mmol/kg; Ssniff Co., Soest, F.R.G.) and distilled water ad libitum for 10 weeks. Control rats were fed the same food supplemented with $MgCl_2$ to an Mg content of 80 mmol/kg dry weight.

Thymocytes were isolated by sieving in RPMI 1640 medium (Seromed, Munich, F.R.G.). The various lymphoma cells were taken after 2–3 intraperitoneal passages.

Determination of phospholipase A_2

Thymocytes (10^8 cells/ml) were incubated at 37° C with 1 µC ^{14}C-arachidonic acid (specific act. = 1 mCi/mmol, New England Nuclear) in Hepes-buffered RPMI 1640 medium, pH 7.4. After 3 hours, the cells were pelleted at 1500 g × 10 min at 4° C and washed three times with cold RPMI 1640 medium. To measure release of ^{14}C-arachidonic acid, 1×10^7 cells/ml were incubated in prewarmed (37° C) RPMI 1640 medium, containing 20 g/l defatted bovine serum albumin, without or with 2 µg/ml ConA. After various times, as indicated in Fig. 1, 0.5 ml cell suspension was centrifuged at 5000 g for 1 min. The pellet and 0.3 ml of the supernatant were taken for measurement of radioactivity by liquid scintillation counting. The results were expressed as released arachidonic acid in percent of incorporated arachidonic acid.

Incorporation of ^{14}C-oleate into PC and PE of intact cells

5×10^7 lymphocytes were preincubated in 2 ml RPMI 1640 medium, buffered with 10 mM Hepes pH 7.4 for 1 hour in the presence or absence of 2 µg ConA/ml. Thereafter, 10 nmol ^{14}C-oleate (spec. activity = 1 mCi/mmol, New England Nuclear) in 10 µl ethanol were added. After a further 1 h incubation at 37° C, 5 ml of methanol, containing unlabelled PC, PI, PE, PS and sphingomyeline, 100 µg of each (Serva, Heidelberg, F.R.G.), where added. The lipids were extracted with chloroform : methanol = 2:1 (V/V). The phospholipids were separated by thin-layer chromatography on TLC silica gel 60 plates (Merck, Darmstadt, F.R.G.) with chloroform : methanol : acectic acid (99 %) : 0.15 M NaCl = 50 : 25 : 8 : 4 (V/V). The phospholipid spots were visualized by exposure to iodine vapor. For identification, pure phospholipids were chromatographed on the same plates. PC and PE were scraped off and counted by liquid scintillation counting.

Determination of acyl CoA : lysophosphatide acyltransferase activity

8×10^8 cells were incubated in 20 ml RPMI 1640 medium with 5 % fetal calf serum without or with 2 µg ConA/ml for 1 hour at

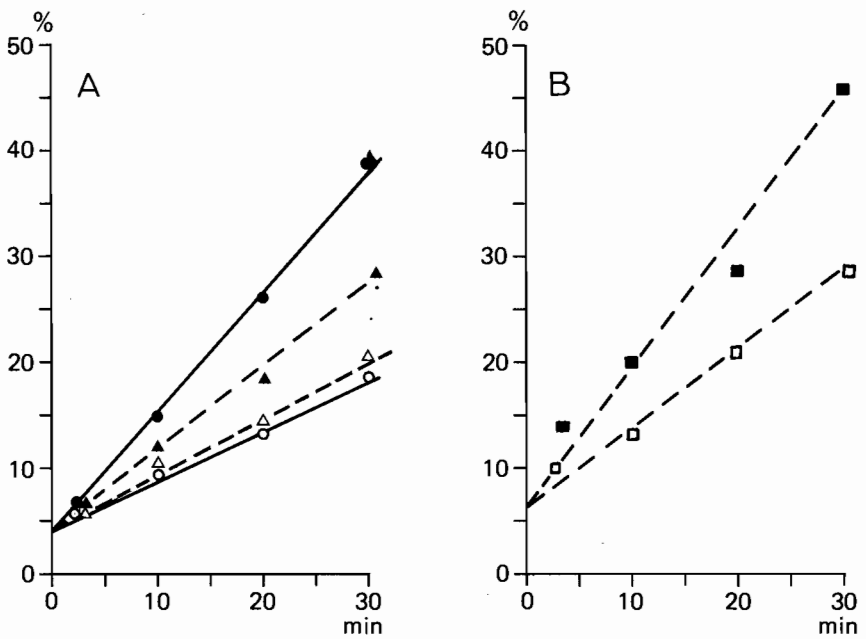

Fig. 1: Effect of Con A on phospholipase A_2 activity in normal thymocytes and lymphoma cells (A) and in Mg-deficient thymocytes (B). Normal cells (o), normal cells with ConA (●), Mg-deficient thymocytes (□), Mg-deficient cells with Con A (■), lymphoma cells (△), lymphoma cells with Con A (▲).

