Pancreatic Phospholipase A_2 – Mediated Enhancement of the Respiratory Burst Response of Human Neutrophils

Julia Müller[#], Marijana Petković[#,*], Jürgen Schiller and Jürgen Arnhold

Institute of Medical Physics and Biophysics, Medical Faculty, University of Leipzig, Liebigstraße 27, D-04103 Leipzig, Germany.
Fax: (+49) 341 97 15 709. E-mail: petm@medizin.uni-leipzig.de

* Author for correspondence and reprint requests

Z. Naturforsch. **56c,** 1150–1156 (2001); received August 14/August 31, 2001

Human Neutrophils, Luminol-Amplified Chemiluminescence, Pancreatic Phospholipase A_2

The aim of this study was to investigate the effects of exogenously added pancreatic phospholipase A_2 ($pPLA_2$) on the production of reactive oxygen species by human polymorphonuclear leukocytes (PMNs). Pancreatic PLA_2 was used because PMNs do not possess a receptor for that enzyme and, therefore, the receptor-mediated effects could be excluded. Respiratory burst activity of PMNs was monitored by luminol-amplified chemiluminescence and the lipid composition of neutrophils after treatment with $pPLA_2$ was determined by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Our results show that the products of the $pPLA_2$ digestion of the PMN membrane – lysophospholipids and the corresponding free fatty acids – significantly enhanced the respiratory burst response of human neutrophils.

Introduction

Human polymorphonuclear leukocytes (shorter: "neutrophils" or "PMNs") are highly specialised immune cells for phagocytosing and killing pathogenic agents and micro-organisms. PMNs are among the first cells that migrate to the site of infection and they are equipped with a wide spectrum of bactericidal agents (Edwards, 1994). Neutrophil NADPH oxidase that is required for the production of all reactive oxygen species (ROS) is activated by a variety of extracellular stimuli (Fantone and Ward, 1982). Besides beneficial effects that PMNs possess in host defence, they also may contribute to tissue damage if the extent of toxic (bactericidal) agents is not sufficiently controlled or changed by modulators found in the extracellular environment of PMNs. For instance, ROS as well as hydrolytic enzymes, released both from the stimulated PMNs, contribute significantly to the tissue injury in heart disease and rheumatoid arthritis (Prasad *et al.*, 1990; Schiller *et al.*, 2000).

Lysophospholids (LPLs) are often generated by the action of phospholipase A_2 (PLA_2) on the *sn*-2 position of various phospholipid species. Neutrophils contain at least two different types of PLA_2: the cytosolic and the secretory form that are activated after cell stimulation (Marshall *et al.*, 2000; review in: Six and Dennis, 2000). The latter one is involved in inflammatory reactions (Murakami *et al.*, 1997). The role of free fatty acids and their metabolites in the regulation of NADPH oxidase activity is well documented (Shiose and Suminoto, 2000), whereas the role of LPLs in PMN functions is not yet sufficiently understood. Exogenous LPLs enhance the production of O_2.-, i.e. NADPH oxidase activity (Ginsburg *et al.*, 1989) and their role as signalling molecules affecting PMN function(s) was postulated.

In this study LPLs were produced in the membrane of PMNs by the addition of pancreatic PLA_2 ($pPLA_2$). PMNs do not possess a specific receptor for this enzyme and, therefore, higher concentration of LPLs as well as free fatty acids for studying the PMN functions can be easily generated. The action of this enzyme results in a release of free fatty acids and the corresponding lysophospholipids that we detected by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) in the PMN membrane. All together, digestion products of phospholipids significantly enhanced the respiratory burst activ-

[#] Both authors contributed equally to this work.

0939–5075/2001/1100–1150 $ 06.00

ity of human PMNs stimulated by chemotactic tripeptide (fMLP) or phorbol ester (PMA).

Materials and Methods

Material

Chemicals for the PMN purification, i.e. Hank's balanced salt solution (HBSS), Ficoll-Hypaque, dextran, heparin, and the stimulators, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) and phorbol myristoyl acetate (PMA) were purchased from Sigma (Deisenhofen, Germany). Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was a product from Boehringer-Mannheim (Germany). Hog pancreas phospholipase A_2 (PLA_2) was purchased from Fluka (Neu Ulm, Germany), showing an activity of 561 U/mg protein (1 U corresponds to the amount of the enzyme that hydrolyses 1 μmol of phosphatidylcholine in 1 min at 37 °C and pH = 8.0).

All solvents (chloroform and methanol), 2,5-dihydroxybenzoic acid (DHB) and trifluoroacetic acid (TFA) were also purchased from Fluka (Germany). All other chemicals were of highest commercially available purity and they were used without any further purification.

Cell preparation

Polymorphonuclear leukocytes (PMNs) were isolated from the heparinised (10 U/ml) blood of healthy volunteers as described elsewhere (Bøyum, 1964). Briefly, after dextran-enhanced sedimentation of erythrocytes, the supernatant was subjected to Ficoll-density gradient centrifugation. The remaining erythrocytes in the pellet were lysed three times for 1 min with distilled water. The resulting cell suspension was centrifuged and neutrophils obtained in the pellet were resuspended in HBSS and stored on ice until use. PMNs were used within 2 h after purification.

Chemiluminescence measurements

All chemiluminescence (CL) measurements were performed on a microplate luminometer MicroLumat LB 96 P (EG & G Berthold, Wildbad, Germany). Freshly prepared PMNs were incubated with hog pancreas PLA_2 for 5 and 20 min. Before addition of the enzyme solution in HBSS (280.5 U/ml, final activity) PMNs were prewarmed at 37 °C for 5 min. PMNs were incubated with $pPLA_2$ and reaction was terminated by spinning-off the PMNs. The cellular pellet was once washed with HBSS and PMNs were resuspended in HBSS.

After preincubation, the corresponding stimulators of the respiratory burst, namely fMLP (10^{-6} M, final concentration) or PMA (10^{-7} M, final concentration) were injected into the cell suspension and the CL response was monitored over 25 min. All CL experiments were repeated with three different cell preparations. CL after stimulation with fMLP or with PMA was measured as described. These results were compared with control PMNs (incubated at 37 °C with HBSS, for 5 and 20 min in the absence of PLA_2). The cell viability after purification and after treatment with $pPLA_2$ was checked by the trypan blue exclusion test.

Extraction of lipids from human neutrophils

PMNs were incubated with $pPLA_2$ as described above for CL measurement. PMNs were spun down and washed once with HBSS. The cellular pellet was resuspended in distilled water in order to decrease the content of inorganic ions in the sample, and the lipids were extracted according to the extraction procedure of Bligh and Dyer (1959) by vigorous mixing with chloroform/methanol mixture. After centrifugation, the chloroform layer was used for the mass spectrometric analysis.

Matrix and sample preparation for MALDI-TOF mass spectrometry

For MALDI-TOF MS, a 0.5 M 2,5-dihydroxybenzoic acid (DHB) solution in methanol containing 0.1% v/v trifluoroacetic acid (TFA) was used. TFA was used because small amounts of TFA improve the signal-to-noise ratio of the lipid spectra (Schiller *et al.*, 1999).

Chloroform was removed from the organic extracts of PMNs prepared as described above by vacuum centrifugation (Centrifugal Evaporator RC 10.10, Jouan, Winchester, Virginia, USA). After that, 20 μl of 0.5 M DHB solution in methanol was added and the sample was vigorously mixed. All samples were applied as 1.8 μl droplets onto the sample plate and were rapidly dried under a warm stream of air.