37° C. Therafter, the cells were resuspended in 10 ml 1 mM Tris-Cl, pH 7.4, for 30 min at 4° C and disrupted by homogenisation in a Potter-Elvehjem with loose fitting pestle by 10 strokes. The homogenate was centrifuged at 10 000 g for 15 min. The supernatant, containing the membrane fraction, was centrifuged at 100 000 g for 1 hour. The pellet was suspended in 2 ml 50 mM potassium phosphate buffer, pH 7.4. The assay for measuring the enzymatic activity contained in 1 ml phosphate buffer, pH 7.4:
60 nmol 1-palmitoyl-Sn-glycero-3-phosphatidylcholine (Sigma, Munich, F.R.G.)
30 nmol ^{14}C-oleoyl-CoA (spec. activity = 50 mCi/mmol, New England Nuclear).

The protein content of the assays amounted to 100 µg. After an incubation of 10 min at 37° C, the reaction was stopped by addition of 1 ml methanol.

The ^{14}C-labelled phospholipids were extracted, chromatographed and counted as described above.

Protein was measured according to *Lowry* et al. [9].

Methylation of phospholipids

Thymocytes (1.5×10^7 cells/ml) were incubated in RPMI 1640 medium with 5 % fetal calf serum at 37° C for 2, 10 and 30 min; after addition of 200 µCi ^{3}H-L-methionine (spec. act.: 89 Ci/mmol, NEN) and 2 µg ConA/ml, as indicated, 0.2 ml aliquots were added to 0.5 ml ice-cold 10 % TCA, containing 10 mM methionine. The suspension was centrifuged at 10 000 g for 10 min. The pellet was washed twice with TCA, containing methionine. The phospholipids were extracted with chloroform/methanol 2/1 (V/V) and chromatographed as described above. Radioactivity was counted in a liquid scintillation counter.

Number of ConA receptors

ConA (Pharmacia, Sweden) was labelled with Na^{125}I (Amersham Buchler, Braunschweig, FRG) after oxidation to I_2 with chloramine-T (Fluka AG, Buchs, Switzerland) according to *McConahey* and *Dixon* [12]. The remaining I_2 was reduced by sodium metabisulfite (Merck AG, Darmstadt, FRG) and removed by dialysis. Aggregated ConA was removed by high-speed centrifugation (25 000 gx1 h). For ConA-binding, 1.5 µg ^{125}I-labelled ConA (spec. activity = 10^{11} cpm/µmol) was added to 10^7 cells in 1 ml RPMI 1640 medium.

After incubation at 37° C for 180 min, the cells were washed three times with 5 ml cold RPMI 1640 medium. Unspecific binding was measured by parallel tests in the presence of 50 µM α-methyl mannoside. The radioactivity of the sedimented cells was counted in a γ-counter (Berthold Co., Wildbad, FRG). The number of ConA binding sites per cell was calculated using Loschmidt's number.

Results

Normal thymocytes

In normal thymocytes, ConA stimulates the release of incorporated ^{14}C-arachidonic acid and thus phospholipase A_2 by a factor of 2.5 (Fig. 1). Simultaneously, the incorporation of ^{14}C-oleic acid into PC and PE is stimulated by ConA with intact cells (Table 1) as well as in PC with a microsome preparation (Table 2). Oleate incorporation into PC was about three times higher than oleate incorporation into PE (Table 1) in accordance with the greater amount og PC in membranes. The effect of ConA was about 2–3 times more marked in the microsome preparation than in intact cells. The addition of lysoPC may have overcome the restriction of enzymatic activity caused by limited substrate availability. The rate of methyl incorporation into PC of intact cells is not constant with time. For a period of up to 10 min, the rate was higher than after that time (Fig. 2), as found by others [16]. The incorporation of methyl groups was not significantly stimulated by ConA.