Mass spectrometry

All MALDI-TOF mass spectra were acquired on a Voyager Biospectrometry workstation (PerSeptive Biosystems, Framingham, MA, USA). The system utilises a pulsed nitrogen laser, emitting at 337 nm. Pressure in the ion chamber was maintained between 1×10^{-7} and 4×10^{-7} Torr. A two stage acceleration device allows to apply delayed extraction conditions (DE-mode), improving both mass resolution and mass accuracy (Hillenkamp *et al.*, 1991). The formed ions were accelerated by a 20 kV accelerating voltage within the ion source. In order to enhance the spectral resolution, the device was used in the reflector mode, so that the total field-free time-of-flight distance was 2 m. An internal calibration was performed by setting the peak of the protonated DHB-matrix to its appropriate value (155.034 Da). To enhance the reproducibility, 128 single shots from the laser were averaged for each mass spectrum and each sample was run in duplicate. The laser power was kept about 10% over threshold to obtain the best signal-to-noise ratio. All lipid spectra (besides those for calibration) were acquired by using the "low-mass gate" at 400 Da to prevent the detector from the saturation by ions arising from matrix peaks (Petković *et al.*, 2001a).

Results and Discussion

LPLs gained considerable scientific interest since they are important intermediates in the synthesis of various PLs, as well as they were found to be important intercellular messengers. LPLs influence numerous cellular functions such as growth and differentiation (Goetzl and An, 1998; Ozaki *et al.*, 1999; Sakai *et al.*, 1994). In studies on the effects of LPLs, these compounds are usually applied exogenously. In this study we tried to mimic "natural conditions" and to produce LPLs in the cell membrane by the addition of $pPLA_2$ to the PMN suspension as well as to investigate the influence of LPLs on the ROS production. This enzyme was chosen among other PLA_2 enzymes since PMNs do not possess a specific receptor for $pPLA_2$. Therefore, the observed effects are most probably mediated exclusively by products of the enzymatic digestion of phospholipids in the neutrophil membrane.

PMNs were incubated with $pPLA_2$ and the MALDI-TOF mass spectra of the corresponding lipid extracts of the cells were recorded. The mass region of the LPLs of control PMNs is presented in Fig. 1a, and the spectra of PMNs incubated for 5 and 20 min with PLA_2 are presented in (b) and (c), respectively. In agreement with the data of Marinetti and Cattieu (1982) certain amounts of LPC 18:0 (peak at m/z = 524.3 and at m/z = 546.3

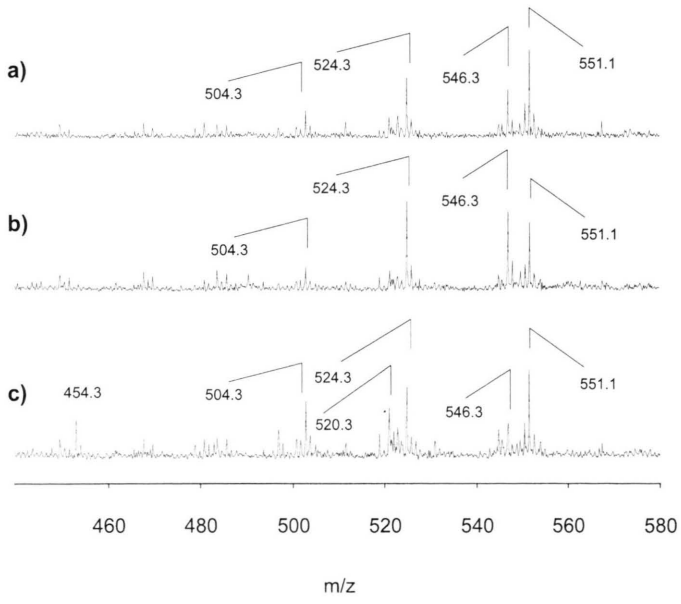

Fig. 1. Positive ion MALDI-TOF mass spectra of unstimulated polymorphonuclear leukocytes (PMNs) (a) and PMNs incubated for 5 (b) and 20 (c) min with pancreatic PLA_2. The mass region of lysophospholipids is presented. Spectra were recorded with 2,5-dihydroxybenzoic acid (DHB) as matrix and represent the average of 128 single laser shots.

for the proton and sodium adduct, respectively) are already present in unstimulated PMNs and the same holds for LPE 18:0 (the corresponding sodium adduct is clearly detectable at *m/z* = 504.3). All lysophospholipids give characteristic peak patterns in their mass spectra. They are described in more details elsewhere (Petković *et al.*, 2001b). For instance, LPE as a neutral lysophospholipid requires one singly positively charged ion to be detectable in the positive ion mode. Therefore, two peaks should arise from the proton (LPE+H) and the sodium adduct (LPE+Na). Besides these peaks, an additional peak is generated by the replacement of one proton by one sodium (LPE-H+2Na). In the spectra of isolated LPE the most intense peak corresponds to the sodium adduct of LPE (LPE + Na). The further two peaks are of much lower intensity and there is great probability that they will be suppressed completely when a complex lipid mixture is analysed (Petković *et al.*, 2001a; Schiller and Arnold, 2000). That is the case in the positive ion MALDI-TOF mass spectra of the lipid extract of human neutrophils, since under all expected peaks corresponding to LPE only a single one is detectable (at *m/z* = 504.3 corresponding to the sodium adduct of LPE 18:0).

Peak intensities in MALDI-TOF mass spectra, however, do not properly reflect the concentration of an analyte, and therefore, an internal reference must be included for comparison of different spectra (Petković *et al.*, 2001b). In our case, the intensity of the matrix peak at *m/z* = 551.1 was used as it was already done for the quantification of other lipid species like triglycerides (Asbury *et al.*, 1999).

According to the relative intensity ratios of the LPL peaks (at *m/z* = 524.3 and *m/z* = 546.3 that correspond to the proton and the sodium adducts of LPC 18:0, respectively) and the intensity of the characteristic matrix peak at *m/z* = 551.1 it is obvious that the intensity of peaks arising from LPC increased after 5 minutes incubation with PLA_2. After 20 min incubation of PMNs with PLA_2, however, the intensity of these peaks decreased (Fig. 1c) while the intensity of the following peaks increased: An additional peak at *m/z* = 454.3 was clearly detectable, and it most probably arises from the proton adduct of LPE 16:0. Additionally, peaks at *m/z* = 496.3 and *m/z* = 518.3 (not labelled in the figure) that correspond to the proton and the sodium adducts of LPC 16:0, respectively, appeared. The identity of the peak at m/z= 520.3 is not completely clear, and it might be attributed to the proton adduct of LPC 18:2, although this is of rather low probability. Finally, a clear increase of the peak at *m/z* = 504.3 corresponding to the proton adduct of LPE 18:0 was observed. This might indicate that LPC 18:0 generated after 5 min in the presence of PLA_2 was reacylated by PMNs after 20 min of incubation, and that other abundant PL species were also digested by PLA_2.

After changes in the LPL region were detected, one would also expect changes in the phospholipid region. This region is, however, crowded by peaks and can be rather difficult analysed. The most intense peaks arise form PC, since (a) this species is the most abundant cellular phospholipid, and (b) it is most easily detectable by MALDI-TOF MS (Schiller *et al.*, 1999). This is the first problem towards the analysis of this mass region. A second problem stems from the rather similar molecular masses of other abundant cellular lipids, mainly PE and PS, that can give a number of overlapping peaks. We were not able to monitor the corresponding changes in that mass region, but due to above described difficulties, we cannot rule changes completely out.

The respiratory burst response of human PMNs was investigated by luminol-amplified chemiluminescence. It is known that PMNs release ROS but also produce them intracellularly (Bender and Van Epps, 1983). Since luminol is a cell membrane permeable agent it detects both extra- and intracellular ROS production (Edwards, 1996; Müller and Arnhold, 2001).