Mg-deficient thymocytes

In the thymocytes from Mg-deficient rats, the basal activity of phospholipase A_2 is about 50 % higher, whereas the activity of the ConA-stimulated phospholipase A_2 is the same as in ConA-stimulated normal thymocytes. Thus, the effect of ConA on phospholipase A_2 in Mg-deficient cells (factor 1.8) is less marked than in normal thymocytes (factor 2.5) (Fig. 1).

The same was found for the ^{14}C-oleate incorporation into PC and PE of intact Mg-deficient cells. Unstimulated ^{14}C-oleate incorporation in Mg-deficient cells was 90 % higher and the effect of

Table 1: Incorporation of ^{14}C-oleate in PC and PE of intact normal and Mg-deficient thymocytes and intact lymphoma cells. The cells were incubated with ^{14}C-oleate in RPMI 1640 medium for 60 min at 37° C without or with 2 µg ConA/ml. Mean ± SD of 5 experiments. The factor of ConA stimulation is given in parentheses.

Phospho-lipid	Addition of ConA	Normal thymocytes	Mg-def. thymocytes	Lymphoma cells
		cpm/mg prot·h		
PC	–	1831 ± 192	3500 ± 378	2746 ± 307
	+	3120 ± 361 (1.7)	4593 ± 537 (1.3)	3412 ± 392 (1.2)
PE	–	750 ± 79	957 ± 105	1100 ± 158
	+	1349 ± 177 (1.8)	1447 ± 182 (1.5)	1500 ± 203 (1.3)

ConA was likewise less pronounced than in normal cells (Table 1).

Measurement of acyltransferase activity in microsomes revealed a lower rate of ^{14}C-oleate incorporation in the preparation of Mg-deficient cells and a reduced effect of ConA as compared with the preparation of normal cells (Table 2), which indicates a smaller amount of acyltransferase. The higher activity of acyltransferase in intact Mg-deficient thymocytes shows that it is operating at a higher level in these cells.

Methyl incorporation was 80 % higher in intact Mg-deficient cells than in normal cells and was not significantly stimulated by ConA (Fig. 2).

T-cell lymphoma cells

Phospholipase A_2 activity in lymphoma cells was only somewhat higher than in normal thymocytes and only negligibly less stimulated by ConA (Fig. 1). An analogous behavior is found for ^{14}C-oleate incorporation in intact lymphoma cells. Oleate incorporation into microsomes of lymphomas was about the same as into the microsomes of normal cells. However, ConA stimulation of ^{14}C-oleate incorporation was reduced as compared to controls (Table 1, 2). Methyl incorporation in lymphoma cells was about the same as in normal cells and not significantly increased by ConA (Fig. 2).

Number of ConA receptors

To find an explanation for the reduced effect of ConA in Mg-deficient and lymphoma cells, the number of ConA binding sites was measured. As shown in Table 3, the number of ConA binding sites was reduced by 10 % in Mg-deficient thymocytes and by 20 % in lymphoma cells.

Discussion

As shown with normal thymocytes, ConA stimulation as a model for signal transmission is associated with an increase in both phospholipase A_2 and acyltransferase. Phospholipid methyltransferase was slightly but not significantly increased. These results are in agreement with comparable findings of other authors [5, 7, 8, 19]. However, there are also contradictory results. Some authors found no effect of ConA on the methylation rate of phospholipids in mouse thymocytes [14], whereas others found ConA stimulation of methylation in human cells [16]. In mouse thymocytes, ConA had no effect on the rate of arachidonic release [13]; whereas, in human blood lymphocytes, ConA stimulated the release of arachidonic acid [13, 17]. Species (and methodological) differences and the inhomogeneity of the cell preparations may be responsible for these discrepancies.