In Fig. 2, a typical CL curve of human neutrophils treated with $pPLA_2$ for 5 min and stimulated afterwards with phorbol ester, PMA, is shown. The chemiluminescence of the corresponding control was also measured and provides by far lower CL intensity. The time of injection of PMA into the PMN suspension is indicated by an arrow in the figure. The typical CL response to PMA stimulation is a monophasic curve with a single maximum and it is generally accepted that CL in this case is caused by intracellular ROS generation (Bender and Van Epps, 1983). It is obvious that incubation of PMNs with $pPLA_2$ results in significantly higher CL response. Significant increase was observed also when the cells were stimulated

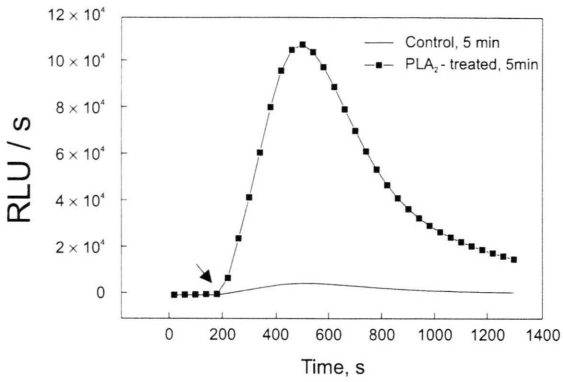

Fig. 2. Luminol-amplified chemiluminescence (CL) of human neutrophils preincubated with pancreatic PLA_2 for 5 min and stimulated afterwards with phorbol myristoyl acetate (PMA). The CL response of the corresponding control (cells incubated at 37 °C for 5 min with buffer, HBSS) is also presented in the figure. Human neutrophils were prior to stimulation with PMA treated with pancreatic PLA_2 and washed with Hank's balanced salt solution (HBSS). The time of injection of PMA into the cell suspension is indicated by an arrow. CL curves are representative data of three independent measurements. "RLU": relative light units.

with the chemotactic tripeptide, fMLP (data not presented). The only difference was that in the case of fMLP-stimulated cells, the typical response is a biphasic curve and after treatment of PMNs with $pPLA_2$, both phases were significantly enhanced (data not shown).

In additional experiments also the overall ROS production was determined by integrating the corresponding CL curves. For each incubation of neutrophils with $pPLA_2$ the corresponding control was also performed in parallel, i.e. cells were incubated at 37 °C for 5 and 20 min with HBSS buffer. Integral RLU / s – values that reflect the overall ROS production – for control cells as well as for those treated with $pPLA_2$ for 5 and 20 min are given in Table I.

The overall ROS production significantly increased after 5 and 20 min incubation with PLA_2 in comparison to the corresponding control. The most pronounced effect was obtained for PMA-stimulated PMNs incubated for 5 min with PLA_2 (an about twenty-fold increase in CL), whereas after 20 min incubation this effect was by far less pronounced (cf. Table I).

In the case of the fMLP-stimulated cells, an about tenfold increase was obtained after 20 min

Table I. Intensities of the luminol-amplified chemiluminescence of PMNs digested with hog pancreas PLA_2. The results are presented as the mean integral RLU / s (relative light units per s) that corresponds to the overall ROS production. Human neutrophils were incubated for 5 and 20 min at 37 °C with $pPLA_2$ or with buffer (control), washed with Hank's balanced salt solution (HBSS) and stimulated with the chemotactic tripeptide, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), or with phorbol ester, phorbol miristoyl acetate (PMA). CL measurements are conducted as described for Fig. 2. The mean values and standard deviations of two independent measurements are given.

	Integral RLU / s (mean ± sd)	
Preincubation of PMNs (37 °C)	fMLP-stimulation	PMA-stimulation
5 min, HBSS	$4.4 \times 10^5 \pm 2 \times 10^4$	$3.2 \times 10^6 \pm 1 \times 10^4$
5 min, $pPLA_2$	$2.9 \times 10^6 \pm 5 \times 10^4$	$6.2 \times 10^7 \pm 2 \times 10^5$
20 min, HBSS	$1.1 \times 10^5 \pm 6 \times 10^3$	$3.1 \times 10^7 \pm 5 \times 10^5$
20 min, $pPLA_2$	$1.1 \times 10^6 \pm 3 \times 10^4$	$3.5 \times 10^7 \pm 1 \times 10^5$

of incubation. After PMNs were incubated with PLA_2 for 5 min and then stimulated with fMLP, the overall ROS production indicated a sixfold increase in comparison to the corresponding control PMNs. This means that under fMLP stimulation conditions a completely different time-dependence in comparison to PMA was observed.

These results are in accordance with the data of Ginsburg *et al.* (1989) that were obtained upon the addition of exogenous LPC and the subsequent evaluation of the PMN respiratory activity by the cytochrome c reduction test. It can be assumed that LPC can act synergistically with calcium and diacylglycerol in the activation of protein kinase C in T-lymphocytes (Asaoka *et al.*, 1991) as well as in other cell types (Huwiler *et al.*, 1997). Since protein kinase C (PKC) plays an important role in signalling pathways leading to the NADPH oxidase activation in human PMNs, we assume that the increase in the amount of LPLs in the membrane would lead to an increased activity of PKC, and concomitantly enhanced ROS production. Additionally, the second product of $pPLA_2$ digestion of phospholipids from the membrane are free fatty acids. Their role in regulation of the NADPH oxidase activity was recently demonstrated (Shiose and Sumimoto, 2000). Due to the "low mass gate" applied for MALDI-TOF mass spectrometry we were not able to detect free fatty acids in the lipid extract of human PMNs and in general, de-

tection of free fatty acids by MALDI-TOF MS is difficult. However, their simultaneous presence is clearly indicated by an increase in the lysophospholipid concentration in the lipid extract of human neutrophils. Therefore, free fatty acids and / or their metabolites also contribute to the observed effects.

We have shown recently that exogenously added LPC acts in a completely different way, i.e. this LPL inhibits under those circumstances in a concentration-dependent manner the CL response of PMA-stimulated as well as the first phase of fMLP – stimulated PMNs, whereas the second phase was enhanced (J. Müller *et al.*, submitted). We have also found that this LPL is incorporated in the membrane of PMNs and therefore, it is of great probability that the effects described in this report are mediated also by the released fatty acids and/or their metabolites. Although the exogenous addition of LPLs might be helpful for elucidating the effects of a single species, treatment of PMNs with $pPLA_2$ is – to our opinion – by far more related to the situation under *in vivo* conditions than the exogenous addition of lysophospholipids.

In conclusion, it has been clearly shown that the treatment of human PMNs with pancreatic phospholipase A_2 leads to the increase in LPL concentration and subsequently to an increase in the production of reactive oxygen species. This approach might become useful for studying the involvement of LPLs in intracellular signalling pathways, since in this way the receptor-mediated – either for PLA_2 or for various further LPLs – pathways can be completely excluded.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG-Grant AR 283/4–1). The authors wish to thank Dr. D. Haferburg (Faculty of Biosciences, University of Leipzig) for his kind help in all aspects of mass spectrometry.

Asaoka Y., Oka M., Yoshida K. and Nishizuka Y. (1991), Lysophosphatidylcholine as a possible second messenger synergistic to diacylglycerol and calcium ion for T-lymphocyte activation. Biochem. Biophys. Res. Commun. **178**, 1378–1385.

Asbury G. R., Al-Saad K., Siems W. F., Hannan R. M. and Hill HH, Jr. (1999), Analysis of triacylglycerols and whole oils by matrix-assisted laser desorption/ionization time of flight mass spectrometry. J. Am. Soc. Mass Spectrom. **10**, 983–991.

Bender J. G. and Van Epps D. E. (1983), Analysis of the bimodal chemiluminescence pattern of stimulated neutrophils by chemotactic factors. Infect. Immun. **41**, 1062–1070.

Bøyum A. (1964), Separation of white blood cells. Nature **204**, 793–794.

Edwards S. W. (1994), Biochemistry and physiology of the neutrophil. Cambridge: Cambridge University Press.

Edwards S. W. (1996), The O_2^- generating NADPH oxidase of phagocytes. Structure and methods of detection. Methods **9**, 563–577.

Fantone J. and Ward P. A. (1982), Role of oxygen-derived radicals and metabolites in leukocyte – dependent inflammatory reactions. Am. J. Pathol. **107**, 397–418.

Ginsburg I., Ward P. A. and Varani J. (1989), Lysophosphatides enhance superoxide responses of stimulated human neutrophils. Inflammation **13**, 163–174.