In intact Mg-deficient rat thymocytes, 1. the basal turnover of phospholipids was increased, and 2. the effect of ConA was reduced. These results may be explained by the changes in Ca^{2+} metabolism in Mg-deficient rat thymocytes. Phospholipase A_2 and PL-methyltransferase are Ca^{2+}-dependent enzymes; their activities are enhanced when the Ca^{2+} influx across the cell membrane, e.g. by A 23187, and the cytosolic Ca^{2+} concentration are increased [7, 8]. Both, Ca^{2+} influx and cytosolic Ca^{2+} concentration, may be increased in Mg-deficient rat thymocytes [4]. The activation by Ca^{2+} of oleate incorporation in intact Mg-deficient cells is still higher, as can be seen by comparing acyltransferase from microsomes with that from intact normal and Mg-deficient cells, because the activity of acyltransferase in the microsomes of Mg-deficient cells is only half that of the microsomes from normal cells.

There may be various reasons for the decreased ConA-stimulation in Mg-deficient cells:

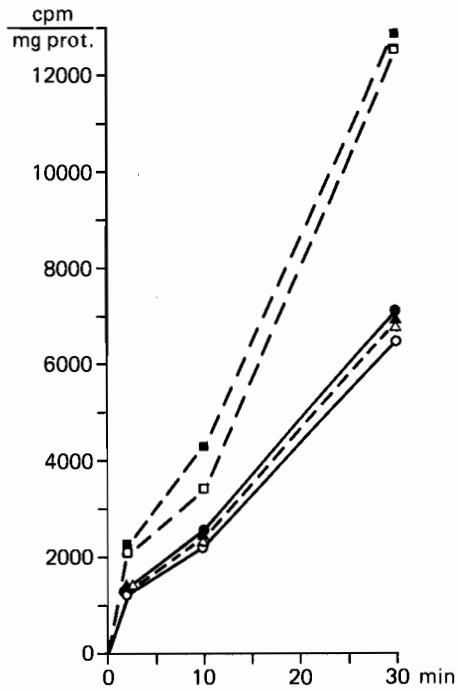

Fig. 2: Methylation of phospholipids in normal and Mg-deficient thymocytes and lymphoma cells. The same symbols as in Fig. 1 were used.

Table 2: Activity of oleoyl-CoA: lysophosphatide acyltransferase. The cells were preincubated without or with 2 µg ConA/ml in RPMI 1640 medium at 37° C for 60 min. After preincubation, microsomes were prepared and tested for acyltransferase activity as described in Methods. Mean ± SD of 5 experiments. The factor of ConA stimulation is given in parentheses.

Phospho-lipid	Addition of ConA	Normal thymocytes	Mg-def. thymocytes	Lymphoma cells
		nmol/mg prot·min		
PC	–	2.67 ± 0.31	1.35 ± 0.22	2.03 ± 0.29
	+	12.34 ± 1.30 (4.6)	4.91 ± 0.52 (3.6)	3.04 ± 0.31 (1.5)

1. The number of ConA-receptors was reduced. However, we cannot decide whether the small reduction in the number of binding sites is sufficient for explaining the lower ConA stimulation.
2. As the basal phospholipid turnover is already enhanced, the additional increase by ConA may be smaller because of a non-linear correlation between PL and Ca^{2+} metabolism.
3. In Mg-deficiency, the number of thymocytes is reduced by necrosis and phagocytosis [1]. Thus, the cell population compared to that of normal rats may have changed, resulting in different properties.

The altered PL metabolism in Mg-deficient thymocytes is irreversible during the experimental period, because the cells were incubated in RPMI 1640 medium with normal Mg^{2+} concentrations. The sum of these alterations may explain the reduced functions of the immune system in Mg deficiency [2, 3].

Phospholipid turnover in intact lymphoma cells is lower than in Mg-deficient cells and hardly stimulated by ConA. As Ca^{2+} in lymphoma cells evidences a change similar to that in Mg-deficient cells and as the Ca^{2+} fluxes are also enhanced in lymphoma cells, one would also expect an increased basal phospholipid turnover in lymphoma cells. However, this did not happen. The question why Ca^{2+} did not have a similar effect in lymphoma cells cannot be answered; the Ca^{2+}-effect may possibly be altered in malignant cells.

The second effect with lymphoma cells is their reduced ConA-stimulation. On testing various lymphomas that developed in different rats, we found different properties with respect to ConA-stimulation measured by ^{3}H-thymidine incorporation. Some lymphomas were not stimulated at all, whereas the cells of other lymphomas were stimulated to a small degree. After a few intraperitoneal passages (4 or more) none of the lymphomas were stimulated by ConA any longer. The reduced stimulation may depend on the reduced number of ConA binding sites. However, the subsequent reactions of signal transmission in the cell membrane after receptor binding may also be abolished.

Table 3: Binding of ^{125}I-labelled ConA to normal and Mg-deficient thymocytes and to lymphoma cells. See Methods for experimental details. Mean ± SD of 3 experiments.

	ConA molecules bound per cell
Normal thymocytes	$(6.02 \pm 0.108) \times 10^6$
Mg-def. thymocytes	$(5.52 \pm 0.131) \times 10^6$
Lymphoma cells	$(4.64 \pm 0.121) \times 10^6$

Literature

[1] *Averdunk, R., Bippus, P. H., Günther, T., Merker, H. J.:* Development and properties of malignant lymphoma induced by magnesium deficiency in rats. J. Cancer Res. Clin. Oncol. **104** (1982) 63 – 73.

[2] *Gaudin-Harding, F.:* Magnésium et système immunitaire: Données recentes. Magnesium-Bull. **3** (1a) (1981) 229 – 236.

[3] *Günther, T., Averdunk, R.:* Reduced lectin stimulation of lymphocytes from magnesium-deficient rats. J. Clin. Chem. Clin. Biochem. **17** (1979) 51 – 55.

[4] *Günther, T., Vormann, J., Merker, H. J., Averdunk, R., Peter, H. W., Wonigeit, K.:* Membrane alterations in magnesium-deficiency-induced malignant T cell lymphoma. Magnesium Exptl. Clin. Res. **3** (1984) 29 – 37.

[5] *Hirata, F., Axelrod, J.:* Phospholipid methylation and biological signal transmission. Science **209** (1980) 1082 – 1090.

[6] *Imai, A., Ishizuka, Y., Nakashima, S., Nozawa, Y.:* Differential activation of membrane phosholipid turnover by compound 48/80 and ionophore A 23187 in rat mast cells. Arch. Biochem. Biophys. **232** (1984) 259 – 268.

[7] *Irvine, R. F.:* How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J. **204** (1982) 3 – 16.

[8] *Lapetina, E. G.:* Regulation of arachidonic acid production: Role of phospholipases C and A_2. Trends in Pharmacol. Sci. **3** (1982) 115 – 118.

[9] *Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.:* Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193** (1951) 265 – 275.

[10] *Majerus, P. W., Neufeld, E. J. Wilson, D. B.:* Production of phosphoinositide-derived messengers. Cell **37** (1984) 701 – 703.

[11] *Mato, J. M., Alemany, S.:* What is the function of phospholipid N-methylation? Biochem. J. **213** (1983) 1 – 10.

[12] *McConahey, P. J., Dixon, F. J.:* A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy **29** (1966) 185 – 189.

[13] *Moore, J. P., Johannsson, A., Hesketh, T. R., Smith, G. A., Metcalfe, J. C.:* Calcium signals and phospholipid methylation in eukaryotic cells. Biochem. J. **221** (1984) 675 – 684.

[14] *Moore, J. P., Smith, G. A., Hesketh, T. R., Metcalfe, J. C.:* Early increases in phospholipid methylation are not necessary for the mitogenic stimulation of lymphocytes. J. Biol. Chem. **257** (1982) 8183 – 8189.

[15] *Nishizuka, Y.:* Turnover of inositol phospholipids and signal transduction. Science **225** (1984) 1365 – 1370.

[16] *Niwa, Y., Sakane, T., Taniguchi, S.:* Phospholipid transmethylation in the membrane of human neutrophils and lymphocytes. Arch. Biochem. Biophys. **234** (1984) 7 – 14.

[17] *Parker, C. W., Kelly, J. P., Falkenheim, S. F., Huber, M. G.:* Release of arachidonic acid from human lymphocytes in response to mitogenic lectins. J. Exp. Med. **149** (1979) 1487 – 1503.

[18] *Tsien, R. Y., Pozzan, T., Rink, T. J.:* T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. Nature **295** (1982) 68 – 71.

[19] *Van den Bosch, H.:* Intracellular phospholipases A. Biochem. Biophys. Acta **604** (1980) 191 – 246.

For the authors: Prof. Dr. T. Günther, Institut für Molekularbiologie und Biochemie, Arnimallee 22, D-1000 Berlin 33