Goetzl E. J. and An S. (1998), Diversity of cellular receptors and functions for the lysophospholipid growth factors lysophosphatidic acid and sphingosine 1-phosphate. FASEB J. **12**, 1589–1598.

Hillenkamp F., Karas M., Beavis R. C. and Chait B. T. (1991), Matrix-assisted laser desorption / ionization mass spectrometry of biopolymers. Anal. Chem. **63**, 1193A-1203A.

Huwiler A., Saudt G., Kramer R. M. and Pfeilschifter J. (1997), Cross-talk between secretory phospholipase A_2 and cytosolic phospholipase A_2 in rat renal mesangial cells. Biochim. Biophys. Acta **1348**, 257–272.

Marinetti G. V. and Cattieu K. (1982), Composition of phospholipids of human leukocytes. Chem. Phys. Lipids **31**, 169–177.

Marshall J., Krump E., Lindsay T., Downey G., Ford D. A., Zhu P., Walker P. and Rubin B. (2000), Involvement of cytosolic phospholipase A_2 and secretory phospholipase A_2 in arachidonic acid release from human neutrophils. J. Immunol. **164**, 2084–2091.

Müller S. and Arnhold J. (2000), Light emission of luminol and its application. In: Chemiluminescence at the Turn of the Millennium – an Indispensable Tool in Modern Chemistry, Biochemistry and Medicine (Albrecht S., Zimmerman T. and Brandl H., ed.). Schweda-Werbedruck-Verlag Dresden, pp. 23–28 (ISBN 3-9807853-0-0).

Murakami M., Nakatani Y., Atsumi G., Inoue K. and Kudo I. (1997), Regulatory functions of phospholipase A_2. Curr. Rev. Immunol. **17**, 225–283.

Ozaki H., Ishii K., Arai H., Kume N. and Kita T. (1999), Lysophosphatidylcholine activates mitogen-activated protein kinases by a tyrosine-dependent pathway in bovine aortic endothelial cells. Atherosclerosis **143**, 261–266.

Petković M., Schiller J., Müller M., Benard S., Reichl S., Arnold K. and Arnhold J. (2001a), Detection of individual phospholipids in lipid mixtures by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: Phosphatidylcholine prevents the detection of further species. Anal. Biochem. **289**, 202–216.

Petković M., Schiller J., Müller J., Müller M., Arnold K. and Arnhold J. (2001b), The signal-to-noise ratio as the measure for quantification of lysophospholipids by matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry. Analyst **126**, 1042–050.

Prasad K., Kalra J., Chandhary A. K. and Debnath D. (1990), Effects of polymorphonuclear leukocyte-derived oxygen free radicals and hypochlorous acid on cardiac function and some biochemical parameters. Am. Heart J. **119**, 538–550.

Sakai M., Miyazaki A., Hakamata H., Sasaki T., Yui S., Yamazaki M., Shichiri M. and Horiuchi S. (1994), Lysophosphatidylcholine plays an essential role in the mitogenic effect of oxidized low density lipoprotein on murine macrophages. J. Biol. Chem. **269**, 31430–31435

Schiller J., Arnhold J., Benard S., Müller M., Reichl S. and Arnold K. (1999), Lipid analysis by matrix-assisted laser desorption and ionization mass spectrometry: A methodological approach. Anal. Biochem. **267,** 45–56.

Schiller J., Benard S., Reichl S., Arnhold J. and Arnold K. (2000), Cartilage degradation by stimulated human neutrophils: Reactive oxygen species decrease markedly the activity of proteolytic enzymes. Chem. Biol. **7**, 557–568.

Shiose A. and Sumimoto H. (2000), Arachidonic acid and phosphorylation synergistically induce a conformational change of $p47^{phox}$ to activate the phagocyte NADPH oxidase. J. Biol. Chem. **275**, 13793–13801.

Six D. A. and Dennis E. A. (2000), The expanding superfamily of phospholipase A_2 enzymes: Classification and characterization. Biochim. Biophys. Acta **1488**, 1–19.